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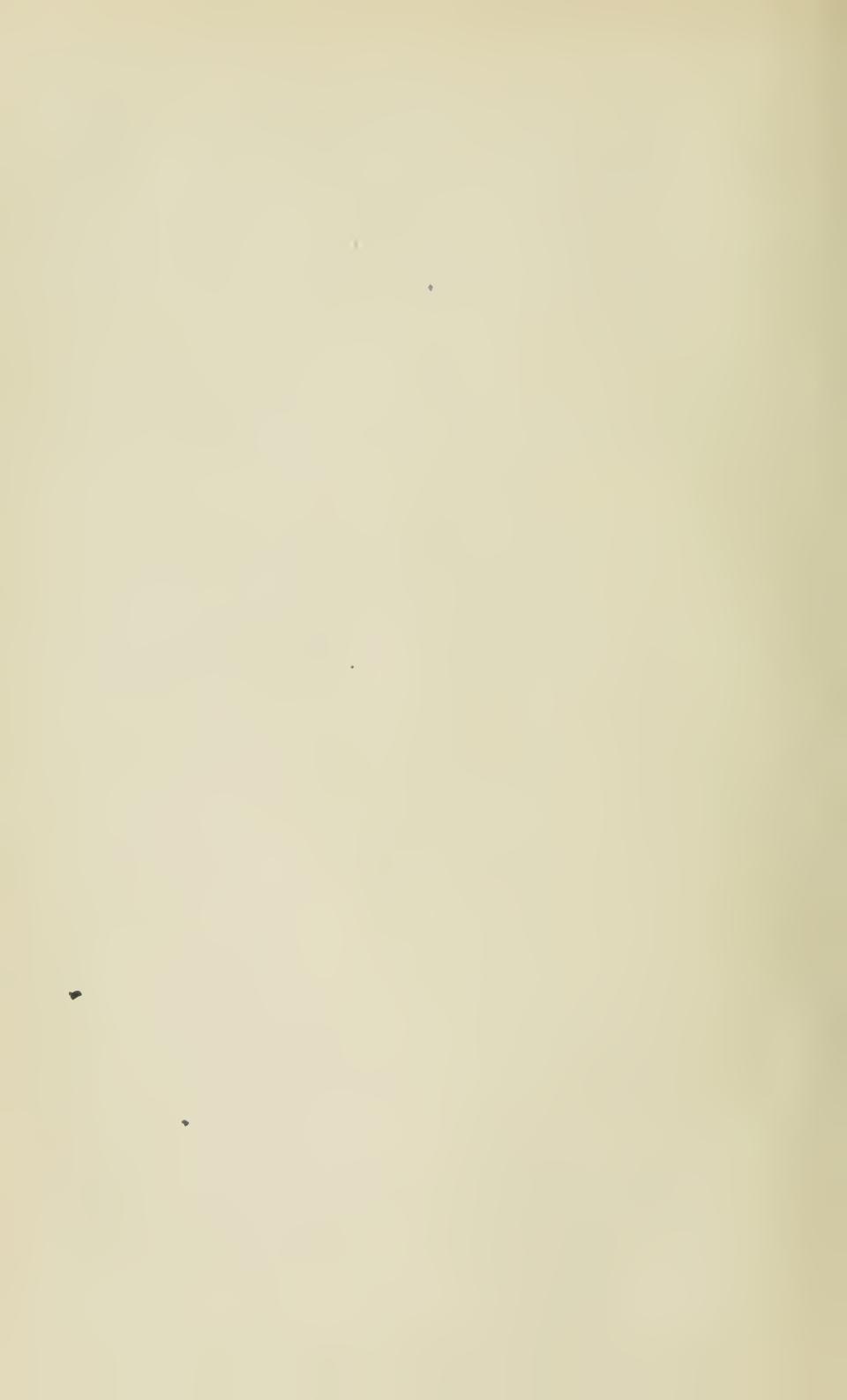
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THE

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NO. I.

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ON THE ACTION OF CHLORAL HYDRATE ON THE  
HEART WITH REFERENCE TO THE SO-CALLED  
PHYSIOLOGICAL PROPERTIES OF HEART MUSCLE.

A. J. CARLSON.

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

I.

THE peculiarities of the heart tissues as revealed by the response of the heart to direct stimulation are generally considered to indicate the properties of the heart muscle, only scant consideration being usually given to the part that the intrinsic nervous system in the heart may play in these reactions. In the reaction of the heart to direct stimulation the influence of extrinsic nerves and nerve endings may possibly be eliminated by section of these nerves and allowing time for degeneration. The cardiac fibres of the vagi may possibly be eliminated by atropin and nicotine, although the point of action of these drugs in the vertebrate heart is still a matter of inference by analogy. According to Hering there is no drug that will abolish the action of the augmentor nerves on the heart without impairing the heart muscle.<sup>1</sup> The fact that the ventricular apex exhibits the reaction characteristic of the heart tissues on direct stimulation may be urged as evidence that the intrinsic nervous tissue is not a factor in these reactions, but even the apex of the ventricle contains the typical nerve plexus or intramuscular nerve net, and in some vertebrates at least, ganglion cells.<sup>2</sup>

<sup>1</sup> HERING : *Archiv für die gesammte Physiologie*, 1903, xcix, p. 250.

<sup>2</sup> CARLSON : *Archiv für die gesammte Physiologie*, 1905, cix, p. 51.

Unless the intrinsic nervous system in the heart walls is thrown out of function it is obvious that any stimulus that affects the muscle cells will at the same time act on the nervous tissue. In fact, the nervous tissue may be more highly excitable than the muscle cells to many forms of stimuli, so that some of the reactions of the heart to direct stimulation may be caused by the stimulation of the nervous tissue alone. It would then seem obvious that "as yet we do not know the properties of the heart muscle in the vertebrates apart from the intrinsic nervous tissue."<sup>1</sup> It may be that the peculiarities revealed by the response of the heart to direct stimulation are due to the peculiar properties of the heart muscle, but it is also possible that they are due to the peculiar properties of the intrinsic nervous tissue in the heart and to the physiological interaction between the two tissues. Direct proofs are lacking in support of either view.

## II.

Harnack,<sup>2</sup> Böhme,<sup>3</sup> and Rohde<sup>4</sup> have recently made important contributions towards the solution of this question. When the heart of the frog or the tortoise is subjected to the action of mono-iodine aldehyde or chloral hydrate it is brought to a standstill in diastole after a primary augmentation of the rhythm, while the heart still retains its excitability and contractility, as shown by its response to direct stimulation. The first action of these drugs is therefore the abolition of automaticity after a primary stimulation. If the drugs are confined to the ventricle, the latter continues to beat in synchrony with the auricles as long as it retains its excitability to direct stimulation. The work of Rohde seems to show that at a certain stage in the action of chloral hydrate on the heart all special peculiarities of response of the heart to direct stimulation are abolished while excitability and contractility of the heart walls still remain. At this stage the heart responds to direct stimulation like an ordinary skeletal or smooth muscle. It is readily tetanized, the characteristic refractory period is not in evidence, and the heart responds to stimuli of gradually increasing strength with contractions of gradually increasing

<sup>1</sup> CARLSON: This journal, 1905, xii, p. 494.

<sup>2</sup> HARNACK: Archiv für Physiologie, 1904, p. 415.

<sup>3</sup> BÖHME: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liii, p. 346.

<sup>4</sup> ROHDE: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liv, p. 104.



amplitude. Rohde's interpretation of these results are that the chloral hydrate paralyzes the intrinsic nervous tissues in the heart before complete paralysis of the heart muscle is effected, and the heart muscle thus separated from the intrinsic nervous tissues responds to direct stimulation much like ordinary skeletal or smooth muscle. The peculiarities in the response of the normal heart to direct stimulation is therefore due to the direct stimulation of the nervous tissues in the heart. Rohde cites experiments of Uexküll (on *Sipuculus*), Bethe (on *Medusæ*), and Magnus (on the intestines) as supporting by analogy this interpretation.

Rohde's experiments are, however, capable of another interpretation. Such a powerful general anæsthetic as chloral hydrate must necessarily act on all the tissues in the heart. It is conceivable that at a certain stage in the action of the chloral hydrate on the muscle cells these cells lose their peculiar properties of refractory state — all or none response and non-tetanizability — while the more fundamental properties of excitability and contractility are retained till a later stage in the action of the drug. In other words, the chloral hydrate may act on the heart muscle itself in a way to alter its response to artificial stimulation. Personally, I do not think that this objection will prove true, but as long as this interpretation remains a possibility the question must be recognized as an open one.

### III.

In the heart of *Limulus*, Rohde's interpretation of the action of chloral hydrate may be put to the experimental test. The function of the intrinsic nervous system in this heart is known. It is, furthermore, without destroying the heart rhythm, possible so to separate the automatic heart ganglion, the nerve plexus, and the heart muscle that the action of a drug may be confined to either tissue without affecting the other. In this way it is a relatively simple matter to determine the point of action of any drug in this heart as well as the intensity or rapidity of action of general protoplasmic poisons, like chloral hydrate, on the muscular and the nervous tissues. An extended series of experiments along this line have been carried out making use of a number of drugs. The general results of this work are soon to be reported. Chloral hydrate was included in the list of drugs studied. The experiments with this drug were carried out in the summer of 1905. The two main points of interest in the action

of this drug on the *Limulus* heart were that the heart ganglion is more sensitive to the drug than the heart muscle, and that the drug has a primary stimulating action on the heart ganglion, while it depresses the heart muscle without any initial stimulation. These characteristics are, however, not peculiar to chloral hydrate, as several drugs act in the same manner on the ganglion and on the muscle. On the appearance of Rohde's paper I decided to repeat some of the experiments with chloral hydrate with a view of determining whether this drug completely paralyzes the nervous tissues in the heart before the muscular tissue is paralyzed. For accounts of the methods of preparing the heart and recording the heart action, in these and similar series of experiments, the reader is referred to one of my previous papers.<sup>1</sup> The drug was used in isotonic solution ( $\frac{6}{10} m$ ), and this was mixed with *Limulus* plasma or sea water in any proportion desired.

## IV.

*Chloral hydrate has a primary stimulating action on the heart ganglion.* The stronger the concentration of the drug, the greater the primary stimulating action. When a solution of the drug is applied to the isolated posterior end of the heart ganglion, the first heart segment being used for recording, the rhythm is greatly augmented both in rate and intensity. This augmentation may approach incomplete tetanus before the depressor phase and ultimate cessation of the ganglionic activity sets in. The augmented ganglionic rhythm is nearly always irregular in the way of alternating strong and weak nervous discharges, grouping of the nervous discharges, etc.

*Chloral hydrate, dissolved in the plasma or sea water surrounding the heart, depresses the excitability and contractility of the heart muscle without primary stimulation.* No strength of the drug was found that would give a distinct increase in the strength of the contraction when confined to the heart muscle (and nerve and nerve-endings in the muscle), but the stronger concentrations of the drug tend to produce an increased tonus in the muscle. This tonus contraction is not accompanied by any increase in the amplitude of the beats, and it does not appear when relatively weak concentrations of the drug are employed. It is therefore evident that chloral hydrate has primarily an opposite action on the two tissues of the heart, — a primary stimulating action on the heart ganglion, and a primary and

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 207.

continuous depressing action on the heart muscle (and nerve endings), while the final action of the drug is the same for both tissues, namely, complete anæsthesia or paralysis. Because of the greater sensitiveness of the heart ganglion to the drug, the primary stimulating action is first in evidence when the chloral hydrate is applied to the whole heart. A similar primary stimulating action of chloral hydrate on the frog's heart is recorded by Rohde. So far, then, the action of this drug is the same on the frog's heart and the *Limulus* heart. In the case of the *Limulus* heart, the primary augmented rhythm following the application of the drug is due to the action of the drug on the automatic ganglion. This may also be the mechanism of the primary stimulation in the vertebrate heart, but this view is an interpretation only; a demonstration of it is so far wanting.

## V.

*When a solution of chloral hydrate is applied to the entire heart, the heart tissues are ultimately paralyzed in the following order: ganglion, nerves, or nerve endings, muscle.* The automatic activity and reflex function of the ganglion appear to be abolished simultaneously. At any rate, I never succeeded in obtaining any reflex response from the ganglion on stimulation of any of the nerves leading from the ganglion to the heart muscle after the ganglionic rhythm had ceased completely in the chloral narcosis. Such reflex responses are always obtained from the ganglion under normal conditions, as I have pointed out in a previous paper.<sup>1</sup> At the stage when the automatic and reflex activities of the heart ganglion are completely suppressed by a solution of chloral hydrate acting on the whole heart, direct stimulation of the nerves leading from the ganglion to the heart muscle still causes contraction of the latter, and when on continued action of the drug the muscle finally ceases to respond to the stimulation of the nerves, direct stimulation of the muscle itself still produces contraction. Accurate time measurements were not made, but in a solution of the drug that abolishes automatism and reflex activity of the ganglion in about ten minutes, the nerves remain excitable for fifteen to twenty minutes, and the heart muscle itself thirty to forty-five minutes longer. There can be, therefore, no question of the accuracy of the above thesis for the *Limulus* heart. I have spoken of the stage when the muscle ceases to respond to the stimulation of the

<sup>1</sup> CARLSON: This journal, 1905, xii, p. 483.

nerves, as the point of paralysis of the nerves. It may be, of course, that it is the nerve endings and not the nerve plexus itself that is completely narcotized, or it is possible that the excitability of the heart muscle itself is so greatly reduced that it no longer responds to the impulses from the nerves, although it can be made to contract by a strong electrical stimulus sent directly through the muscle cells. But these possibilities have no bearing on the question before us, for no

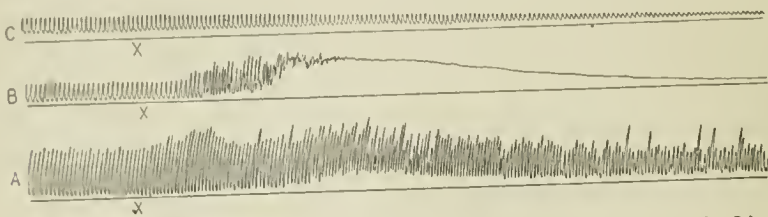


FIGURE 1. — Two-fifths the original size. Tracings from the anterior end of the *Limulus* heart. *A*, heart ganglion isolated posteriorly, recording end of heart bathed in plasma; *X*, chloral hydrate and plasma in the proportion of 1 to 50, applied to the ganglion, showing primary stimulation of the ganglion. *B*, heart prepared as in *A*; *X*, chloral hydrate and plasma in proportion of 1 to 15 applied to the ganglion, showing primary stimulation and tetanus of the ganglion. *C*, ganglion extirpated in the first two heart segments, the lateral nerves being left intact; *X*, chloral hydrate and plasma in proportion of 1 to 15 applied to the ganglion free recording end, showing gradual diminution in the amplitude of the contraction.

matter where the paralysis is located at this stage, it is obvious that on direct stimulation of the heart the stimulation of the intrinsic nerve plexus takes no part in the results.

## VI.

The results on the *Limulus* heart are therefore in complete agreement with Rohde's interpretation of the mechanism of the change in the response of the vertebrate heart to direct stimulation during the different stages of chloral hydrate narcosis. In the *Limulus* heart the results are a matter of demonstration, not of interpretation. But in so far as the action of this drug appears to be identical in the *Limulus* and the frog's heart, it seems to me, these results in *Limulus* lend some support to Rohde's theory. I have pointed out in a previous paper some of the changes in the response of the *Limulus* heart to direct stimulation following extirpation of the ganglion. Complete paralysis of the ganglion by a drug is, of course, equivalent to extirpation.

According to a preliminary statement by Howell, Mr. Schultz, one of Howell's students, has failed to confirm Rohde's results, particularly as regards the abolition of the refractory period of the heart tissue at a certain stage of the chloral hydrate narcosis, Howell maintaining that the heart tissues retain the property of a marked refractory period, even after subjection to the action of chloral hydrate.<sup>1</sup> It is difficult to understand how contradictory results should be obtained in a matter that may be determined by so simple and direct experiments. If chloral hydrate abolishes the marked latent period of the heart tissues this action is probably gradual, so that one may find that the refractory condition remains in the earlier stages of the narcosis, while in the later stages it is less in evidence. The refractory state is, of course, not peculiar to the heart tissues. In the heart of invertebrates, and at least some of the vertebrates, it is a condition of reduced excitability only, and not a state of inexcitability.<sup>2</sup>

<sup>1</sup> HOWELL: *Journal of the American Medical Association*, 1906, xlvi, p. 1670.

<sup>2</sup> CARLSON: *Zeitschrift für allgemeine Physiologie*, 1904, iv, p. 259; *This journal*, 1906, xvi, p. 72.

## THE INFLUENCE OF OSMOTIC PRESSURE ON THE IRRITABILITY OF SKELETAL MUSCLE.

By WALTER J. MEEK.

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

THE experiments reported in this paper were undertaken at the suggestion of Professor Carlson, with a view to determine whether the effect of hypertonicity and hypotonicity on muscle cells is the same as on ganglion cells. Carlson has shown that in the case of the *Limulus* heart and the tortoise auricle hypertonicity of the surrounding medium depresses the rate and decreases the amplitude of the beat, while hypotonicity acts as a primary stimulus to the rhythm and increases the amplitude of the beats.<sup>1</sup> In the *Limulus* heart the action is the same on the heart ganglion and on the heart muscle. In the case of the latter the action might be either on the muscle cells or on the motor nerve endings. There is no way of deciding between the two possibilities, since no drug is known that will paralyze the motor nerve endings in the heart without injuring the heart muscle itself. In order to clear up this point it was decided to try the effect of hypotonic and hypertonic solutions on curarized skeletal muscle. The problem has been, first, to find out whether hypertonicity and hypotonicity act in the same way on skeletal muscle as on heart muscle, and, secondly, with the aid of curare, to determine whether these effects were due to action on the muscle cells or the motor nerve endings.

The gastrocnemius muscles of frogs were used, and the solutions were applied by perfusion. The frogs were either pithed or curarized. The gastrocnemius was freed from the bone at its point of attachment. The tibia was then fastened in a clamp to support the limb, while the body of the frog was fastened on a board ventral side up. The removal of the muscle was done with some care to avoid injuring or compressing the arteries and veins of the region. The gastrocne-

<sup>1</sup> CARLSON: This journal, 1906, xv, pp. 357-370.

mius was then attached by its lower end to a muscle lever in the usual way.

For perfusion, a small three-way cannula was introduced into the right systemic arch of the aorta. The arch of the aorta was used in preference to the dorsal aorta merely for the sake of convenience. The left aortic arch was ligatured. The cannula was connected by rubber tubes with funnels containing the desired solutions. By means of clamps on these tubes the different solutions could be thrown in at will. Perfusion was often aided by compressing the tubes at intervals. The level of the liquid was maintained at a height of about 40 cm. Enough blood vessels are ruptured in the preparation of the frog to prevent any great œdema, and it was not found necessary to sever the vena cava for this purpose.

The time of perfusion usually varied from twenty minutes to an hour or even more. By perfusion time here, we mean the time elapsing before the effect of the solutions becomes noticeable. The rapidity and degree of perfusion may be told by the change of color in the muscle due to the washing out of the blood.

Two kinds of hypertonic solutions were used in these experiments. The first was a double-strength Ringer's solution, and the second a normal Ringer's solution to which  $\frac{1}{4}$  cane sugar had been added. The results were identical for the two solutions, the only difference in action being that the sugar solution seems a little more rapid in action.

Hypotonicity was obtained by dilution of Ringer's solution with distilled water.

The muscle was in all cases directly stimulated by a single shock from the secondary coil of an induction machine. Uniformity of time was secured by use of a metronome to break the primary circuit. Stimuli of sub-maximal strength were always used. This was necessary in order to have the muscle show an immediate increase or decrease in its curve should the excitability or contractility be affected. The height of the contraction was taken as the measure of the effect of the solutions. The stimuli were thrown in at the rate of about 32 per minute.

In all the experiments the mode of procedure was to perfuse for some time with a Ringer's solution and then record the height of contraction for a given sub-maximal stimulus. The strength of the stimulus was then kept unchanged through the remainder of the experiment. The height of the curve under these conditions was taken

as the standard for comparing those obtained with hypertonicity and hypotonicity.

#### THE INFLUENCE OF HYPERTONICITY.

Hypertonicity depresses contractility and excitability<sup>1</sup> without primary stimulation.—In all cases the amplitude of the muscular contraction is diminished by perfusing with a hypertonic solution. For

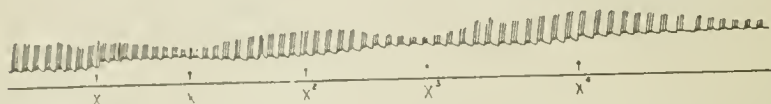


FIGURE 1.—One-third the original size. Tracing from curarized gastrocnemius showing depressing effect of hypertonicity. Perfusion with Ringer's solution. Double strength solution admitted at  $x$ ,  $x'$ , and  $x''$ . Isotonic solution admitted at  $x'''$  and  $x''''$ . Wide intervals show that drum was stopped for one minute.

a short time after the hypertonic solution is admitted the amplitude of contraction remains unchanged, but soon after the solution begins to wash out the Ringer's of normal strength the height of contraction begins gradually to decrease.

The time required for the solution to take effect depends on the rapidity of perfusion and the condition of the muscle. In muscle that has been well washed out with Ringer's solution and where perfusion is rapid, the curve begins to fall in a few seconds after the hypertonic solution is thrown in. Fig. 1 shows a period of about two minutes before the effect is noticeable, but often the decrease begins in less time.

If the perfusion is continued, the excitability and contractility of the muscle is lowered until no contraction whatever is obtained for the given stimulus. This ordinarily takes place in from five to ten minutes, and the depression is maintained until the hypertonic solution is removed.

There is some evidence that the decrease in excitability and contractility is directly proportional to the concentration of the solution, as noted by Carlson for the heart. No attempt was made to prove this point definitely. The fact, however, that the effect of the hypertonicity always shows itself in a gradually descending curve would indicate that the muscle decreased in excitability and contractility as

<sup>1</sup> The changes in the response of the muscle to direct stimulation are referred to as changes in excitability and contractility, as it is not plain which of these two factors is involved.



the plasma in the intercellular spaces or the substance of the muscle cells becomes more hypertonic.

Recovery of the muscle after replacing the hypertonic solution with isotonic Ringer's is always gradual, and usually much slower than the depression. This is invariably true if the muscle has been brought to the point where the contraction has been entirely abolished. In case the contraction has been only slightly diminished recovery takes place quickly. The height of the curve after recovery is seldom as great as at the beginning. If perfusion is long main-



FIGURE 2.—One-half the original size. Tracing from curarized muscle to show stimulating action of hypotonicity and depression by hypertonicity. Perfusion with Ringer's solution. First contractions while muscle was perfused with isotonic solution. Hypotonic solution admitted at  $x$  and hypertonic at  $x'$ .

ained, there seems to be some kind of a permanent injury to the muscle. After recovery a second perfusion causes a second depression, and this may be repeated several times.

Hypertonic solutions sometimes cause contracture of the muscle, although this does not seem to be a necessary accompaniment of the depressor effects. It is possibly due to the salt solution employed, and thus has little bearing on the hypertonicity.

Figs. 1, 2, and 3 illustrate these several facts. In Fig. 1 we have at the beginning contractions of a fairly regular height with perfusion by an isotonic solution. Ringer's solution of double strength was thrown in at  $x$ . For a space of two minutes the curve maintains its height, but then it begins to fall, until it is restored by putting in an isotonic solution at  $x'$ . This is repeated three times. A contracture is also noticeable. In Fig. 3 the hypertonic cane sugar solution admitted at  $x$  quickly reduced the height of contraction to zero with a decided contracture. Isotonic Ringer's solution (Fig. 3 B) brought back the excitability and contractility, but the curve did not again reach its original height.

Fig. 1 is from a tracing made with a curarized muscle. The results after the use of curare are apparently identical with those obtained on non-curarized muscle. Many experiments were made on this point and no exceptions were noticed. This result is of importance in

locating the point of action of the hypertonic solution. The action is undoubtedly on the muscle cells themselves.

### THE INFLUENCE OF HYPOTONICITY.

Hypotonicity increases the excitability and contractility of skeletal muscle without any primary depression. — In all cases the effect of hypotonicity is directly the opposite of hypertonicity; that is, it in-

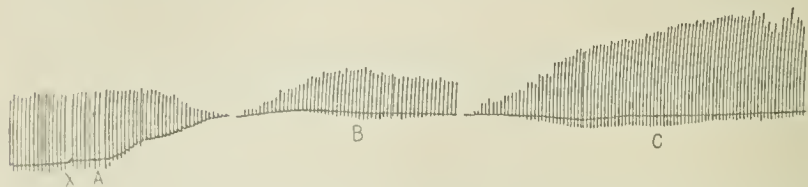


FIGURE 3.— One-half the original size. Tracing from non-curarized muscle. First contractions in *A* while muscle was perfused with isotonic Ringer's solution. Ringer's solution +  $\frac{m}{4}$  cane sugar admitted at *x*. The contractions decrease to zero. The space between *A* and *B* equals two minutes during which isotonic Ringer's solution was perfused. Contractions returned in *B*, but not to original weight. Space between *B* and *C* equals five minutes. The contractions returned to zero with isotonic perfusion. Hypotonic solution was then admitted with the stimulation resulting shown in *C*.

creases the height of the muscular contraction. The ease with which a hypotonic solution brings a muscle back to its former condition after hypertonicity was soon observed. This, however, was not taken as a final test of the stimulating action of hypotonicity. As before, the muscle was washed out with Ringer's of normal strength and then the hypotonic solution admitted. The amplitude of contraction under these conditions soon begins to increase. This continues until a maximum is reached, which is maintained until fatigue comes on.

The increase in excitability and contractility develops gradually but slowly when compared to the decrease under hypertonicity. In a well-perfused muscle it frequently takes one to five minutes to become noticeable, and the maximum may appear much later than this.

Hypotonic solutions of whatever degree of strength act as stimulants. The augmentation seems to be directly proportional to the dilution. The tracing reproduced in Fig. 2 illustrates this fact. After contraction had been abolished by the hypertonic solution, it was brought back to an average height of about 12 mm. by a solution of normal strength, while a hypotonic solution of one-half strength increased this height to about 25 mm. This is an increase roughly

proportional to the dilution, for the normal solution may be considered as hypotonic in comparison with the hypertonic which preceded it.

The length of time that a muscle will maintain an increased amplitude after perfusion with a hypotonic solution was not determined. In one case it remained for about five minutes. It is undoubtedly more or less temporary.

Figs. 2, 3, and 4 illustrate the above points for hypotonicity. In Fig. 3 it will be noted that after a partial recovery from hypertonicity by the use of Ringer's solution of normal strength, hypotonicity raises the curve above the height at the beginning of the experiment. Fig. 4 shows an increase in amplitude without a preceding depression from hypertonicity.

Figs. 2 and 4 reproduce tracings from curarized muscles. As in the case of hypertonicity, paralyzing the nerve endings has no effect on the action of the solution. The result here confirms the statement already made, that the action is directly on the muscle cells.

It is not the purpose of this paper to enter at length upon theoretical considerations concerning the phenomena described. We are certain from the experiments with curare that the muscle cells are directly affected, but the mechanism by which this is brought about is not at all clear. Carlson has pointed out that the effect of the solutions is probably not due to increase or decrease of pressure on the cell membranes with consequent loss or imbibition of water. As suggested by him, it may be due to changes brought about by alteration in the permeability of the cell walls. Muscle undoubtedly works best in the long run under normal osmotic conditions. For a time it is evident it might accomplish more under the stimulation of hypotonicity, but this doubtless would be only temporary, and possibly would be followed by absolute injury.

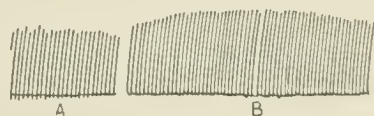


FIGURE 4. — One-half the original size. Tracing from curarized muscle to show the effect of hypotonicity. *A* shows height of contraction during perfusion with isotonic Ringer's solution. *B* shows curve after perfusing with hypotonic solution.

#### SUMMARY.

1. Hypertonicity depresses contractility and excitability without primary stimulation. In all cases perfusion with double strength Ringer's solution or Ringer's solution + isotonic cane sugar diminishes the amplitude of the muscular contraction.

2. Hypotonicity increases the excitability and contractility without primary depression. In all cases the effect of hypotonicity is the opposite of hypertonicity; that is, the height of the muscular contraction is increased.

3. Curarized skeletal muscle gives the same reactions to hypotonicity and hypertonicity as uncurarized. The point of action is therefore on the muscle cells.

# THE EFFECT OF SUBCUTANEOUS AND INTRAVENOUS INJECTIONS OF SOME SALINE PURGATIVES UPON INTESTINAL PERISTALSIS AND PURGATION.

By JOHN AUER.

[From the Rockefeller Institute for Medical Research, New York.]

A DETAILED consideration of the extensive literature on this subject is not necessary, as Hay's monograph<sup>1</sup> gives a critical summary of the work accomplished up to 1884, and Eckardt's dissertation<sup>2</sup> brings this matter almost up to date. The most important references, however, will be found in the discussion of the experimental results obtained in this investigation.

## THE EFFECT OF SUBCUTANEOUS INJECTIONS UPON PURGATION.

Rabbits, ranging in weight between 1500 and 2500 grams, were used. The injections were invariably made into the subcutaneous tissue of the lumbar region, and the site moderately massaged afterwards. The animals were then placed in separate cages, and the fæces collected at certain intervals (see below for details). They were fed on cabbage, turnip, carrots, and oats; no water was given.

In order to gain some idea concerning the amount and character of the fæces passed by rabbits when kept confined in separate cages, five rabbits were observed for a number of weeks. It was found that the twenty-four hour amount varied between nothing and sixty grams; the average for forty-eight determinations was nineteen grams, but this average means very little when the surprising range above noted is considered. As a rule, the fæces were hard, dry, and well formed; but occasionally unformed masses were found, together with normal pellets. More frequently than unformed fæces, soft, discrete,

<sup>1</sup> HAY: *Journal of Anatomy and Physiology*, 1881, xvi, p. 593; *Ibid.*, 1882, xvii, p. 435.

<sup>2</sup> ECKARDT: *Inaugural Dissertation*, Giessen, 1905.

caked together pellets were seen; these also did not compose the entire output, but only a small part of it. It may be added here that the passage of soft masses of pellets has been observed a number of

## FEBRUARY 15.

		One hour after injection.	Total, 5 hours.	Total, 24 hours.
Rabbit 1 . . . .	male	0 fæces.	0	0
Rabbit 2 . . . .	female	3 gm.; soft, caked.	3 gm.	5 gm.
Rabbit 3 . . . .	male	1 gm.; dry.	1 gm.; dry.	1 gm.
Rabbit 4 . . . .	female	1 gm.; dry.	1 gm.; dry.	1 gm.
Control . . . .	male	1 gm.; dry.	1 gm.; dry.	1 gm.
Control . . . .	female	14 gm.; dry.	14 gm.; dry.	16 gm.

Rabbits 1 to 4, inclusive, received 15 c.c.  $\frac{m}{3}$  (4 per cent) sodium sulphate solution, subcutaneously; rabbits 5 and 6 were controls.

## FEBRUARY 16.

		3 hours after injection.	Total, 6 hours.	Total, 29 hours.
Rabbit 5 . . . .	male	0 fæces.	0	Lost.
Rabbit 6 . . . .	female	0 fæces.	0	3 gm.; dry.
Rabbit 7 . . . .	male	0 fæces.	4 gm.	7 gm.; dry.
Rabbit 8 . . . .	female	0 fæces.	0	9 gm.; dry.

Rabbits 5 to 8, inclusive, received 15 c.c. of a 25 per cent sodium sulphate solution, subcutaneously.

times during some other investigation, and that they were preceded and followed by perfectly normal, hard, dry, well-formed scybala.

*a* Sodium Sulphate ( $\text{Na}_2\text{SO}_4 + 10 \text{H}_2\text{O}$ ). — Twenty-four experiments were made; in eight of them only the twenty-four hour output was inspected and weighed; in the other sixteen the amount and character were determined more frequently. The amount injected, irrespective of the size of the animal, was 15 c.c., and the solutions

used were 4 per cent ( $\frac{m}{8}$ ) and 25 per cent in strength. In none of the series was purgation noted; and by purgation is here understood the passage of soft and unformed fæces in amounts exceeding that which normal animals might conceivably pass. Many of the animals showed a constipation after the injections. The specimen series on the preceding page will illustrate these statements.

*b Sodium Phosphate* ( $\text{Na}_2\text{HPO}_4 + 12 \text{H}_2\text{O}$ ). — Only eight experiments were made, the solution used being  $\frac{m}{8}$  (4.5 per cent).

## FEBRUARY 19.

	1.5 hours after injection.	Total, 5 hours.	Total, 24 hours.
Rabbit 3 . . . .	0 fæces.	1 gm.; dry.	18 gm.; a few soft pellets.
Rabbit 4 . . . .	20 gm.; dry.	31 gm.; dry.	61 gm.; dry.
Rabbit 5 . . . .	8 gm.; dry.	9 gm.; dry.	22 gm.; dry.
Rabbit 6 . . . .	8 gm.; dry.	8 gm.; dry.	26 gm.; dry.
15 c.c. $\frac{m}{8}$ (4.5 per cent) sodium phosphate, subcutaneously.			

## MARCH 3.

		1 hour after injection.	Total, 6 hours.	Total, 24 hours.
Rabbit 13 . . . .	male	0	0	16 gm.; dry.
Rabbit 14 . . . .	male	10 gm.; dry.	13 gm.; dry.	34 gm.; some unformed masses.
Rabbit 15 . . . .	male	0	1 gm.; dry.	14 gm.; dry.
Rabbit 16 . . . .	male	a few pellets; dry.	2 gm.; dry.	6 gm.; dry.
15 c.c. (4.5 per cent) sodium phosphate, subcutaneously.				

In this series No. 14 passed some unformed fæces between six and twenty-four hours after the injection; the pellets passed before this, during the first six hours, were normal in appearance and consistency, and the amount not greater than occasionally found in controls (see Rabbit 6 in table of February 15). As this occurred in only one

rabbit out of eight, and as normal rabbits now and then pass unformed masses, it seems permissible to assume that the sodium phosphate had nothing to do with the purgation.

No. 4 shows an exceptional output, especially during the first five hours; there was no purgation, however, for the pellets were dry and well formed.

*c* **Sodium Citrate.**— Only four experiments were made.

FEBRUARY 20.

	One hour after injection.	Total, 3 hours.	Total, 21 hours.
Rabbit 1 . . .	9 gm.; a few soft pellets, others dry and hard.	12 gm.	31 gm.; some pellets soft.
Rabbit 2 . . .	7 gm.; dry.	7 gm.	37 gm.; dry.
Rabbit 7 . . .	1.5 gm.; a few soft pellets, rest dry.	10 gm.	31 gm.; a few soft pellets.
Rabbit 8 . . .	0	0	26 gm.; dry.
15 c.c. $\frac{m}{8}$ sodium citrate, subcutaneously.			

No purgation occurred; the few soft pellets which were found in the output of two rabbits (Nos. 1 and 7) may be considered normal.

*d* **Magnesium Sulphate** ( $\text{MgSO}_4 + 7 \text{H}_2\text{O}$ ).— In a previous communication<sup>1</sup> it has been pointed out on the basis of a large series of experiments that the subcutaneous injection of 25 per cent solutions, in 9 to 15 c.c. doses, produced in no instance purgation. It was therefore not considered necessary to repeat these experiments.

#### THE EFFECT OF INTRAVENOUS INJECTIONS UPON PURGATION.

Practically, all the rabbits used in the sodium sulphate and phosphate series received the injection into the ear vein. In the magnesium sulphate series the solutions were given through the jugular vein.

*a* **Sodium Sulphate.**— See next page.

These experiments show clearly that the intravenous injection of sodium sulphate, in the doses and concentrations mentioned, does not produce purgation; on the contrary, seven out of the eight experiments show a definite constipation lasting at least six hours, which may legitimately be ascribed to the action of the salt injected.

<sup>1</sup> MELTZER and AUER: This journal, 1905, xiv, p. 366.



MARCH 9.

	1st hour.	2d hour.	Total, 6 hours.	Total, 25 hours.
Rabbit 13 . . .	0	0	0	20 gm.; dry.
Rabbit 14 . . .	0	0	1 dry pellet.	1 gm.; dry.
Rabbit 15 . . .	0	0	0	4 gm.; dry.
Rabbit 16 . . .	2 gm.; 1 soft pellet.	0	4 gm.; dry.	26 gm.; dry.
2 c.c. $\frac{m}{4}$ (4 per cent) sodium sulphate, intravenously.				

MARCH 13.

	1st hour.	Total, 4 hours.	Total, 7 hours.	Total, 24 hours.
Rabbit 13 . . .	0	0	1 gm.; dry.	9 gm.; dry.
Rabbit 14 . . .	0	0	0	0
Rabbit 16 . . .	A few dry pel- lets.	7 gm.; dry.	8.5 gm.; dry.	10.5 gm.; dry.
Rabbit 15 . . .	0	0	0	11 gm.; dry.
2 c.c. of a 25 per cent sodium sulphate, intravenously.				

*b* Sodium Phosphate.— See next page.

The result of the series of March 8th is perfectly clear: a constipation which lasted at least six hours; that of the March 7th series requires a few words of explanation. Rabbits 14 and 16 passed soft masses within twenty minutes after the injection of the phosphate into the ear vein. These masses were composed of discrete, small, soft, caked together pellets. The early appearance of these masses, their form as agglomerations of small, soft pellets, the passage of perfectly dry, hard, and well-formed pellets in good quantities after some hours, all seem to point to the assumption that these soft masses were present in the colon of the animals before injection of the salt into the circulation.

MARCH 7.

	2-hour total.	During 3d hour.	During 4th hour.	During 5th hour.
Rabbit 13 . . .	0	0	0	1 soft pellet.
Rabbit 14 . . .	6 gm.; soft, caked together.	5 gm.; soft, caked.	0	12 gm.; dry.
Rabbit 15 . . .	0	A few dry pellets.	A few dry pellets.	8 gm.; dry.
Rabbit 16 . . .	9 gm.; soft, caked.	A few soft pellets.	A few <i>dry</i> pellets.	5 gm.; a few soft pellets.
	Total, 5 hours.	Total, 23 hours.		
Rabbit 13 . . .	1 soft pellet.	22 gm.		Fæces passed after the fifth hour were all dry and well formed.
Rabbit 14 . . .	23 gm.	23 gm.		
Rabbit 15 . . .	8 gm.	24 gm.		
Rabbit 16 . . .	14.5 gm.	45 gm.		
2 c.c. $\frac{m}{8}$ (4.5 per cent) sodium phosphate, intravenously.				

MARCH 8.

	1 hour.	Total, 5 hours.	Total, 6 hours.	Total, 24 hours.
Rabbit 9 . . .	0	0	0	12 gm.; dry.
Rabbit 10 . . .	0	0	0	0
Rabbit 11 . . .	0	0	3 gm.; dry.	22 gm.; dry.
Rabbit 12 . . .	0	0	0	22 gm.; dry.
2 c.c. $\frac{m}{8}$ (4.5 per cent) sodium phosphate, intravenously.				

c **Magnesium Sulphate.** — In a series of experiments already published<sup>1</sup> most of the rabbits showed no sign of purgation; the fæces passed during observation (two to four hours) were dry, and small in

<sup>1</sup> MELTZER and AUER: This journal, 1906, xv, p. 387.

amount. A few passed small quantities of caked pellets. The magnesium sulphate was injected into the jugular vein, the concentration ranging between 1.7 per cent and 25 per cent, the amount between 1 and 80 c.c. As this series was quite extensive (49 rabbits); no further experiments were made with this salt.

#### THE EFFECT OF SUBCUTANEOUS AND INTRAVENOUS INJECTIONS UPON PERISTALSIS.

The rabbits were narcotized, usually by morphine hydrochlorate; 1 to 2 c.c. of a 1 per cent solution proved amply sufficient when injected subcutaneously, though in some instances 5 c.c. were used. In a few experiments the animals were kept lightly under ether, or magnesium sulphate, 1.5 grams, per kilo body weight, was injected subcutaneously. After analgesia had set in, the abdomen was opened in the median line from the ensiform process to the pubis, and the abdominal flaps held apart by means of hooks. As some of the experiments were carried out without using a saline bath, the exposed gut was covered with absorbent cotton cloths saturated with 0.9 per cent salt solution at 39° to 40°; loss of heat was prevented largely by wrapping the animal in cotton and by frequently renewing the warm cotton covering the intestines. In others the abdomen was opened under saline solution at 39° to 40°. No glass plate was used to cover the gut in order to rule out this source of possible stimulation. As the loops floated more or less on the surface of the saline solution, the exposed surfaces were moistened frequently. The cæcum being so large in rabbits, a moderate dislocation of this organ was frequently necessary in order to bring the duodenum and transverse colon into view; the descending colon was seen only exceptionally; the ascending colon, and practically the entire cæcal loop were always visible.

During the experiment the gut was handled as little as possible, for it was found that any manipulation of the intestines usually induced or increased the movements.

The salts tested were injected subcutaneously, either in the thigh or pectoral region; moderate massage was used to aid the absorption of the injected fluid; the amount injected was usually 15 c.c. of an  $\frac{1}{8}$  solution. When given intravenously, either the lateral ear vein or the jugular vein were used, the dose ranging between 1 and 10 c.c. of an  $\frac{1}{8}$  solution; with magnesium sulphate a 25 per cent solution was invariably employed.

Twenty experiments were made in all. As the different salts, with the exception of magnesium compounds, produced in the main apparently the same effects, one description will suffice. When injected subcutaneously there usually occurred within fifteen to forty minutes an increase in the movements of the small gut. This did not begin everywhere at the same time. The duodenal loop usually showed the increase first, later the jejunum and ileum. The movements were of the pendular type, and were never as active in the lower small intestines as in the duodenum. No peristalsis of the cæcum was ever seen in this series; the ascending and transverse colon occasionally showed some moderate peristaltic waves; the descending colon was only seen when distended with gas, and then strong peristaltic waves swept down this portion of the gut before the injection of the purgative saline, the incorporation of the salt not noticeably increasing the motion. Associated with the increased pendular movements of the duodenum, this portion of the canal became fuller and rounder, due to a liquid material.<sup>1</sup> As this material gradually traversed the duodenal circuit and passed into the upper loops of the small intestine, distending them moderately, the swaying motions of these loops became more marked. This filling of the duodenal loop was frequently observed; it took place gradually, and was apparently independent to some extent of any action exerted by the saline injected. After one to two hours the liquid usually reached the upper loop of the jejunum, distending the flattened, tape-like coils of this part of the gut, the distension definitely increasing the motions.

As already stated, the chief type of motion noted in the small intestine was the swaying pendular one. Strong constrictions were seen at times, but these remained local and relaxed swiftly; they were possibly due to some local irritation. If the gut was practically empty and collapsed, a condition frequently found in the lower small intestine, the injected saline usually caused a moderate squirming, vermicular motion of some of these flattened loops, not of all. This motion became pendular as soon as the loops were distended. These gut movements, produced by the subcutaneous injection of salines (sodium sulphate, sodium phosphate, sodium citrate), were by no means continuous; periods of activity alternated with periods of almost absolute rest. Nor did the entire gut move at the same time; the duodenum might, for instance, show active pendular movements while the lower small intestine was quiet, or the jejunum

<sup>1</sup> MACCALLUM: This journal, 1904, x, p. 263.

might exhibit good pendular swaying with little or no motion in the ileum.

The duration of the intestinal motions produced was difficult to estimate, but it may be stated that their vigor was usually perceptibly diminished an hour after the onset.

The intravenous injection of 2 to 6 c.c. of the solutions had the same effect upon intestinal movements as their subcutaneous incorporation; the increase, however, occurred much earlier, usually a few minutes after the injection, and the duration of these increased motions was much shorter, about ten to fifteen minutes. During this time the gut was not continuously active, but periods of good pendular movements alternated with periods of rest.

Attempts to increase intestinal activity by summation of saline action were not successful; the effect of a subcutaneous injection of 15 c.c. of  $\frac{m}{8}$  sodium sulphate solution was not increased, at least, not in vigor, by a subsequent injection of 15 c.c. sodium phosphate subcutaneously; the same result was obtained when sodium phosphate and sulphate were injected intravenously or when intravenous and subcutaneous injections were combined.

It must be definitely understood that the increased intestinal motions which have been described in no case even approached the vigor and effectiveness of the peristalsis induced by intravenous injection of physostigmin sulphate or barium chloride. The intestinal movements produced by the salines seemed ineffective to cause purgation, the more so, as the cæcum with its stored up masses of soft material always appeared inert. That this impression proved correct has already been set forth.

The intravenous injection of magnesium sulphate not only did not produce any peristalsis, but stopped whatever movements the gut showed. This effect will be considered more fully at another time.

It seems hardly necessary to state that the intestines were always observed at least thirty minutes before any of the salts were injected, and the behavior of the gut during this period of time served as basis for judging the effect of the injection.

These experiments show clearly that subcutaneous and intravenous injection of sodium sulphate, sodium phosphate, sodium citrate, and magnesium sulphate do not produce purgation, at least not with the doses employed. On the contrary, a definite constipation was caused in some of the series, especially with sodium sulphate.

The movements of the small intestine, especially the duodenum,

were definitely increased by the subcutaneous or intravenous injection of the salts mentioned, magnesium sulphate excepted. The cæcum never exhibited any peristalsis under the conditions described.

That the subcutaneous and intravenous injection of sodium sulphate and magnesium sulphate does not purge is stated by most experimenters. Hay,<sup>1</sup> in his monograph, modified this by noting that when the injections were made over the abdomen and caused irritation moderate purgation resulted. This has, however, not been corroborated by Ellenberger,<sup>2</sup> nor by Eckardt.<sup>3</sup> Hay used only sodium and magnesium sulphate, but Eckardt employed sixteen different salts, among them sodium phosphate, lithium citrate, and the sulphates mentioned. A recent observer,<sup>4</sup> however, writes that "all those salts which act as purgatives when introduced into the stomach or intestine have the same action when injected subcutaneously or intravenously." MacCallum states that the purgative effect with subcutaneous injection usually occurs after one hour, that the amount passed in six hours is two to six times the normal quantity, and that the fæces are sometimes semi-fluid. No experiments are quoted, and no weights are given. The salts used included those employed in the experiments described in this paper; the dosage was the same. MacCallum's results, as far as purgation is concerned, are therefore in striking disagreement with those reached by Hay, Eckardt, and in this research.

Concerning the effect of subcutaneous and intravenous injections upon visible peristalsis there can be no doubt, however, that MacCallum is substantially correct in his statement that peristalsis is increased. There is one exception, though, so far, to his general statement. Magnesium sulphate or chloride does not have this action.

As already described, sodium sulphate, phosphate, and citrate definitely though moderately increase the movements of portions of the small gut, chiefly the duodenum; occasionally the colon shows fair peristalsis; the cæcum never showed any motility. MacCallum<sup>5</sup> merely states that the intestines show strikingly increased motion,

<sup>1</sup> MATTHEW HAY: *Journal of anatomy and physiology*, xvi, p. 593; xvii, pp. 64-67.

<sup>2</sup> ELLENBERGER: *Archiv für wissenschaftliche und praktische Thierheilkunde*, xiii, p. 1.

<sup>3</sup> P. A. ECKARDT: *Inaugural Dissertation*, Giessen, 1905, p. 74.

<sup>4</sup> J. B. MACCALLUM: *This journal*, 1903, x, p. 102.

<sup>5</sup> MACCALLUM: *Loc. cit.*, pp. 102, 103.

without mentioning the different reaction of the various parts of the gut.

There are few references in the literature regarding the effect of subcutaneous or intravenous injections of salines upon visible peristalsis. Laborde<sup>1</sup> saw increased peristalsis after injecting magnesium chloride intravenously, without subsequent purgation. Rabuteau<sup>2</sup> obtained no increased peristalsis and no purgation after intravenous incorporation of the same salt.

Summing up, it may therefore be said that the subcutaneous and intravenous injection of magnesium sulphate and chloride, sodium sulphate, phosphate, and citrate does not produce purgation in rabbits. Applied in the same way, these substances, with the exception of magnesium salts, produce a moderate but definite increase in the peristalsis of parts of the gut.

Magnesium salts injected subcutaneously or intravenously do not cause increased intestinal peristalsis in rabbits. The use of salines subcutaneously or intravenously, in human therapeutics, as suggested by MacCallum,<sup>3</sup> is not warranted by the experimental evidence.

Peristalsis and purgation are not synonymous terms; increased peristalsis may occur during constipation (see sodium sulphate series); and Leubuscher,<sup>4</sup> among others, saw no increased peristalsis during purgation.

It gives me pleasure to express my sincere thanks to Dr. S. J. Meltzer for suggesting this piece of work, and for his continued helpful interest during its progress.

<sup>1</sup> LABORDE: *Gazette hebdomadaire de médecine et de chirurgie*, 1879, p. 352.

<sup>2</sup> RABUTEAU: *Gazette médicale*, 1879, No. 29.

<sup>3</sup> MACCALLUM: *Loc. cit.*, p. 109.

<sup>4</sup> LEUBUSCHER: *VIRCHOW'S Archiv für pathologische Anatomie*, 1886, civ, p. 104.

# ON THE INFLUENCE OF NEUTRAL SALTS UPON THE RATE OF SALIVARY DIGESTION.

BY JANE BOIT PATTEN AND PERCY G. STILES.

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THE conditions which favor or retard the action of enzymes have been much studied, but much remains to be done before general statements can be made. The number of enzymes already known and their wide divergences of behavior compel the investigator to specialize narrowly, and warn him that his findings may not be applied in other cases than those actually studied. A great collection of data concerning these matters is to be desired. The influence of reaction upon many enzymes has been observed with care, but the effect of neutral salts, particularly in high concentration, has been very scantily recorded.

In this paper the authors present the general results of about six hundred comparative trials in which the influence of some common neutral salts upon the rate of starch digestion by ptyalin was tested. The relation of this enzyme to acids and alkalies has been thoroughly worked out. The principal experiments concerning neutral salts have been performed by Grützner,<sup>1</sup> by Kübel,<sup>2</sup> and by Cole.<sup>3</sup> In all cases attention has been directed mainly to the effects produced by somewhat minute quantities of salts.

It is of great interest to ascertain how far there is any parallel between the influence of various salts or ions upon the activities of living protoplasm, and that which they exert upon the performance of enzymes. We know that there is such a similarity in the case of salts like the cyanides, which are extremely toxic, or those which obviously change the state of aggregation of proteids, like compounds of mercury and copper. But we find it hard to trace such resem-

<sup>1</sup> GRÜTZNER: Archiv für die gesammte Physiologie, 1902, xci, p. 195.

<sup>2</sup> KÜBEL: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 276.

<sup>3</sup> COLE: Journal of physiology, 1904, xxx, p. 202.



blances among the metals of the alkali, and alkaline earth groups. In other words, we see no reason to believe that an optimum mixture of salts for the preservation of protoplasm (*i. e.*, a Ringer's mixture), will be an optimum solution for enzyme action, though it would not be strange to find it so.

It has been a common observation that most salts in small quantity accelerate the digestion of starch by ptyalin, but that all salts in higher concentration retard it more or less. Cole has shown that this accelerating action is very striking when the starch paste and saliva used as a control are both dialyzed. The ferment is comparatively inert until the electrolyte is supplied. When the starch and saliva are not subjected to dialysis it must be recognized that we do not begin with a salt-free mixture. Enough electrolytes may be present in the control to insure a maximal speed of hydrolysis, and when we add more and more we may see merely a progressive slowing. In other cases we may still observe an acceleration with the lower concentrations. As we did not dialyze our materials we always began with exceedingly active mixtures, and saw much less than did Cole of the acceleration phase.

There is one difficulty attending all experiments of this kind which must be borne in mind. This is the probable alteration of the starch by the salts, especially when the solution approaches saturation. Cole has pointed out that the slowing of digestion may not be due wholly to the restraint of the enzyme, but also to the incipient precipitation of the starch. This factor becomes important when such salts as ammonium sulphate and ammonium nitrate are used. These salts notably increase the opalescence and the settling of starch, and correspondingly delay its digestion. A general survey of our results leads to the belief that, if we except these ammonium salts, we need not assign a large place to this secondary influence. If it were a prominent one there should be other cases in which the digestive action is somewhat suddenly checked as the concentration increases. The fairly even sweep of the descending curve favors rather the idea that the salt mainly affects the enzyme and not the starch. Another fact which points in the same direction is the superior retarding power of some monovalent salts (*e. g.*, lithium chloride) as compared with certain divalent salts (*e. g.*, calcium chloride). The effect upon colloids is supposed to be a function of the valency.<sup>1</sup>

The extreme sensitiveness of ptyalin to H and OH ions is in

<sup>1</sup> HARDY and WHETHAM: cited by Cole, *loc. cit.*

marked contrast to its tolerance of many salts. It is accordingly necessary to have a neutral solution, or at least one of a constant reaction throughout each series of trials. Our usual procedure has been to salt the starch, then acidify with one or two drops of hydrochloric acid, shake with a little powdered calcium carbonate, and then to add the saliva, thus making the mixture neutral or very weakly alkaline. Of course the use of many salts, like those of iron and aluminum, is interdicted, because we cannot obtain neutral solutions.

The retarding effects which we observe with neutral salts are not due to the progressive destruction of the enzyme but to its inhibition. This point was readily determined. For if we keep salted saliva for twenty-four hours, or more, we find it about as active as saliva which has but just received the addition of the salt. Moreover, salted saliva may be made more active by dilution or by dialysis.

In the routine of our experiments we made use of a paste of arrow-root of one per cent strength. For each trial 20 c.c. of paste was placed in a beaker. In some cases we dissolved in each beaker a weighed quantity of salt. In other instances we saturated a large volume of starch-paste and mixed the saturated paste with unsalted paste in the proportions required to carry out the series. To each sample was added 5 c.c. of dilute saliva. It was found desirable to have the beakers of a uniform size, for the digestion seems to be hastened when the surface exposed to the air is increased. The starch and saliva were stirred together for a few seconds, and then as a rule the beaker was not touched except to dip from it at frequent intervals drops for the iodine test. At one period in our work we changed our practice and placed our beakers in a cradle shaken by clock-work. This seemed to make no difference in the relative results, and we abandoned the plan. The end-point was recorded, as in Cole's work, when the color of our iodine solution was no longer deepened by the addition of the digesting mixture.

The original records were of the times elapsing between the pouring in of the saliva and the disappearance of the erythrodextrin color. For convenience in tabulating we reduced these to the form of rate-values. In each series we have represented the speed of the control (*i. e.*, the reciprocal of the time) by 100, and calculated the relative speeds of the salted mixtures as compared with this standard. In the table below we present the general facts in regard to the influence of several salts upon the rate of salivary digestion. The salts which

retard less are placed above those which have a more marked hampering effect. But the order cannot be quite fixed, for it is not the same at different concentrations.

In addition to the trials summarized in the table, we made somewhat less accurate experiments with several other salts. Among

Salt.	Concentration.								
	0	$\frac{\text{Mol.}}{4}$	$\frac{\text{M.}}{2}$	M.	2 M.	3 M.	4 M.	5 M.	6 M.
MgSO <sub>4</sub> . .	100	138	128	115	106	105*	....	....	....
NH <sub>4</sub> Cl . .	100	....	110	95	80	76	....	....	76*
NH <sub>4</sub> Ox . .	100	106*	....	....	....	....	....	....	....
CaCl <sub>2</sub> . .	100	200†	133	80	31	12	5	0*	....
BaCl <sub>2</sub> . .	100	152	113	76*	....	....	....	....	....
KCl . . .	100	100	88	64	31	18*	....	....	....
KBr . . .	100	....	85	67	19	....	....	13*	....
NaCl . . .	100	....	72	51	29	15	....	....	7*
KI . . . .	100	....	72	45	3	....	....	....	....
KNO <sub>3</sub> . .	100	71	65	53	41	34*	....	....	....
K <sub>2</sub> SO <sub>4</sub> . .	100	70	62*	....	....	....	....	....	....
KClO <sub>3</sub> . .	100	62	39*	....	....	....	....	....	....
Na <sub>2</sub> SO <sub>4</sub> . .	100	58	35	25*	....	....	....	....	....
LiCl . . .	100	20	4	1	0†	....	....	....	....

\* Saturated.                      † Not saturated.

these were magnesium chloride, the benzoates of ammonium, sodium, and potassium, and the formates of ammonium and sodium. Ammonium sulphate and ammonium nitrate were tried but set aside because of their peculiar effect upon the starch noted above.

Examining the data expressed in quantitative form in the table, and referring to the additional notes which are rather qualitative in their nature, we may call attention to the following facts.

1. Ptyalin does its work with surprising ease in the presence of many salts in high concentration. This is even the case with some

saturated solutions, notably those of magnesium sulphate and ammonium chloride. Grützner stated that magnesium sulphate and sodium sulphate are specific poisons for ptyalin, but Cole failed to confirm this, and we saw no sign of such an action.

2. The most striking instances of accelerating effects were obtained with salts of magnesium, calcium, and barium — belonging to a natural chemical group. But with increasing concentration these salts diverge in their behavior, and calcium chloride at last restrains the enzyme, which never becomes true of magnesium sulphate.

3. When we compare the retarding influence of salts of ammonium, potassium, and sodium, we find the ammonium compound much less active in checking the digestion than either of the other two. There seems to be no marked difference between the sodium and the potassium salts. Sodium chloride retards somewhat more than does potassium chloride, and this order holds for the sulphates, but seems to be reversed for the benzoates.

4. Comparing the halogen compounds of potassium we find for the stronger solutions the same order which Grützner and Kübel established for the lower concentrations, namely, that the chloride retards less than the bromide which in turn retards less than the iodide. Here we should refer to Grützner's observation, that the fluoride distinctly accelerates the amylolytic action at every stage up to saturation (which with sodium chloride is reached at a concentration of  $\frac{m}{4}$ ). The favorable influence of the fluoride is unexpected, since it is generally considered the most poisonous of the halogens toward protoplasm.

5. Another anomaly appears in the almost complete inhibition of the enzyme by lithium chloride in moderate amounts. This salt is probably the most harmless of all foreign compounds toward certain living tissues, but it checks starch digestion almost as quickly as copper sulphate.

We made a considerable number of parallel experiments in which the commercial Taka-diastrase was employed instead of saliva. Taka-diastrase is prepared from the mould, *Aspergillus oryzae*. It is a powerful agent, and in most cases we found it even more resistant than ptyalin toward neutral salts, but we cannot say whether this resistance is a property of the ferment or whether it is due to protective materials accompanying it. Organic matter of many kinds lessens the sensitiveness of ptyalin to acid,<sup>1</sup> and may well increase its tolerance for neutral salts.

<sup>1</sup> COLE: *Loc. cit.*, p. 206.

The authors conclude this research with an increased sense of the individuality of ions, and of the difficulty of explaining their properties by mathematical functions. In our experiments we have used but one salt at a time. It is suggested that a study of variously proportioned mixtures of two or more salts as affecting the rate of enzyme action should be very fruitful of information regarding "toxic and antitoxic" characters.

## THE COMPOSITION OF BONE IN OSTEOMALACIA.<sup>1</sup>

BY FRANCIS H. MCCRUDDEN.

[From the Laboratory of Biological Chemistry of the Harvard Medical School.]

THE pathological changes which occur in osteomalacia are still the subject of dispute. Most pathologists, including Virchow, have considered the bone changes in this disease to be essentially halisteresis, — that is, solution of the inorganic constituents of bone, — resembling the chemical processes which occur when bone is placed in acid. On the other hand, Cohnheim has declared that the process consists of, first, the taking up of bony tissue, including organic and inorganic constituents alike, by osteoclasts, and then the laying down of new osteoid tissue. According to Cohnheim, the bones are, until well past adolescence, the seat of an uninterrupted and coincident apposition and absorption of material, and in the case of osteomalacia, as well as in rickets, the osteogenic substance laid down is free from lime.<sup>2</sup> Von Recklinghausen's discovery of osteoblasts in the osteoid tissue of osteomalacia<sup>3</sup> is in accord with Cohnheim's theory and lends considerable weight to it.

The author, in considering the results of his investigations of the metabolism in osteomalacia,<sup>4</sup> decided that in this disease there is absorption of the bony tissue, and apposition of new tissue which, unlike normal bone, is poor in lime and especially rich in magnesium, and also in an organic substance containing much sulphur and little phosphorus, — a substance somewhat similar in its chemical composi-

<sup>1</sup> The expenses for the analyses were contributed in part by the Procter Fund for the Study of Chronic Diseases.

<sup>2</sup> O. COHNHEIM: Lectures on General Pathology, translated by A. B. McKee, 1889, ii, p. 632.

<sup>3</sup> VIERORDT: Osteomalacie, in Nothnagel's *Specielle Pathologie und Therapie*, iii, pt. 2, p. 123.

<sup>4</sup> GOLDTHWAIT, PAINTER, and OSGOOD and MCCRUDDEN: This journal, 1905, xiv, p. 389.

tion to the normal organic matrix of bone. This theoretical conclusion, which does not essentially depart from Cohnheim's view, it was the object of the following investigation to test.

Pieces of two horse ribs which had been affected by the characteristic changes of osteomalacia were analyzed, the analysis including determinations of the sulphur and phosphorus content which seemed to be absent in other similar investigations, and for comparison pieces of two normal horse ribs were similarly analyzed.

The percentage compositions were as follows :

	Osteomalacia.		Normal.	
	I	II	I	II
CaO	20.09	18.35	33.48	33.12
MgO	0.50	0.46	0.11	0.10
P <sub>2</sub> O <sub>5</sub>	16.55	16.00	23.66	23.22
S	0.35	0.38	0.11	0.09
Total CaO, MgO, and P <sub>2</sub> O <sub>5</sub>	} 37.14	34.81	57.25	56.44

Evidently, in this case of osteomalacia there is a decrease in the calcium contents of the diseased bone and an increase in its magnesium contents, results which confirm the older analyses.<sup>1</sup> There is also an increase in the sulphur contents and a decrease in the phosphorus contents, — facts previously unobserved. The inorganic material of the bone as a whole is diminished in amount.

Clearly these facts are in complete accord with the indications of the author's metabolism investigations, and with Cohnheim's theory which is based upon anatomical evidence. At the same time they weigh against the theory of simple haliteresis, in that, while the calcium is relatively but slightly diminished, some forty per cent, the magnesium and sulphur are relatively very considerably increased, some three to four hundred per cent in these two samples of bone.

It seems, then, highly probable that in osteomalacia at least two

<sup>1</sup> ROLOFF: VIRCHOW'S Archiv, xxxvii, 433; HUPPERT: Archiv der Heilkunde, 1866, 1867, viii, 345; CHABRIÉ: Les phénomènes chimiques de l'ossification, Paris, 1895, p. 65.

processes occur in the diseased bone,—the one an absorption of calcium and phosphorus, and the other an apposition of magnesium and of material, probably organic, which is rich in sulphur.

The author wishes to express his thanks to Dr. W. F. Whitney, Curator of the Warren Museum, for allowing him the use of the diseased bone, and to Prof. William T. Councilman, who examined the specimen microscopically and pronounced it to be osteomalacia.



THE RATE OF DIFFUSION OF THE INORGANIC SALTS  
OF THE BLOOD INTO SOLUTIONS OF NON-ELEC-  
TROLYTES AND ITS BEARING ON THE THEORIES  
OF THE IMMEDIATE STIMULUS TO THE HEART  
RHYTHM.

BY WILLEY DENIS.

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

IT has been shown by Carlson<sup>1</sup> that the heart ganglion of *Limulus*, when isolated from the heart muscle at the posterior end of the heart, though still in connection with the muscle at the anterior end, will continue in activity in an isotonic (gram-molecular) solution of cane sugar from sixty to ninety minutes, while in a bath of gram-molecular glycerine solution the ganglionic rhythm ceases in from five to ten minutes, and with a gram-molecular urea solution in from two to three minutes. The above results are taken from values obtained with ganglia in good condition. When the initial condition of the ganglia is poor, viz., the rhythm slow and irregular, a standstill is obtained in a much shorter time. Carlson has pointed out that the above results tend to support the view that the blood salts form only the condition, not the cause, of heart activity, and that "the immediate stimulus to the rhythm is some substance or process within the cells themselves." The theory is, therefore, put forward that the non-electrolytes (cane sugar, glycerine, urea) by diffusion into the tissue may alter the permeability of the cell wall, and by diffusion into the cells themselves alter the equilibrium relations of the cell contents. Further, the diminished concentration of the electrolytes in the inter-cellular spaces probably causes a diffusion of the electrolytes out from the cells, thus causing a further departure from the normal condition of the automatic tissue. The above interpretation emphasizes the importance of the direct action of the non-electrolytes on the automatic tissues in bringing about the cessation of rhythm. The view that

<sup>1</sup> CARLSON: This journal, 1906, xvi. p. 221.

the standstill of the heart in isotonic solutions of non-electrolytes is directly due to the dilution of the blood salts, requires that the rate of diffusion of these blood salts into solutions of sugar, glycerine, and urea be directly proportional to the length of time that the heart continues in activity in these non-electrolytes. It has been shown by Arrhenius<sup>1</sup> and also by Abegg<sup>2</sup> that the diffusion of aqueous solutions of electrolytes through aqueous solutions of non-electrolytes, is slower than when the diffusion takes place through water, and in general, the more non-electrolyte or electrolyte added to the solution through which the diffusion takes place the slower will be the rate of diffusion. Further, it has been shown by these chemists that on adding a non-electrolyte or an electrolyte to water the rise in viscosity and the retardation of diffusion of an aqueous solution of an electrolyte through the liquid are roughly proportional.

As no data concerning the rate of diffusion of the salts of the blood through gram-molecular solutions of cane sugar, urea, or glycerine were available, I have, at the suggestion and under the direction of Professor Carlson, made a number of measurements of the relative rate of diffusion of normal solutions of sodium chloride, potassium chloride, calcium chloride, and magnesium chloride through gram-molecular solutions of the above non-electrolytes.

As only relative results were sought, no attempt was made to work under constant temperature conditions. During the course of the experiments which were conducted during a period of several weeks, the room temperature varied from 22.5° to 30° C. In every determination carried out by the second method, at least three duplicates were made and the average value used.

The two methods employed were: First, Scheffer's<sup>3</sup> modification of Graham's original jar method. The inner cylinders employed were 8 cm. high by 2.5 cm. wide; the outer cylinders were 50 cm. high by 9 cm. wide. From sixty to seventy cubic centimetres of the normal salt solutions were run into the inner cylinder by means of a burette, and 2000 c.c. of the liquid through which the diffusion was to take place was slowly run into the outer cylinder through a finely pointed siphon. The normal salt solutions used were prepared from chemically pure material, and were standardized by titration with standard four-tenths nitrate of silver, using potassium chromate as indi-

<sup>1</sup> ARRHENIUS: *Zeitschrift für physikalische Chemie*, 1892, x, p. 51.

<sup>2</sup> ABEGG: *Ibid.*, 1893, xi, p. 248.

<sup>3</sup> SCHEFFER: *Berichte der deutschenchemischen Gesellschaft*, 1882, xv, p. 788.

cator. When the experiment was complete, a glass plate fastened to a rod was slipped over the mouth of the inner cylinder, the whole removed from the outer cylinder, carefully rinsed with distilled water, and the contents of the inner cylinder made up to a volume of

TABLE I.

a.	b.	c.	d.	c-d.	e.	f.
KCl . . .	water	0.55290	0.41321	0.13976	136.00	0.01860
NaCl . . .	water	0.43269	0.32695	0.10574	135.75	0.01780
CaCl <sub>2</sub> . .	water	0.39285	0.38550	0.00720	136.75	0.01310
MgCl <sub>2</sub> . .	water	0.65821	0.64872	0.00949	137.00	0.00105
KCl . . .	urea	0.57622	0.45560	0.11756	143.50	0.01600
NaCl . . .	urea	0.43890	0.33410	0.10480	144.25	0.01580
CaCl <sub>2</sub> . .	urea	0.38100	0.33830	0.04270	141.75	0.00790
MgCl <sub>2</sub> . .	urea	0.61110	0.60515	0.00604	140.75	0.00069
NaCl . . .	glycerine	0.43890	0.33090	0.10900	144.50	0.01010
CaCl <sub>2</sub> . .	glycerine	0.39190	0.36520	0.0267	144.50	0.00320
MgCl <sub>2</sub> . .	glycerine	0.65820	0.65330	0.0049	144.25	0.00051

a. Diffusing solution (normal solution employed in every case).

b. Solution through which the diffusion took place (gram-molecular solutions employed in every case).

c. Weight in grams of salt in diffusion cylinder at the beginning of the experiment.

d. Weight in grams of salt in diffusion cylinder at the end of the experiment.

c-d. Weight in grams of salt which diffused out during the experiment.

e. Time in hours during which the experiment was continued.

f. Per cent of total salts which diffused out in one hour.

250 c. c., aliquot portions of which were then titrated with four-tenths nitrate of silver, using potassium chromate as indicator.

The results obtained by this method are shown in Table I.

In the second method used glass tubes 20.4 cm. long and .7 cm. wide were employed. At either end the tubes were bent at an angle of 90° and fastened horizontally to a stone shelf supported by masonry. The horizontal portion of the tube being completely filled with the solution through which the diffusion was to take place, 5 c. c. of the normal solution of the salt under investigation was slowly

poured in at one end of the tube by means of a finely pointed pipette, and at the other end about 2 gr. nitrate of silver in the form of crystals was added. This method was suggested by Carlson. The formation of silver chloride when the foremost ions of the diffusing salts meet takes place at first in the form of a line not more than .5 m.m. broad, so that the end point obtained is very definite. The

TABLE II.

a.	b.	c.	a.	b.	c.
MgCl <sub>2</sub> . . .	cane sugar	1.00	NaCl. . . .	cane sugar	1.08
MgCl <sub>2</sub> . . .	glycerine	3.40	NaCl . . . .	glycerine	5.10
MgCl <sub>2</sub> . . .	urea	4.80	NaCl . . . .	urea	6.90
MgCl <sub>2</sub> . . .	water	6.10	NaCl. . . .	water	13.30
CaCl <sub>2</sub> . . .	cane sugar	1.04	KCl . . . .	cane sugar	1.15
CaCl <sub>2</sub> . . .	glycerine	4.80	KCl . . . .	glycerine	5.20
CaCl <sub>2</sub> . . .	urea	6.80	KCl . . . .	urea	8.50
CaCl <sub>2</sub> . . .	water	8.60	KCl . . . .	water	16.50

a. Solution which diffused (normal solution used in every case).  
 b. Solution through which diffusion took place (gram-molecular solution used in every case).  
 c. Rate of diffusion as compared to the rate of diffusion of normal MgCl<sub>2</sub> through gram-molecular cane sugar, this value being taken as equal to 1.

time of diffusion was taken as the period elapsing between the addition of the salt solution and the first appearance of the white precipitate. The distance of diffusion was measured as the space between the point on the tube at which the precipitate first appeared and the point to which the tube was filled before adding the salt solution.

The results obtained are given in Table II.

The above results are therefore not contradictory to the view that the more rapid cessation of the heart rhythm in isotonic urea solutions as compared with the results obtained with solutions of cane sugar is directly due to the relatively rapid diffusion of the blood salts out from the intercellular spaces when the heart is immersed in the urea solution. On the other hand, according to the theory of Carlson,

the rate of diffusion into the tissues of the non-electrolyte composing the bath forms an important factor in regard to the length of time for which the ganglionic rhythm may be continued. We know that through water, at least, urea diffuses at a much more rapid rate than does cane sugar, while the rate of diffusion of glycerine occupies a more or less intermediate position. Nernst<sup>1</sup> has pointed out that the resistance to diffusion increases with increasing molecular weight, and has further calculated the absolute force of diffusion  $K$  into water for cane sugar and for urea, using the coefficient of diffusion for the latter substance as measured by Scheffer<sup>2</sup> and for cane sugar the coefficient of diffusion  $k$  calculated by Stefan<sup>3</sup> from Graham's measurements. The values calculated by Nernst are as follows:

	$t$	$k$	$K$
Urea . . . . .	7.5''	0.810	$2.5 \times 10^9$ Kg.
Cane sugar . . . . .	9.0''	0.312	$67.0 \times 10^9$ Kg.

In this connection the experiments of Hedin<sup>4</sup> should be mentioned as showing clearly the more rapid diffusion of isotonic solutions of urea through dead animal tissues as compared with the rate of diffusion of isotonic solutions of cane sugar.

In Hedin's experiments solutions of cane sugar, glycerine, and urea were allowed to diffuse through portions of the excised intestines of cows from which all fat and as much as possible of the serous coat had been removed and which had been soaked over night either in isotonic sodium chloride or mannite solution. In order that the cells of the tissue might not be injured the outer liquid used for experiments with non-electrolytes was isotonic sodium chloride, for experiments with electrolytes isotonic (five per cent) mannite. Hedin's results are given in Table III.

There appears, then, to exist some direct relation between the rate of diffusion of the blood salts into these non-electrolytes and the length of time the heart rhythm is maintained in isotonic solutions of the same. On the other hand, there appears to exist a similar direct relation between the rate of diffusion of these non-electrolytes through water or dead animal membranes and the rapidity with which they bring the automatic heart ganglion to a standstill. These facts make

<sup>1</sup> NERNST: *Zeitschrift für physikalische Chemie*, 1888, ii, p. 613.

<sup>2</sup> SCHEFFER: *Ibid.*, p. 401.

<sup>3</sup> STEFAN: *Wiener Sitzungsberichte*, 1879, lxxix, p. 161.

<sup>4</sup> HEDIN: *Archiv für die gesammte Physiologie*, 1900, lxxviii, p. 205.

it impossible on this line of inquiry to determine whether the standstill of the heart in isotonic solutions of these non-electrolytes is due to the dilution of the inorganic salts in the plasma surrounding the cells or to a direct action of the non-electrolytes on the automatic

TABLE III.

Diffusing into 0.95 per cent NaCl.		Diffusing into 0.5 per cent mannite.	
Substance diffusing.	Permeability.	Substance diffusing.	Permeability.
Cane sugar . . . . .	1.00	KCl . . . . .	1.92
Glycerine . . . . .	1.20	NaCl . . . . .	1.75
Urea . . . . .	1.48	CaCl . . . . .	1.34
		K <sub>2</sub> SO <sub>4</sub> . . . . .	1.44
		MgSO <sub>4</sub> . . . . .	1.93
		Na <sub>2</sub> SO <sub>4</sub> . . . . .	1.31

tissue, the latter view being the one especially emphasized by Carlson. That the dilution of the electrolytes in the plasma surrounding the automatic cells is not the only factor in causing the cessation of the heart rhythm in solutions of non-electrolytes appears to be clearly shown by Carlson in the fact that the heart ganglia in good condition continue in activity in the sugar solution for a much longer time than the ganglia in poor condition. This difference in the reaction of the hearts must, in part at least, be referred to differences in permeability of the automatic cells to the non-electrolytes as well as to the substances within the cells themselves. It is obvious, however, that this does not amount to a demonstration, as the heart in poor condition may have a relatively lower excitability and may in consequence be brought to a standstill in a less dilution of the blood salts than hearts in good condition, assuming that these blood salts constitute the immediate stimulus to the rhythm.

In conclusion, attention is called to the fact that in my own results as well as in those of Hedin, there is a noticeably more rapid rate of diffusion of the primarily stimulating salts, sodium chloride and potassium chloride, as compared to the rate of diffusion of the

depressor salts, viz., the chlorides of calcium and magnesium,<sup>1</sup> which would suggest that the stoppage of the *Limulus* heart ganglia in isotonic solutions of non-electrolytes may in part be due to the relatively greater concentration of the salts of calcium and magnesium in the cells and intercellular spaces.

<sup>1</sup> CARLSON: This journal, 1906, xvi, p. 378.

# ADRENALIN GLYCOSURIA, AND THE INFLUENCE OF ADRENALIN UPON NITROGENOUS METABOLISM.

BY FRANK P. UNDERHILL AND OLIVER E. CLOSSON.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

OF the more recent investigations dealing with the nature of adrenalin glycosuria may be cited the work of Noël Paton,<sup>1</sup> who has concluded that the character of the nitrogenous metabolism in this experimental condition is closely allied to that occurring in ordinary diabetes.

v. Noorden<sup>2</sup> has demonstrated that in diabetes in man there may be a significant rise in the ammonia of the urine due to the production of acidosis resulting from the increased formation of various acids. Paton has carried out experiments with the rabbit and dog in which the relation of urea nitrogen and ammonia nitrogen to the total nitrogen has received especial attention. He found that after the subcutaneous introduction of adrenalin there is a markedly increased production of ammonia at the expense of urea, that the sugar is not entirely derived from the breaking down of proteids; that on an insufficient diet the decomposition of proteid is markedly increased, and concludes that "the proteid metabolism under adrenalin thus behaves as it does in ordinary diabetes."

## I. THE INFLUENCE OF SUBCUTANEOUS INJECTIONS OF ADRENALIN CHLORIDE UPON NITROGENOUS METABOLISM IN THE DOG.

In an earlier paper<sup>3</sup> from this laboratory it was pointed out that the mechanism of adrenalin glycosuria is somewhat different from that produced by a diversity of other drugs. In the present investigation the influence of adrenalin upon the distribution of various urinary forms of nitrogen has been repeated with the dog.

<sup>1</sup> PATON: *Journal of physiology*, 1903, xix, p. 286.

<sup>2</sup> v. NOORDEN: *Pathologie des Stoffwechsels*, Berlin, 1893.

<sup>3</sup> UNDERHILL: *Journal of biological chemistry*, 1905, i, p. 113.



Full-grown bitches kept in the usual metabolism cages were fed upon a fixed diet of lean meat, cracker meal, and lard. The animals were catheterized daily, precautions being taken against the possibility of bladder infection.<sup>1</sup> Decomposition of the urine voided during the day was prevented by the use of toluol. Sugar estimations were made according to the Allihn method. Total nitrogen of the urine and fæces was determined by the Kjehldahl-Gunning method; urea, ammonia, and creatinin nitrogen by the corresponding methods of Folin.<sup>2</sup> Whenever sugar was present in the urine Mörner's<sup>3</sup> modification of Folin's method was employed for the estimation of urea. The adrenalin preparation used for the injections was the colorless "adrenalin chloride, 1 to 1000" supplied by Parke, Davis & Co.

*Experiment 1.*—On the 20th of February, 1906, a bitch of 14.8 kilos was placed on a diet containing 7.64 gm. of nitrogen and consisting of 140 gm. of lean meat, 160 gm. of cracker meal, and 26 gm. of lard. The food was given in two equal portions morning and night. On February 25th the nitrogen content of the diet was changed to 7.68 gm. In the morning of the same day the animal received a subcutaneous injection of 10 c.c. of adrenalin chloride which appeared to have little effect upon the general behavior of the dog. The urine of the injection day contained 5.6 gm. of dextrose, with a faint trace of coagulable proteid. Legal's test for acetone and Gerhardt's test for diacetic acid proved negative. The following morning (February 26th) a second subcutaneous injection of 10 c.c. of adrenalin chloride was given, with the subsequent appearance in the urine of 6.9 gm. of dextrose and a trace of coagulable proteid. The presence of acetone or diacetic acid could not be detected. Polarimetric determination indicated 7 gm. of dextrose. After fermentation of the urine with yeast no reducing substances could be found. On the two following days of the experiment the urine was dextrose-free, but contained small quantities of coagulable proteid. The animal appeared to be in good condition, and throughout the experiment devoured her food with no apparent loss of appetite.

In the appended table is given the distribution of the various forms of nitrogen determined.

From the results given in Table I it is evident that the repeated subcutaneous injection of adrenalin chloride was not attended by any significant disturbance in the proportion of the urinary constituents determined.

<sup>1</sup> Cf. UNDERHILL and CLOSSON: *Ibid.*, 1906, ii, p. 51.

<sup>2</sup> FOLIN: This journal, 1905, xiii, p. 45.

<sup>3</sup> MÖRNER: *Skandinavisches Archiv für Physiologie*, 1903, xiv, p. 297.

*Experiment 2.* — A bitch of 14.4 kilos was placed on a diet composed of 140 gm. of lean meat, 160 gm. of cracker meal, and 26 gm. of lard, and containing 7.75 gm. of nitrogen, on the 27th of March, 1906. On March 31st the animal received a subcutaneous injection of 10 c.c. of adrenalin chloride. During the day a portion of the food was vomited and the urine became slightly contaminated. The trace of vomit was removed in great measure by filtration through absorbent cotton. A small quantity of coagulable proteid was present in the filtered urine and 10.15 gm. of dextrose. The next injection of adrenalin was given on April 6th, on which day only half the daily portion of food was given, that of the morning meal. The injection was made several hours later, and no vomiting occurred. The urine contained a trace of coagulable proteid and 4.76 gm. of dextrose. A third administration of 20 c.c. of adrenalin chloride (divided into two injections of 10 c.c. each given in the morning and afternoon) was made on the 10th of April, and only the morning portion of food given. The urine of this day was free from coagulable proteid, but contained 14.8 gm. of dextrose. The following day the bitch refused to eat and lay quietly in the bottom of the cage. The urine contained neither proteid nor sugar. After the 11th of April the entire diet for the day was greedily devoured. Urine collected subsequently to the end of the experiment (April 16th) was free from both sugar and proteid.

The composition of the urine covering the period from March 27th to April 17th is given in Table II.

Leaving out of consideration the first adrenalin injection period in which the urine was slightly contaminated with vomit, it is obvious that in Experiment II no marked change occurs in the percentage composition of the urinary constituents determined when large and repeated injections of adrenalin are administered subcutaneously to the dog. In the second and third injection periods the influence of the insufficient diet is readily seen from the balance table for the corresponding periods. This table shows that when the animal was on an inadequate diet adrenalin induced an increased proteid metabolism. This inference seems justified in view of the fact that the nitrogen output, especially on the second day of the third injection period, is higher than one would expect in conditions of simple starvation. Control experiments have been carried out under conditions similar to those in Experiment II, but without the administration of adrenalin to ascertain the influence of insufficient diet for a limited period (one to two days) on the relation of various forms of nitrogen found in the urine. Since no disturbance in the percentage composition of the urine was observed, it may be fairly concluded that the failure of

TABLE I.  
COMPOSITION OF THE URINE.

FORE PERIOD.													
Date.	Body weight.	Volume.	Specific gravity.	Reaction to litmus.	Total N.	Urea N.	Ammonia N.	Creatinin N.	P <sub>2</sub> O <sub>5</sub> .	Sugar.	Urea N.	Ammonia N.	Creatinin N.
	kilos.	c.c.			gm.	gm.	gm.	gm.	gm.	gm.	%	%	%
Feb. 20	14.8	340	1.020	acid	6.33	5.19	0.30	0.145	1.44	....	82.0	4.7	2.3
21	14.9	500	1.016	acid	6.66	5.62	0.36	0.157	1.56	....	84.3	5.4	2.3
22	14.9	420	1.020	acid	5.94	5.06	0.37	0.167	0.95	....	85.1	6.2	2.8
23	14.9	450	1.015	acid	6.00	5.07	0.30	0.170	0.95	....	84.5	5.0	2.8
24	14.9	440	1.022	acid	7.31	6.18	0.38	0.171	1.09	....	84.2	5.1	2.3
ADRENALIN INJECTION PERIOD.													
25 <sup>1</sup>	14.5	550	1.020	acid	7.39	6.28	0.38	0.186	1.25	5.6	85.0	5.1	2.5
26 <sup>1</sup>	14.6	330	1.030	acid	6.01	4.99	0.36	0.166	1.06	6.9	83.3	6.0	2.7
27	14.5	320	1.020	acid	4.82	4.05	0.27	0.157	1.19	none	84.0	5.6	3.2
28	14.6	280	1.022	acid	5.40	4.52	0.34	0.145	0.61	none	83.7	6.3	2.6

		1 Injection of 10 c.c. of adrenalin chloride.	
NITROGEN BALANCE.			
		Fore Period.	Adrenalin Injection Period.
Nitrogen intake	{	Urine . . . . . 38.20	. . . . . 30.72
		Faeces . . . . . 32.27	. . . . . 23.62
Nitrogen output	{	. . . . . 2.40	. . . . . 4.67
		<u>34.67</u>	<u>28.29</u>
Balance.		+3.53	+2.43

TABLE II.  
COMPOSITION OF THE URINE.

FORE PERIOD.													
Date.	Body weight.	Volume.	Specific gravity.	Reaction to litmus.	Total N.	Urea N.	Ammonia N.	Creatinin N.	P <sub>2</sub> O <sub>5</sub> .	Sugar.	Urea N.	Ammonia N.	Creatinin N.
	kilos	c.c.			gm.	gm.	gm.	gm.	gm.	gm.	%	%	%
March 27	14.3	710	1.010	acid	6.24	5.25	0.37	0.192	1.00	....	84.1	5.9	3.0
28	14.4	690	1.012	acid	6.00	5.09	0.29	0.187	1.05	....	84.8	4.8	3.1
29	14.4	540	1.011	acid	5.97	4.90	0.30	0.190	0.98	....	82.1	5.0	3.2
30	14.3	580	1.015	acid	6.22	5.30	0.29	0.189	1.00	....	85.2	4.7	3.0
FIRST ADRENALIN INJECTION PERIOD.													
31 <sup>1</sup>	13.9	500	1.032	acid	8.06	7.26	0.37	0.205	1.24	10.15	90.0	4.6	2.5
April 1	14.0	460	1.020	acid	7.01	5.89	0.41	0.178	1.66	none	84.0	5.8	2.5
2	14.1	660	1.014	acid	6.28	5.31	0.45	0.182	1.41	none	84.5	7.1	2.9
3	14.3	510	1.015	acid	6.48	5.29	0.47	0.160	0.91	none	81.6	7.2	2.5
4	14.4	400	1.018	acid	6.15	5.01	0.40	0.167	0.76	none	81.4	6.5	2.7
5	14.4	550	1.011	acid	5.90	4.91	0.35	0.162	0.88	none	83.2	5.9	2.7
SECOND ADRENALIN INJECTION PERIOD.													
6 <sup>1</sup>	13.7	650	1.028	acid	6.77	5.62	0.36	0.206	1.28	4.76	83.0	5.3	3.0
7	13.9	460	1.017	acid	5.72	4.67	0.37	0.167	0.97	none	81.6	6.5	2.9
8	13.8	320	1.020	acid	5.82	4.90	0.44	0.169	1.06	none	84.1	7.5	2.9
9	14.0	300	1.020	acid	5.70	4.90	0.38	0.167	1.11	none	86.0	6.7	2.9

THIRD ADRENALIN INJECTION PERIOD.

10 <sup>1</sup>	13.5	450	1.028	acid	6.39	5.13	0.49	0.173	0.97	14.80	80.3	7.6	2.7
11	13.7	370	1.026	acid	8.01	6.76	0.26	0.186	1.40	none	84.4	3.2	2.3
AFTER PERIOD.													
12	13.7	360	1.022	acid	7.23	6.16	0.41	0.170	1.05	....	85.2	5.6	2.3
13	13.8	420	1.018	acid	7.53	6.35	0.43	0.169	0.96	....	84.3	5.8	2.2
14	13.7	430	1.020	acid	7.74	6.82	0.41	0.173	1.11	....	88.1	5.2	2.2
15	13.8	530	1.025	acid	7.31	6.40	0.33	0.176	1.13	....	87.5	4.5	2.4
16	13.7	310	1.020	acid	6.33	5.49	0.30	0.173	0.80	....	86.7	4.7	2.7

1 Day of injection.

NITROGEN BALANCE.

		Fore period.		First adrenalin injection period.		Second adrenalin injection period.		Third adrenalin injection period.		After period.	
Nitrogen intake	. . . . .	31.00		46.50	27.13	38.75					
Nitrogen output	{ Urine . . . . .	21.43	39.88	24.01	14.40	36.14					
	{ Feces . . . . .	1.50	3.07	3.38	0.76	3.50					
Balance	. . . . .	+5.07	42.95	27.39	15.16	39.64					
			+3.55	-0.26	-11.28	-0.89					

adrenalin to produce changes in the percentages of the ammonia and urea nitrogen cannot be ascribed to the conditions of experimentation. A further evidence in favor of this conclusion is that the insufficient diet of itself would have a tendency to cause an increase in the proportion of ammonia nitrogen and a decrease in the percentage of urea, as Folin<sup>1</sup> has demonstrated in man, and which has been similarly observed in this laboratory for the dog.<sup>2</sup>

The apparent discrepancies between the results of Paton and those of the present investigators led the writers to subject Paton's paper to a critical examination. On page 297, Experiment XIII, the following data are recorded:

Diet.	Nitrogen contained.	
	Per cent.	Total.
Oatmeal . . . 150 gm.	2.15	3.22
Milk . . . 500 c.c.	0.366	1.83
Water . . . 600 c.c.	....	....
		<u>4.05</u>

Obviously there is a miscalculation; the total nitrogen of the diet amounting to 5.05, not 4.05 as recorded. That this is not merely a typographical error may be ascertained from the nitrogen balance given on page 298 of Paton's paper, where the figures 4.05 instead of 5.05 have been used throughout. In the calculations given adrenalin appears to cause an excretion of nitrogen exceeding the intake, but by eliminating the error no such excess of output of nitrogen over intake is to be observed. This error does not, however, invalidate Paton's conclusion that adrenalin caused an increased excretion of nitrogen, for from the data given and with the error eliminated there still remains a smaller excess of the nitrogen taken in over that eliminated during the adrenalin period than in the fore or after periods.

From the same experiment, Table VI, page 298, we reproduce the following:

Day.	Nitrogen of Urine.				
	Total in grams.	Urea N in grams.	Per cent of urea N.	NH <sub>3</sub> N in grams.	Per cent of NH <sub>3</sub> N.
1	2.15	....	....	.216	5.4
2	2.15	....	....	.216	5.4

<sup>1</sup> FOLIN: This journal, 1905, xiii, p. 117.

<sup>2</sup> Unpublished experiments.

It is readily seen that the per cent of ammonia nitrogen should be 10, and not 5.4.

Again, in Table V, page 296, the following occurs:

Day.	Nitrogen of Urine.				
	Total in grams.	Urea N in grams.	Per cent of urea N.	NH <sub>3</sub> N in grams.	Per cent of NH <sub>3</sub> N.
3	2.10	1.94	88	.120	5.7
4	3.32	1.98	85	.145	6.2
9	3.83	2.99	78	.466	14.7

The percentages given for urea and ammonia nitrogen on the fourth day are obviously wrong, for the urea nitrogen should be 59 per cent and the ammonia nitrogen 4.3 per cent. On the ninth day the percentage of ammonia nitrogen is 12.1 instead of 14.7 as given.

Without attempting to account for these errors which have led us to subject all of the experimental data to a critical review, we cannot avoid calling attention to some other aspects of Paton's work which seem to demand criticism. Thus in Experiment XI, page 294, he writes: "The weather was warm at the time, and on the fifth, sixth, seventh, and eighth days the urine was distinctly alkaline and appeared to have undergone ammoniacal decomposition." However, these urines were not included in the table. "Since special precaution was not taken to prevent ammoniacal decomposition, it was considered advisable to make another series of observations in which the urine was caught in a solution of hydrochloric acid." Experiment XII then follows, in which the three errors noted above occur and to which alone his conclusions can apply, for in Experiment XIII adrenalin failed to produce any greater disturbance in the relation of urea nitrogen and ammonia nitrogen to total nitrogen than occurred on any two consecutive days of the fore period according to the figures given.

Paton explains the large excretion of ammonia in his experiments by assuming that the sugar formed sets up a condition similar to acidosis. He bases this assumption upon experiments<sup>1</sup> in his laboratory in which it was shown that the subcutaneous injection of large quantities of dextrose into the dog produces an increased excretion of ammonia as a result of the formation of various toxic substances. In recently published experiments<sup>2</sup> we have, however, failed to obtain such results after the subcutaneous injection of dextrose into dogs.

<sup>1</sup> SCOTT: *Journal of physiology*, 1902, xxviii, p. 107.

<sup>2</sup> UNDERHILL and CLOSSON: *Loc. cit.*

Furthermore, from a recent investigation on the distribution of the various urinary forms of nitrogen in diabetes mellitus, we quote the following: "Die Harnstoffausscheidung ist bei Diabetes normal, auch wenn die Ammoniakzahlen eine (nicht erhebliche) Vermehrung zeigen."<sup>1</sup> v. Noorden<sup>2</sup> shows that only when the condition of the diabetic patient approaches coma is the percentage of urea greatly diminished. From these facts it would appear that, even assuming all of Paton's analytical data to be correct, a strict comparison of adrenalin glycosuria with ordinary diabetes is certainly not justified, for in the latter case a typical falling off in urea nitrogen claimed by Paton for the experimental diabetes is rarely realized.

## II. THE ABILITY OF THE ORGANISM TO UTILIZE DEXTROSE IN ADRENALIN GLYCOSURIA.

The glycosuria following adrenalin administration is attributed by Paton to a failure on the part of the tissues to utilize dextrose. If this hypothesis is correct after the injection of large quantities of dextrose into a dog with adrenalin glycosuria, one would expect the administration to be followed by the reappearance in the urine of a great portion of the introduced sugar. Moreover, if the condition induced by the subcutaneous injection of adrenalin is at all analogous to ordinary diabetes, the dextrose given should not be utilized.

The following experiment bears upon this point:

*Experiment 3.*—A bitch of 8 kilos on a fixed diet received 20 c.c. of adrenalin chloride subcutaneously, resulting in the excretion of 11.5 gm. of dextrose in the urine. Two days later the same quantity of adrenalin chloride was administered and 187 c.c. of a 30 per cent dextrose solution (7 gm. per kilo) were introduced subcutaneously. The total elimination of dextrose in the urine was 17.6 gm. A second experiment was carried out with another animal under similar conditions resulting in the appearance of only the merest trace of dextrose in the urine.

Previous experiments<sup>3</sup> in this laboratory have shown that only a very small quantity of dextrose appears in the urine, even when as much as 7 gm. per kilo are administered subcutaneously to the dog. Since in the above experiment adrenalin alone sufficed

<sup>1</sup> SATTÀ: Beiträge zur chemischen Physiologie, 1905, vi. p. 373.

<sup>2</sup> v. NOORDEN: *Loc. cit.*

<sup>3</sup> UNDERHILL and CLOSSON: *Loc. cit.*



to call forth an excretion of 11.5 gm. of dextrose and adrenalin and 56 gm. of dextrose together caused the elimination of only 17.6 gm. of dextrose, it may be concluded that the interference of adrenalin with the normal retention of dextrose is minimal at most. Nor in view of these results can the experimental conditions induced by adrenalin be compared to the condition that obtains in ordinary diabetes where excess of carbohydrate is speedily eliminated.

### III. THE NATURE OF ADRENALIN GLYCOSURIA.

Since Blum's<sup>1</sup> discovery of adrenalin glycosuria various attempts have been made to explain the mechanism involved. Thus the drug has been improperly placed<sup>2</sup> in the category with such glycosuria-producing substances as carbon monoxide, morphine, strychnine, etc., which have been shown to produce the appearance of sugar in the urine by an action upon respiratory factors.<sup>3</sup> Again it has been claimed that in this experimental condition the pancreas is more or less involved.<sup>4</sup>

From the mass of experimental data to be found in the literature certain well-defined observations have been repeatedly confirmed. That the reducing body present in the urine after the parenteral administration of adrenalin is dextrose is well established, and that a true hyperglycæmia occurs has been shown by Metzger,<sup>5</sup> Zuelzer,<sup>6</sup> Vosburgh and Richards,<sup>7</sup> Paton,<sup>8</sup> and Underhill.<sup>9</sup> According to several investigators (Blum, Herter, Paton), the intensity of the glycosuria caused by adrenalin is in a measure at least dependent upon the quantity of carbohydrates in the body, although Blum claims that glycosuria may be induced in dogs and rabbits after a fast so prolonged that all the glycogen has disappeared from the liver. Pflüger,<sup>10</sup>

<sup>1</sup> BLUM: Deutsches Archiv für klinische Medicin, 1901, lxxi, p. 146.

<sup>2</sup> HAMMARSTEN: Text-book of physiological chemistry, 1904, p. 256.

<sup>3</sup> Cf. UNDERHILL: *Loc. cit.*

<sup>4</sup> HERTER: Medical News, 1902, xxx, p. 865; HERTER and WAKEMAN: VIRCHOW'S Archiv für pathologische Anatomie, 1902, clxix, p. 479; *Ibid.*, American journal of the medical sciences, 1903, cxxv, p. 46.

<sup>5</sup> METZGER: Münchner medicinische Wochenschrift, 1902, p. 478.

<sup>6</sup> ZUELZER: Berliner klinische Wochenschrift, 1901, p. 1209.

<sup>7</sup> VOSBURGH and RICHARDS: This journal, 1903, ix, p. 35.

<sup>8</sup> PATON: *Loc. cit.*

<sup>9</sup> UNDERHILL: *Loc. cit.*

<sup>10</sup> PFLÜGER: Archiv für die gesammte Physiologie, 1902, xci, p. 119.

however, has shown the great difficulty of obtaining dogs with glycogen-free livers by inanition alone.

The repeated administration of adrenalin even in well-fed dogs is not always followed by glycosuria, as the appended experiment shows.

Date. 1906	Injection of adrenalin chloride in c.c.	Dextrose in urine in grams.
Feb. 25	10	5.6
" 26	10	6.9
Mar. 5	10	none
" 6	10	4.5
" 7	5	none
" 15	10	none
" 16	10	none
" 17	10	trace
" 18	10	none
" 19	20	none

This confirms observations recorded by Herter,<sup>1</sup> Loeper and Crouzon,<sup>2</sup> and Paton.<sup>3</sup>

The study of the influence of adrenalin upon the glycogen content of the liver has shown that "in acute adrenalin poisoning, if the animal does not die too rapidly, the glycogen in the liver is diminished in amount," while "in chronic adrenalin poisoning the amount of glycogen in the liver is not necessarily altered."<sup>4</sup> This would appear to account for the failure of glycosuria after repeated doses of adrenalin, as in the experiment given above. A certain tolerance seems to be established. Doyon, Morel, and Kareff<sup>5</sup> have demonstrated that adrenalin causes a diminution of glycogen of the liver accompanied by a rise in the sugar content of the blood. Moreover, adrenalin<sup>6</sup> perfused through surviving livers stimulates sugar formation.

That the pancreas ordinarily plays a rôle in adrenalin glycosuria is not established. Although Herter's<sup>7</sup> observations would seem to in-

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

<sup>2</sup> LOEPER and CROUZON: *Archives de médecine expérimentale*, 1904, xvi, p. 83; Abstract in *Comptes rendus de la société de biologie*, 1903, lv, p. 1376.

<sup>3</sup> PATON: *Loc. cit.*

<sup>4</sup> PATON and DRUMMOND: *Journal of physiology*, 1904, xxxi, p. 92.

<sup>5</sup> DOYON, MOREL, and KAREFF: *Journal de physiologie*, 1905, p. 998.

<sup>6</sup> IWANOFF: *Zentralblatt für Physiologie*, 1905, xix, p. 891.

<sup>7</sup> HERTER: *Loc. cit.*

dicate such a function on the part of this organ, the results of Doyon, Morel, and Kareff,<sup>1</sup> are opposed to it.

The influence exerted by adrenalin upon the tissues has been studied by Drummond<sup>2</sup> and Elliott.<sup>3</sup> According to the former the toxic action of adrenalin is most distinctly seen in the glandular organs, and particularly the liver and kidney. Elliott, on the other hand, demonstrates "that its single characteristic is the aptness to stimulate plain muscle and gland cells that are or have been in functional union with sympathetic nerve fibres."<sup>4</sup> He shows that only where sympathetic nerve fibres exist does adrenalin have an action.

From the facts given it appears to the writer that the influence of adrenalin in causing glycosuria can be explained most readily by assuming that it acts directly upon the nervous elements, probably through the sympathetic system. In no other way is it likely that such small quantities injected would have such pronounced effects. The stimulation of the sympathetic nerves leading to the liver or to other storehouses of carbohydrate may be sufficient to cause these organs to throw out their stored up material in the form of dextrose. While it lasts the stimulation is probably intense and a greater quantity of dextrose is formed and discharged into the circulation than can be cared for immediately. As fast as it is returned to the liver to be transformed into glycogen, it is rejected, owing to the stimulation of the liver cells by the adrenalin. It would appear from this that two processes may be involved, — one causing the transformation of glycogen into dextrose, the other preventing the reverse process. Blum<sup>5</sup> in one of his first papers on adrenalin glycosuria put forth the view that it resembled piqûre rather than pancreatic diabetes. The action upon the nervous system appears to the writer to be the essential point. The resulting stimulation of the liver cells in connection with these nerve fibres causes an increased output of dextrose, and whether this output of dextrose is due to an increased formation or to a diminished storage of dextrose is a purely secondary matter.

<sup>1</sup> DOYON, MOREL, and KAREFF: *Loc. cit.*

<sup>2</sup> DRUMMOND: *Journal of physiology*, 1904, xxxi, p. 81.

<sup>3</sup> ELLIOTT: *Ibid.*, 1905, xxxii, p. 401.

<sup>4</sup> Cf. v. FREY: *Sitzungsberichte der Würzburger physikalische-medizinische Gesellschaft*, 1905.

<sup>5</sup> BLUM: *Archiv für die gesammte Physiologie*, 1902, xc, p. 617

## SUMMARY.

No evidence has been obtained that the distribution of the nitrogen — in the forms of ammonia nitrogen, urea nitrogen, creatinin nitrogen — is altered by the subcutaneous administration of adrenalin in dogs. The results of Paton in which the ammonia nitrogen of the urine was increased and the urea nitrogen decreased have not been confirmed.

The ability of the organism to utilize dextrose subcutaneously administered in adrenalin glycosuria is not seriously impaired.

Repeated doses of adrenalin finally fail to produce glycosuria, thus giving evidence of the establishment of a certain degree of tolerance, as already noted by other investigators.

It is suggested that the mechanism of adrenalin glycosuria is essentially of nervous origin, and acts through the intermediation of the sympathetic nervous system which is called into play. This activity is in part directed upon the sugar-storing organs, causing them either to relinquish their supply of dextrose-producing substances, or preventing the storage of glycogen, owing to the stimulation of the cells, or both, thereby causing hyperglycæmia and glycosuria.

Adrenalin glycosuria bears no relation to ordinary diabetes in man.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE  
MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.  
E. L. MARK, DIRECTOR. No. 180.

THE REACTIONS OF EARTHWORMS TO SALTS: A STUDY  
IN PROTOPLASMIC STIMULATION AS A BASIS OF  
INTERPRETING THE SENSE OF TASTE.

BY G. H. PARKER AND C. R. METCALF.

WHAT transpires when a stimulating solution causes a reaction in an organism is not easily stated, and may differ even fundamentally in different cases, but with increased knowledge as to the nature of solutions, the complete analysis of such instances becomes more nearly possible, and experimentation can be directed with greater definiteness toward solving such problems. The object of the work recorded in this paper was to attack this question in a well circumscribed case, and for this purpose a class of relatively simple salts, the chlorides of sodium, potassium, lithium, and ammonium, were selected as stimuli, and the common earthworm, *Allolobophora fætida*, as the reacting organism. That this animal is strongly chemotropic had already been attested by Loeb ('94, p. 262).

The salts used were the purest obtainable commercial products, the so-called c. p. salts, which were subsequently still further purified by recrystallization. We are under obligations to Professor C. R. Sanger, of the Harvard Chemical Laboratory, for aid in getting sodium chloride free from sulphate, and to his assistant, Mr. M. L. McCarthy, for the preparation of pure lithium chloride. The solutions were made up in distilled water, and titrated against a mol. solution of silver nitrate, with potassic chromate as an indicator, and corrected till they were within 2 per cent of agreement with the standard solution. The dilutions made from the stock solutions of salts were also titrated against a silver solution, that possible mistakes in mixing might be checked.

The method employed in testing the worms was very similar to that used by Loeb (:02), and especially by Braeuning (:04), on the frog. The worms were thoroughly rinsed in tap-water till they were cleaned externally of foreign matter, and each worm was then suspended from the tail-end by a silk thread. The thread was attached to the worm by being passed through its tail-end on a needle, and then tied in a loose knot. As might have been expected from the work of Norman (:00), the worms reacted very slightly, if at all, to this method of suspension. The worms thus prepared and numbered were kept singly in small, open glass vessels of tap-water. They were always handled by means of the silk thread, as this method proved far more satisfactory than any other tried.

The apparatus with which the tests were made, which is shown in the accompanying figure, consisted of a base with an upright wooden post to which a pivoted arm was fixed. One of the ends of the arm was notched so that the silk thread carrying a worm could be inserted into it, and the other end was used as a handle by which the arm could be moved so as to raise the worm or lower it into the solution contained in a glass vessel on the base.

When a test was to be made, a worm was taken up by its thread and attached to the arm of the apparatus. The superfluous water was drained from it, and, after it had lengthened fully, it was lowered gently but quickly into the solution to the depth of the anterior edge of the clitellum. As the tip of the worm cut the surface of the solution, a stop-watch was started, and when as a result of the contraction of the worm the tip withdrew from the solution the watch was stopped. The interval of time thus recorded to fifths of a second was taken as the reaction time for that particular experiment. From some of the less stimulating solutions the worms did not withdraw for a considerable period; after two minutes had expired such experiments were discontinued, and the worm was taken out of the solution. Records of this kind of result, which in the whole series of trials was relatively rare, are indicated in the tables by an asterisk, thus, 120\*. After the worm under ordinary circumstances had withdrawn from the solution, it was rinsed in tap-water, returned to its glass, and allowed to rest at least five minutes before another trial with it was made. After twenty or thirty such trials, even with periods of rest of at least five minutes, it was found that the worms gradually decreased in their reactivity, and it was decided for the sake of uniformity to use no worm for more than twelve reactions.

To make these tests with the four solutions usually employed in as uniform a way as possible, four worms were used in each set of trials, and the solutions were made to follow the sequence indicated by the numbers in Table I.

TABLE I.

The numbers in this table show the sequence in which the solutions were used in testing four worms in any set of reactions.

Number of the worm.	SALTS.			
	NaCl.	KCl.	LiCl.	NH <sub>4</sub> Cl.
1	1	5	9	13
2	14	2	6	10
3	11	15	3	7
4	8	12	16	4

It will be seen by inspecting this table that each solution was used on each worm, and further that each solution had first place with some one worm. By averaging the reaction times thus obtained for each salt, a measure of its capacity to stimulate was arrived at in which exhaustion as a disturbing factor was eliminated. In this way results were obtained that admitted of direct comparison. As a matter of fact, it was found that the series given in Table I could be repeated three times on one set of worms without calling forth obvious evidence of exhaustion, and the tables of actual results contained in the Appendix represent this procedure. So far as the reaction times were concerned, the particular sequence in which the salts were used was found to be immaterial provided that after each test the worm was kept for at least five minutes in tap-water. In the actual experiments the sequence given in Table I (NaCl, KCl, LiCl, NH<sub>4</sub>Cl) was used, but experience showed that any other sequence was equally good. Although the tables given in the Appendix were originally made up in the order given in Table I, that order, for ease of comparison, has been changed to the one established by the relation of the reaction times themselves. But, as already stated, there is not the least reason to suppose that the sequence now given in these tables would not have yielded the same results. The reaction times were taken during March, April, and May.

Notwithstanding the care exercised in the management of the worms, it was found that some individuals presented striking and characteristic differences as compared with others, as Braeuning (1904, p. 177) has already stated to be the case with frogs. These differences are to be attributed to the different physiological states of the worms. Since, however, each solution was used on each worm, such differences offer no difficulty in the interpretation of single

TABLE II.

Average reaction times in seconds of *Allolobophora fætida* to solutions of various strengths of the chlorides of potassium, lithium, ammonium, and sodium, and to distilled water.

Salts.	m.	$\frac{m}{10}$ .	$\frac{m}{15}$ .	$\frac{m}{25}$ .	$\frac{m}{50}$ .	$\frac{m}{100}$ .	$\frac{m}{500}$ .	H <sub>2</sub> O.
KCl	0.2	26.87	32.05	45.85	55.45	41.57	29.88	} 20.02
LiCl	0.2	11.37	15.12	30.25	37.80	36.13	17.27	
NH <sub>4</sub> Cl	0.2	2.70	6.48	11.35	17.73	23.05	12.18	
NaCl	0.2	0.25	0.2	0.2	0.25	1.53	7.43	

tables, but they do render the comparison between tables less reliable, though, as a general inspection of the results will show, the error thus introduced cannot be said to vitiate the final conclusions.

The strengths of solutions used were mol.  $\frac{m}{10}$ ,  $\frac{m}{15}$ ,  $\frac{m}{25}$ ,  $\frac{m}{50}$ ,  $\frac{m}{100}$ , and  $\frac{m}{500}$ , and tests were made with distilled water.

The detailed results of these experiments are contained in Tables VIII to XV in the Appendix, the averages from which are given in Table II.

On inspecting this table it will be seen that the mol. solutions of all four salts were so vigorously stimulating that the worms always withdrew within two-tenths of a second and, consequently, it is impossible for us to state whether at this concentration one salt was a more effective stimulus than another. In all the remaining sets of solutions the average reaction times were different for each salt, and consequently the salts can be arranged in sequences in accordance with their stimulating capacities. These sequences agree in all the concentrations from  $\frac{m}{10}$  to  $\frac{m}{500}$ . Beginning with the salt giving the shortest reaction time, this sequence is sodium, ammonium, lithium, and potassium.



Each salt likewise shows a characteristic series for its own concentrations. In sodium the reaction times in concentrations from mol. to  $\frac{m}{50}$  inclusive are probably all minimal, the slight deviations observed being obviously accidental. At  $\frac{m}{100}$  a real lengthening of the time takes place, and this is increased at  $\frac{m}{500}$ , but even at this dilution it is not so slow as in distilled water.

Ammonium at mol. concentration gives a minimal reaction time, but the time lengthens at  $\frac{m}{10}$  and continues to lengthen progressively to  $\frac{m}{100}$ . At  $\frac{m}{500}$  it shortens considerably, to become again longer for distilled water.

The series for lithium starts at a minimal reaction time for the mol. solution, which is followed by a series of progressively increasing periods to a maximum at  $\frac{m}{50}$  or  $\frac{m}{100}$ , after which it quickens at  $\frac{m}{500}$  to a time somewhat more rapid than that for distilled water. No two-minute records were obtained from any of the worms in the sodium or ammonium solutions, but in the lithium solutions one was observed, namely, at concentration  $\frac{m}{50}$  (Appendix, Table XII).

The series for potassium also begins with a minimal reaction time at a mol. concentration, and progressively lengthens at each concentration to a maximum at  $\frac{m}{50}$ . At  $\frac{m}{100}$  it perceptibly shortens and again at  $\frac{m}{500}$ , but not so much so as in distilled water. The potassium series contains three two-minute interrupted records, one at  $\frac{m}{25}$  and two at the maximum  $\frac{m}{50}$  (Appendix, Tables XI and XII).

When the series for the different salt solutions are compared, it will be seen that each series shows one maximum, and that in accordance with the reaction times of these maxima the salts fall into a sequence agreeing with that shown by the reaction times of any concentration between  $\frac{m}{10}$  and  $\frac{m}{500}$ , *i. e.*, the shortest maximum reaction time is shown by sodium chloride (7.43 sec.);<sup>1</sup> the next by ammonium (23.05 sec.); the next by lithium (37.80 sec.); and the longest by potassium (55.45 sec.). Since these reaction times may be taken as indications of the degree of stimulation, it is clear, from what has been shown, that the most stimulating solution is that of sodium followed in sequence by ammonium, lithium, and potassium.

Earthworms contain such a large proportion of water that it is quite natural to suppose that changes in their fluid contents due to osmotic pressure might be a means of stimulation.

It is conceivable that the extraction of water from the skin of the

<sup>1</sup> It is probable that the reaction time to sodium chloride is longer in solutions weaker than  $\frac{m}{500}$ , but these were not tested.

worm, or the infiltration of this material into the animal might stimulate the integumentary sense organs in such a way as to result in the reactions already recorded. Under such conditions stimulation ought not to occur in solutions that are isotonic with the fluids of the worm, but any solution whose osmotic pressure is either greater or less than that of the fluids of the worm might be stimulating. Moreover, under such circumstances the reaction time ought to show some relation to the disparity between the osmotic pressure of the fluids of the worm and that of the stimulating solution, in that the

TABLE III.

Dissociation per thousand molecules and osmotic pressure in atmospheres of the various strengths of solutions of the chlorides of lithium, sodium, ammonium, and potassium. The table was calculated from the data given by Kohlrausch and Holborn ('98).

Strengths of solutions.	Dissociation per thousand.				Osmotic pressure in atmospheres.			
	LiCl.	NaCl.	NH <sub>4</sub> Cl.	KCl.	LiCl.	NaCl.	NH <sub>4</sub> Cl.	KCl.
<i>m</i>	625	675	746	748	36.40	37.52	39.11	40.16
$\frac{m}{10}$	818	839	851	853	4.07	4.12	4.15	4.15
$\frac{m}{15}$	842	859	874	873	2.75	2.77	2.80	2.80
$\frac{m}{25}$	867	880	895	893	1.67	1.68	1.70	1.70
$\frac{m}{50}$	898	908	919	915	0.85	0.85	0.86	0.86
$\frac{m}{100}$	923	932	939	934	0.43	0.43	0.43	0.43
$\frac{m}{500}$	961	967	970	965	0.09	0.09	0.09	0.09

time should lengthen as these two factors approximate each other and shorten as they diverge.

Table III gives the calculated osmotic pressures in atmospheres of the solutions used in the preceding experiments and a comparison of this table with Table II, in which the reaction times of the worms to these solutions are given, will show something of the value of this factor in stimulation. It will be seen at once in Table III that the osmotic pressures for any set of four equimolecular solutions are fairly close, and yet the reaction times to these solutions, as shown in Table II, are often quite far apart; thus at  $\frac{m}{100}$  the osmotic pressures of the four solutions are practically identical, but the reaction times to these are such as to make them easily distinguishable. Moreover,

if the amount of osmotic pressure were the effective factor in stimulation, the sequence of the salts in any set of solutions where this sequence could have been made out should have been potassium, ammonium, sodium, and lithium, but, instead of that, at all concentrations in which a sequence was observable, it was sodium, ammonium, lithium, and potassium. It is thus clear that differences of osmotic pressure are not the controlling factors in these reactions.

Notwithstanding what has been said, it cannot be denied that osmotic pressure is an important factor in determining the state of an earthworm. That these animals change their volumes by giving out water or taking it up in accordance with the condition of the surrounding medium is shown by the following experiment. The anterior ends of a number of earthworms were cut off and the worms were kept under normal conditions till by the healing of the wound their mouths were closed. They were then placed in shallow, dis-

TABLE IV.

Percentage change in volume in *Allolobophora fetida* on being transferred from distilled water to various concentrations of potassium chloride in water, where the worms remained 24 hours.

Strength of sol. of KCl.	m.	$\frac{m}{10}$ .	$\frac{m}{15}$ .	$\frac{m}{25}$ .	$\frac{m}{50}$ .	$\frac{m}{100}$ .	$\frac{m}{200}$ .
Percentage loss in volume of the worms.	60	22	19	14	9.9	7.0	6.6

tilled water, where they remained for several days till their digestive tubes were entirely free from waste. Eight sets each of five such worms were then measured volumetrically by removing the superficial moisture from the worms and passing them into a narrow graduated tube containing a known volume of water. This operation was repeated from time to time till the worms showed an approximately constant volume, which happened usually within about twenty-four hours. After their volumes were thus determined, one set of worms was put into each of the seven solutions of potassium chloride used in the preceding experiments, and, after twenty-four hours, their volumes were again ascertained by measuring the worms in the graduated tube, using in place of distilled water the solutions in which they had been retained. The eighth set was kept in distilled water as a check. The volume of the eighth set remained practically constant, and the changes in the volumes of the other seven sets are given in Table IV.

The worms put in the mol. solution contracted vigorously, discharged droplets of a thick, whitish secretion, which clung to their integument, and died in a few minutes. At the end of twenty-four hours they were much shrivelled. In all the other solutions the worms lived over a day without showing any evidence of deterioration, though in the  $\frac{m}{10}$  solution they were almost constantly in motion, as though continually stimulated.

All the living worms, during the twenty-four hours that they were in the solutions, discharged, probably from the skin, a small amount of slimy secretion. This must have reduced their volumes somewhat, but, since this secretion on being collected never measured as much as one per cent of the total volume of the worms, it was disregarded, for it was evident from the facts already given that even allowing for this slight loss, there was still abundant evidence to show that earth-worms, like frogs (Braeuning, :04, p. 173), change their volume by taking in and giving out water.

Although this whole process, which goes on rather slowly, is probably not the occasion of the stimulation due to salt solutions, its first steps, or something very like them, we believe, are unquestionably concerned in this operation. In many cases the reactions of the worms were relatively so quick that the organs stimulated must have been very superficial in position, probably in the epidermis, and the transfer of materials to these organs from the surrounding solution or the reverse is probably one of the causes of stimulation rather than the more general transfer between the deeper parts of the body and the exterior. If some such physical operation is not assumed, it is difficult to explain why distilled water of the stock that was used in making up the solutions was more stimulating than some of the solutions themselves, such as ammonium chloride  $\frac{m}{100}$ , lithium chloride  $\frac{m}{25}$  to  $\frac{m}{100}$ , or potassium chloride,  $\frac{m}{10}$  to  $\frac{m}{500}$  (Table II). Worms lived in this distilled water for over a week without apparent deterioration, and we never observed any injurious effects such as might be attributed to very minute amounts of metallic impurities (Nägeli's oligodynamic effects). We therefore believe that the stimulating effect of distilled water as compared with that of certain salt solutions is to be attributed to some such physical cause as osmotic pressure or even simple diffusion. Loeb (:02, p. 261) has shown that it is probable that the entrance of water into the skin of a frog is stimulating to the integumentary sense organs and its exit quieting, and that the reverse is true for motor nerves, — a conclusion confirmed by Mathews (:05);

and Braeuning (1904, p. 172), who seems not to have known of Loeb's work, also lays stress on this method of stimulation in the reactions of frogs to salt solutions. While these physical transfers of materials may be looked upon in a certain sense as stimuli, the real stimulation is probably due to the changes produced by them in the concentration of the substances dissolved in the protoplasm.

Although these physical processes doubtless play some part in this form of stimulation in the earthworm, it is obvious, from what has been stated, that they cannot account for the characteristic and striking differences in the reaction times of these animals for the four sets of salts tested. These differences must be explained in some other way, and are probably dependent upon the action of some of the ionic constituents of the solutions.

Since the degrees of dissociation for the four salts are fairly close for each strength of solution used (Table III), and since all four salts are chlorides, it follows that their different stimulating effects cannot well be attributed to the action of the common anion, chlorine. Moreover, if this ion were the effective stimulating material, the sequence of the salts from the standpoint of stimulation ought to be for most of the sets of solutions ammonium, potassium, sodium, and lithium. Since the sequence is sodium, ammonium, lithium, and potassium, it is obvious that the chlorine ions have no effect in determining this sequence.

To explain this differential effect in stimulation there remain the four cations, sodium, ammonium, lithium, and potassium, and the undissociated molecules. Of these the sodium ions or sodium chloride molecules or both must be responsible for the very stimulating quality of the sodium solutions. That the sodium ions are very probably the stimulating element is seen from the fact that the solutions of all other sodium salts that we tested (sodium nitrate, sulphate, and acetate) called forth the same quick reaction that sodium chloride did. This is well seen in sodium acetate in comparison with sodium chloride, for which the reaction times are given in Table V.

From these observations we conclude that the effective stimulating element in the sodium solution is the sodium ion and not the undissociated molecule of salt. In a similar way we have been led to a corresponding conclusion regarding the other salts, and we therefore believe that the characteristic reaction times for the four salts tested depend primarily upon their cations. Since these cations are all univalent, valence cannot be called upon as a means of explaining the

observed differences in stimulation, as in fact Loeb (:02, p. 262) has already shown in the frog. Such stimulation must depend upon the specific properties of the cations in question.

In passing from the stronger to the weaker solutions, the number of cations diminishes, stimulation is less, and the reaction times

TABLE V.

The reaction times in seconds of four earthworms, *Allolobophora foetida*, to strengths of sodium chloride and of sodium acetate from  $\frac{m}{25}$  to  $\frac{m}{400}$ . While sodium chloride at  $\frac{m}{25}$  is saltish, and at  $\frac{m}{50}$  is slightly salty and the corresponding sodium acetate solutions have a slight taste, all other solutions ( $\frac{m}{100}$  to  $\frac{m}{400}$ ) were to us indistinguishable from distilled water.

No. of the worm.	$\frac{m}{25}$ .		$\frac{m}{50}$ .		$\frac{m}{100}$ .		$\frac{m}{200}$ .		$\frac{m}{400}$ .	
	NaCl.	CH <sub>3</sub> COONa.	NaCl.	CH <sub>3</sub> COONa.	NaCl.	CH <sub>3</sub> COONa.	NaCl.	CH <sub>3</sub> COONa.	NaCl.	CH <sub>3</sub> COONa.
29	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	14.8	17.6
30	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	4.2	8.0
31	0.2	0.2	0.4	0.2	0.2	0.2	0.2	0.2	0.4	0.6
32	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	9.4	6.8
Averages	0.2	0.2	0.25	0.2	0.2	0.2	0.2	0.2	7.2	8.25

lengthen (Table II). After the maximum time in any series has been reached (ammonium  $\frac{m}{100}$ , lithium  $\frac{m}{50}$  to  $\frac{m}{100}$ , potassium  $\frac{m}{50}$ ), the increase in rate in the still weaker solutions is due, we believe, to double stimulation caused in part by the cations, and in part by a more physical action of the solution as a whole, a form of stimulation that is probably seen in its purity in the reaction to distilled water. It seems to us therefore that while the different reaction times of the worms are dependent primarily on stimulation by the cations of the particular solutions, they are also partial products of certain other stimuli which, however, in themselves would not give differential effects.

The reactions of the worms dealt with in this paper are not inappropriately ascribed to the sense of taste, and it is well known that the

four salts here used are stimuli for the salty taste in man. Kahlenberg ('98) and Höber und Kiesow ('98) have pointed out that this salty taste is dependent upon the anion chlorine and that a similar taste is excited by the closely related ions bromine and iodine. It is perfectly clear, however, that there is a wide gap between the condition in man and that in earthworms, for, while we can taste solutions of common salt only to about a  $\frac{1}{50}$  dilution, earthworms react vigorously to dilutions ten times as great,  $\frac{1}{500}$ . Not only is this difference in sensitiveness characteristic for man and the earthworm, but the two organisms in this case react to opposite classes of ions. In man, as already mentioned, the salty taste of the four salts used in our experiments is unquestionably due to the common anion chlorine; in the earthworm, as we have demonstrated, the characteristic stimulation is due to the cations sodium, ammonium, lithium, and potassium; in other words, for the earthworm the solutions tested do not represent a homogeneous group corresponding to the salty taste in man, but the worm reacts with striking difference to these four solutions. Thus it is clear that the primary stimulus for man is the anion, for the earthworm the cation of the salts tested.

Although physiologists are inclined to describe the salty taste as a homogeneous taste and to look upon chlorine, bromine, and iodine ions as the homologous stimulus for it, such a view is only partly true. If any one tests by taste the four solutions that we used, he can with a very little practice come to distinguish them with perfect certainty. All four solutions at mol. strength taste characteristically salty, but the taste of each one has a slight but peculiar flavor which allows that one to be distinguished from the rest. This specific character of the saltiness, which has already been recognized by Höber (:02, p. 183), must be due to cationic stimulation, — a feature which, as we have just seen, is greatly emphasized in earthworms. We believe therefore that while the organs of taste in man are stimulated chiefly by the anions of the four salts studied, they are also stimulated slightly by the cations; thus the positive charge as well as the negative charge on ions may be stimulating, as Loeb (:02, p. 262) has demonstrated in the frog. It is not impossible that in the earthworm, where the cations are the chief stimuli, the anions may also play a subordinate rôle, but of this we have no conclusive evidence.

Since the same salts that give rise to an almost homogeneous stimulation in man produce such diverse effects in *Allolobophora*, we must conclude that the occasion of the differences is to be sought

for in the different constitution of the protoplasm of the two organisms. While the living substance of the organs of taste in *Allolobophora* is of such a composition that it is open to easy stimulation by the cations sodium, ammonium, lithium, and potassium, and is not especially stimulated, if at all, by the anion chlorine, that of the gustatory organs of man is so constituted that it is easily stimulated by the anion chlorine and very feebly affected by the four cations named. In other words, we are driven to assume a high degree of protoplasmic differentiation in the gustatory end-organs of these two animals, and the idea, often implied in recent biological work, that protoplasm exhibits uniformity in its vital reactions to such relatively simple reagents as solutions of electrolytes, appears to be untenable. Our observations lead to the view that the composition of living substance is so immensely complex, and the possibilities of its differentiation are so great, that sweeping generalizations as to its relation to chemical stimuli are to be accepted with the utmost caution.

If the terminal protoplasm of the gustatory organs of various animals may be differentiated to the extent that this discussion suggests, it follows that different animals in accordance with their different chemical environments might be expected to present different modes of stimulability, *i. e.*, to be differently adapted to chemical stimulation. To test this proposition, the salt solutions employed in the preceding experiments were tried as stimuli on a species of earthworm from a different environment from that in which *Allolobophora* lives. *Allolobophora*, as is well known, is found almost exclusively in horse manure. The second worm tested, *Helodrilus caliginosus* (Sav.), is found almost always in garden soil, and hence must have encountered a different set of solutions from those which influence *Allolobophora*. The reaction times of *Helodrilus* to  $\frac{m}{50}$  solutions of the chlorides of sodium, ammonium, lithium, and potassium were taken by Mr. Owen Bryant, to whom we are indebted for the use of Table VI.

An inspection of Table VI will show that in all four sets of experiments the average reaction times, though steadily shortening as the spring advanced, give grounds for a distribution of the salts in the invariable sequence potassium, ammonium, sodium, and lithium. This sequence was so different from that obtained by us with *Allolobophora* that Mr. Bryant tried on *Allolobophora* the very solutions with which he obtained the results on *Helodrilus*, and found that they gave the sequence recorded in our earlier experiments, namely, sodium,



ammonium, lithium, and potassium. It thus appears that these salts are stimulating to the manure-inhabiting *Allolobophora* in the sequence sodium, ammonium, lithium, and potassium, and to the earth-inhabiting *Helodrilus* in the order potassium, ammonium, sodium, and lithium. We have here undoubtedly two conditions of stimulation dependent in all probability upon environmental adaptation and exhibiting again the capacity of protoplasm for this kind of differentiation.

TABLE VI.

Average reaction times in seconds of the earthworm *Helodrilus caliginosus* (Sav.) to  $\frac{1}{50}$  solutions of the chlorides of lithium, sodium, ammonium, and potassium. Each average is based on twelve readings, four from each of three worms, as described on p. 57.

Date.	Source of worms.	$\frac{m}{50}$ KCl.	$\frac{m}{50}$ NH <sub>4</sub> Cl.	$\frac{m}{50}$ NaCl.	$\frac{m}{50}$ LiCl.
April 1	Cohasset, Mass.	4.19	6.81	18.56	20.39
" 6	" "	3.67	4.87	17.73	32.32
" 18	Arlington, "	2.28	2.67	7.43	8.61
" 27	Cohasset, "	1.18	1.65	1.77	3.67

Regarded from the standpoint of adaptation, the sense of taste may be looked upon as one of the means of distinguishing between a favorable and an unfavorable chemical environment, or, in its restricted form as a means of distinguishing wholesome from unwholesome food. From this standpoint injurious or poisonous substances ought to be tasted in very weak solutions, *i. e.*, the toxicity and the sapidity of a substance ought to be related. It is therefore interesting to observe how very reactive *Allolobophora* is to solutions of sodium chloride, a substance which, as Ringer ('95), Loeb (:00, :05), Cushing (:01), Ostwald (:05), and others have pointed out, is in its pure state especially injurious to many animals and animal tissues. These injurious effects can be largely counteracted by the addition of other substances, especially calcium salts, to the solutions—a procedure which tends to produce what Loeb has called a balanced physiological salt solution.

If taste is related to toxicity in the way suggested, we ought to find an increase in the reaction times of *Allolobophora* to solutions of sodium chloride containing, for instance, calcium. The results of a set of trials to test this question are shown in Table VII.

It is clear from these results that so far as the sense of taste in *Allolobophora* is concerned, calcium ions do not counteract sodium ions, and that these experiments give no support to the view that what will alter the toxicity of a substance will correspondingly change its taste. Nevertheless it must not be forgotten that silver and mercury ions can be tasted in greater dilutions than hydrogen ions,

TABLE VII.

Reaction times in seconds of *Allolobophora* to solutions of sodium chloride and calcium chloride singly and combined.

Number of the worm.	Number of the trial.	NaCl $\frac{m}{100}$ .	CaCl <sub>2</sub> $\frac{m}{100}$ .	NaCl $\frac{m}{100}$ CaCl <sub>2</sub> $\frac{m}{100}$ . <sup>1</sup>	NaCl $\frac{m}{200}$ CaCl <sub>2</sub> $\frac{m}{200}$ . <sup>2</sup>
33	1	9.2	17.8	6.2	13.6
	2	8.4	14.2	9.0	16.4
	3	10.0	11.2	10.2	17.8
34	1	0.2	7.2	0.2	0.2
	2	0.2	7.8	0.2	0.2
	3	0.2	5.8	0.2	0.2
35	1	7.2	11.2	6.8	12.2
	2	6.6	9.4	7.4	10.6
	3	5.8	12.6	5.0	8.2
Averages . . . .		5.3	10.8	5.0	8.8

<sup>1</sup> This mixture was made by adding to 5 c.c. of normal sodium chloride 5 c.c. of normal calcium chloride and 490 c.c. of water.

<sup>2</sup> This mixture was made by adding to 2.5 c.c. of normal sodium chloride 2.5 c.c. of normal calcium chloride and 495 c.c. of water.

and hydrogen ions in greater dilutions than hydroxyl ions, and that the heavy metal ions are usually more poisonous than hydrogen ions and hydrogen ions more poisonous than hydroxyl ions. Whether, however, such a rule holds generally is still to be ascertained. But, however this may be, it is obvious, from what has already been shown, that the sense of taste in animals, as exhibited by their reactions to electrolytes, necessitates the conception of fundamentally diverse conditions in the protoplasm of their gustatory organs.

SUMMARY.

1. *Allolobophora foetida* withdraws from solutions of the chlorides of sodium, ammonium, lithium, and potassium varying in strength from mol. to  $\frac{m}{500}$  at rates that show these salts to be stimulating in the sequence (beginning with the most energetic) of sodium, ammonium, lithium, and potassium. The worms withdraw quickly from distilled water that gives no evidence of oligodynamic action.

2. *Allolobophora* is stimulated in part by the physical action of the salt solutions (osmotic pressure, diffusion, etc.), in part possibly by the chlorine anions (but of this there was no conclusive evidence), and principally by the cations sodium, ammonium, lithium and potassium. The differential stimulation depends not upon valence or the sign of the electric charge, but upon the specific character of the cations.

3. To man all four solutions taste salty, but with slight characteristic differences; the salty taste is due to the anion chlorine, the characteristic flavor to the cations sodium, ammonium, lithium, and potassium.

4. In *Allolobophora* the chief stimulus is the cation, in man the anion; hence the gustatory protoplasm of these two organisms must be chemically differentiated in these two directions.

5. In the manure-inhabiting *Allolobophora* the four solutions are stimulating in the order sodium, ammonium, lithium, and potassium; in the earth-inhabiting *Helodrilus* the same solutions are stimulating in the order potassium, ammonium, sodium, and lithium; hence these reactions are indicative of a probable adaptation of the gustatory protoplasm of these worms to their own chemical environments.

6. The reactions of *Allolobophora* to solutions of sodium chloride are not influenced by solutions of calcium in such a way as to show that the sapidity of sodium chloride is related in any close way with its well-known toxicity.

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## APPENDIX.

The seven following tables give in detail the reaction times to fifths of a second of 28 earthworms (*Allolobophora foetida*), four of which were used with each set of the seven dilutions of the four salts tested. The dilutions were mol.  $\frac{m}{10}$ ,  $\frac{m}{15}$ ,  $\frac{m}{25}$ ,  $\frac{m}{50}$ ,  $\frac{m}{100}$ , and  $\frac{m}{500}$ . The actual sequence in the use of the salts was NaCl, KCl, LiCl, and NH<sub>4</sub>Cl.

but for convenience of reference this sequence has been changed in the tables (see p. 57).

The eighth table gives in detail the reaction times of four earthworms (*Allolobophora fætida*) to distilled water.

TABLE VIII.

Worms 1 to 4 were tested, each one three times in each of the four mol. solutions and withdrew in every case in less than 0.2 second. As this was the minimum unit of time used for records, their reaction times have been set down as 0.2, and are given in the table in condensed form only.

Temperature of the solutions, 19° C.

No. of the worm.	<i>m</i> NaCl.	<i>m</i> NH <sub>4</sub> Cl.	<i>m</i> LiCl.	<i>m</i> KCl.
1, 2, 3, 4	0.2	0.2	0.2	0.2

TABLE IX.

Temperature of the solutions, 21° C.

Number of the worm.	$\frac{m}{10}$ NaCl.	$\frac{m}{10}$ NH <sub>4</sub> Cl.	$\frac{m}{10}$ LiCl.	$\frac{m}{10}$ KCl.
5	0.2	2.6	5.4	14.2
	0.2	1.6	8.6	8.0
	0.2	3.6	15.4	33.6
6	0.2	3.2	10.8	22.6
	0.2	2.8	15.4	16.2
	0.2	2.8	6.0	7.4
7	0.2	2.4	11.2	70.8
	0.2	2.4	22.6	46.8
	0.2	4.2	10.8	27.6
8	0.8	2.2	10.6	22.4
	0.2	2.0	13.2	24.6
	0.2	2.6	6.4	28.2
Averages	0.25	2.70	11.37	26.87

TABLE X.  
Temperature of the solutions, 20° C.

Number of the worm.	$\frac{m}{15}$ NaCl.	$\frac{m}{15}$ NH <sub>4</sub> Cl.	$\frac{m}{15}$ LiCl.	$\frac{m}{15}$ KCl.
9	0.2	9.8	6.0	12.6
	0.2	2.4	4.0	13.4
	0.2	16.4	9.8	60.0
10	0.2	6.6	10.8	9.6
	0.2	3.0	8.8	24.8
	0.2	5.4	16.2	8.4
11	0.2	9.2	10.2	90.6
	0.2	3.0	42.8	46.0
	0.2	2.0	6.4	37.6
12	0.2	3.2	19.6	20.8
	0.2	6.8	32.4	21.0
	0.2	10.0	14.4	39.8
Averages	0.2	6.48	15.12	32.05

TABLE XI.  
Temperature of the solutions, 19° C.

Number of the worm.	$\frac{m}{25}$ NaCl.	$\frac{m}{25}$ NH <sub>4</sub> Cl.	$\frac{m}{25}$ LiCl.	$\frac{m}{25}$ KCl.
13	0.2	8.4	32.0	103.0
	0.2	31.2	37.0	120.0*
	0.2	9.2	17.2	22.0
14	0.2	10.6	15.4	32.4
	0.2	5.6	19.6	38.2
	0.2	7.0	63.4	12.4
15	0.2	9.4	26.4	19.4
	0.2	10.4	36.6	12.8
	0.2	18.4	41.4	117.8
16	0.2	11.4	22.0	21.6
	0.2	4.8	22.4	16.8
	0.2	9.8	29.6	33.8
Averages	0.2	11.35	30.25	45.85

\* An asterisk indicates that at the end of two minutes the worm had not withdrawn from the solution, and that in consequence the experiment was interrupted at that moment.

TABLE XII.

Temperature of the solutions, 19° C.

Number of the worm.	$\frac{m}{50}$ NaCl.	$\frac{m}{50}$ NH <sub>4</sub> Cl.	$\frac{m}{50}$ LiCl.	$\frac{m}{50}$ KCl.
17	0.2	15.6	52.0	103.2
	0.2	16.0	47.8	38.6
	0.2	7.2	19.0	63.6
18	0.2	14.8	15.0	120.0*
	0.2	18.4	120.0*	51.8
	0.4	18.2	27.0	28.6
19	0.2	27.6	22.8	120.0*
	0.2	12.8	68.2	20.2
	0.2	15.6	25.8	47.2
20	0.6	15.0	18.4	18.8
	0.2	26.6	9.8	26.6
	0.2	25.0	27.8	26.8
Averages	0.25	17.73	37.80	55.45

\* An asterisk indicates that at the end of two minutes the worm had not withdrawn from the solution, and that in consequence the experiment was interrupted at that moment.

TABLE XIII.

Temperature of the solutions, 20.5° C.

Number of the worm.	$\frac{m}{100}$ NaCl.	$\frac{m}{100}$ NH <sub>4</sub> Cl.	$\frac{m}{100}$ LiCl.	$\frac{m}{100}$ KCl.
21	0.2	10.6	16.4	6.0
	0.2	3.4	7.8	29.2
	0.2	9.4	39.0	38.6
22	0.2	60.8	15.0	60.8
	0.2	31.2	28.0	31.2
	0.2	21.0	107.4	21.0
23	5.0	18.0	96.6	12.2
	0.2	13.0	14.4	100.4
	5.0	59.6	19.8	39.0
24	6.6	26.2	31.0	15.8
	0.2	7.4	45.2	112.0
	0.2	16.0	13.0	32.6
Averages	1.53	23.05	36.13	41.57

TABLE XIV.  
Temperature of the solutions, 19° C.

Number of the worm.	$\frac{m}{500}$ NaCl.	$\frac{m}{500}$ NH <sub>4</sub> Cl.	$\frac{m}{500}$ LiCl.	$\frac{m}{500}$ KCl.
25	0.2	19.0	17.8	28.2
	0.2	12.8	10.4	36.8
	0.2	12.2	23.0	40.0
26	4.0	9.2	16.0	38.2
	5.0	16.0	17.4	36.0
	8.0	19.0	21.2	33.4
27	8.0	8.6	17.4	36.6
	18.6	9.4	18.6	54.4
	12.0	14.0	23.0	19.0
28	8.6	4.2	12.8	5.4
	7.6	10.4	14.2	9.6
	16.8	11.4	15.4	21.0
Averages	7.43	12.18	17.27	29.88

TABLE XV.

Reaction times of four earthworms (*Allolobophora foetida*) to distilled water. The worms used had been previously employed for the reactions recorded in Table XIV. Temperature of the water, 19° C.

Number of the worm.	First trial.	Second trial.	Third trial.	Averages.
25	12.6	25.4	14.8	17.6
26	20.0	25.4	25.0	23.5
27	15.2	40.6	36.0	30.6
28	6.0	6.6	12.6	8.4
General average . . . . .				20.02

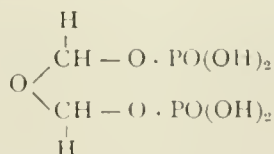


## EXPERIMENTS ON THE PHYSIOLOGICAL ACTION AND METABOLISM OF ANHYDRO-OXYMETHYLENE-DIPHOSPHORIC ACID (PHYTIN ACID).

BY LAFAYETTE B. MENDEL AND FRANK P. UNDERHILL.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

IN 1903 Hart and Andrews<sup>1</sup> reported a series of investigations which led them to the conclusion that commercial feeding stuffs of vegetable origin do not contain appreciable quantities of phosphorus in organic combination. Germinated grains are rich in forms of soluble organic phosphorus; and germination does not, as is generally assumed, transform organic phosphorus into inorganic form. With this conclusion observations of Schulze and Castoro<sup>2</sup> are in substantial accord. A subsequent study by Patten and Hart<sup>3</sup> demonstrated that practically all of the soluble phosphorus of wheat bran is of an organic nature, and that the organic compound exists in the bran as a magnesium-calcium-potassium salt of a phospho-organic acid. The free acid was found to correspond to the formula  $C_2H_4P_2O_6$ ; and Patten and Hart regard it as probably identical with a compound isolated by Posternak<sup>4</sup> from various seeds and other plant parts, and described as anhydro-oxymethylene diphosphoric-acid:



<sup>1</sup> HART and ANDREWS: American chemical journal, 1903, xxx, p. 470; also New York Agricultural Experiment Station, Bulletin 238.

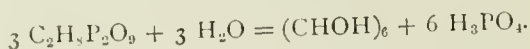
<sup>2</sup> SCHULZE and CASTORO: Zeitschrift für physiologische Chemie, 1904, xli, p. 476.

<sup>3</sup> PATTEN and HART: American chemical journal, 1904, xxxi, p. 564.

<sup>4</sup> POSTERNAK: Comptes rendus de la société de biologie, 1903, lv, p. 1190.

Posternak introduced the name "phytin" for this acid, but the term has lately been used commercially to designate an acid calcium-magnesium salt of the organic compound.

The phospho-organic acid here referred to is doubtless the same substance which several investigators have met with in various plant materials in the form of its salts. Pfeffer<sup>1</sup> probably detected a similar salt as early as 1872. Various workers in Schulze's laboratory separated the characteristic compound whose properties Posternak later described.<sup>2</sup> The free acid obtained by Patten and Hart is a thick, transparent liquid of yellowish-brown color. It is readily soluble in water and alcohol, and has a sharp acid taste. Its alkali salts are freely soluble in water; the calcium and magnesium salts are somewhat soluble in water, the barium and strontium salts sparingly soluble. The free acid is tetrabasic, and forms two salts: one normal, the other an acid salt. Heated with concentrated mineral acids, it is broken up quantitatively into inosit and phosphoric acid, according to the following equation:



Strong solutions of the acid are quite stable, and do not show the presence of phosphoric acid after keeping for many days.<sup>3</sup>

The compound just described, containing over twenty-six per cent of phosphorus, is of interest physiologically, because it occurs as a constituent of the food of both man and animals. Through the co-operation of Mr. E. B. Hart, we have attempted to supplement the extensive investigation conducted at the New York Agricultural Experiment Station (Dr. W. H. Jordan, Director) on the rôle of this phosphorus compound in metabolism. Commercial phytin has lately been introduced into medical practice with somewhat extravagant claims which demand careful experimental verification.<sup>4</sup> The investigations of Röhmann and his pupils<sup>5</sup> have suggested that the phos-

<sup>1</sup> PFEFFER: *Jahrbücher für wissenschaftliche Botanik*, 1872, viii, p. 147.

<sup>2</sup> PALLADIN: *Zeitschrift für Biologie*, 1894, xxxi, p. 199; SCHULZE and WINTERSTEIN: *Zeitschrift für physiologische Chemie*, 1896, xxii, p. 90; WINTERSTEIN: *Berichte der deutschen chemischen Gesellschaft*, 1897, xxx, p. 2299.

<sup>3</sup> According to a personal communication from Mr. E. B. HART.

<sup>4</sup> Thus LOEWENHEIM: *Berliner klinische Wochenschrift*, 1904, p. 1221, hails phytin as "das mächtigste Anregungsmittel des Stoffwechsels, das die Medizin je zur Verfügung gehabt hat."

<sup>5</sup> RÖHMANN: *Berliner klinische Wochenschrift*, 1898, No. 36; MARCUSE: *Archiv für die gesammte Physiologie*, 1897, lxvii, p. 373; STEINITZ: *Ibid.*, 1898,

phorus of compounds, like casein and vitellin, is retained in the organism better than corresponding quantities of inorganic phosphate fed with ordinary proteids. Similar conclusions are afforded by the studies of Cronheim and Müller<sup>1</sup> on growing children. It must be admitted, however, that the conditions determining the metabolism of phosphorus compounds are still too imperfectly understood to permit such satisfactory generalizations as apply in the case of nitrogen and some other elements.<sup>2</sup> This is likewise true of the present significance of lecithins and other organic phosphorus compounds.

The experiments of Gilbert and his collaborators<sup>3</sup> indicate that in the case of the dog, rabbit, and guinea-pig the salts of anhydrooxymethylene-diphosphoric acid are scarcely toxic when administered per os. Fatal results followed doses of 50 mgm. per kilo, given intravenously; 200 mgm., intraperitoneally; 2500 mgm., subcutaneously; and 9500 mgm. per os. Sécheret claims that the proteolytic enzymes of the alimentary tract do not alter the acid in vitro; and he concludes that it is probably transformed by the intestinal epithelium, so as to be finally excreted as inorganic phosphoric acid. The acid is not a protoplasmic poison; it does not check the growth of the Eberth- or tubercle bacillus or *Penicillium*. Finally, Sécheret states that small doses of the salts of the phospho-organic acid, taken per os in man, induce an increased excretion of nitrogen by stimulating tissue metabolism and simultaneously effecting an increased retention of phosphorus in the body. Without reviewing the purely clinical reports<sup>4</sup> which have been published, reference may be made to the observation of Scofone<sup>5</sup> that phytin phosphorus is well ab-

lxxii, p. 75; ZADIK: *Ibid.*, 1899, lxxvii, p. 1; LEIPZIGER: Inaugural Dissertation, Breslau, 1899; EHRLICH: Inaugural Dissertation, Breslau, 1900.

<sup>1</sup> CRONHEIM and MÜLLER: *Zeitschrift für diätetische und physikalische Therapie*, 1903, vi.

<sup>2</sup> *Cf.*, for example, the conclusions reached in Tigerstedt's laboratory, especially EHRSTRÖM: *Skandinavisches Archiv für Physiologie*, 1904, xiv. The literature on the metabolism of phosphorus has recently been briefly reviewed by ALBU and NEUBERG: *Physiologie und Pathologie des Mineralstoffwechsels*, Berlin, 1906, p. 134.

<sup>3</sup> GILBERT and POSTERNAK: *La médication phosphorée*, Paris, 1903; GILBERT and LIPPMANN: *La presse médicale*, Septembre, 1904; G. SÉCHERET: *Thèse de Paris*, 1904. We have seen an abstract of the last paper only, in the *Jahresbericht für Tierchemie*, 1904, xxxiv, p. 729.

<sup>4</sup> *E.g.* LOEWENHEIM: *Berliner klinische Wochenschrift*, 1904, p. 1221; FÜRST: *Centralblatt für Kinderheilkunde*, 1904, Heft 11; DAMBRE: *Contribution à l'étude de la médication phosphorée. La Phytin*. *Thèse de Toulouse*, 1905.

<sup>5</sup> SCOFONE: *Abstract in Biochemisches Centralblatt*, 1905, iii, p. 606.

sorbed, being excreted again in good part as earthy phosphate in the urine. Giacosa<sup>1</sup> has also noted that phytin does not reappear as such in the urine; neither could its decomposition product inosit be detected.

The most exhaustive study of phytin yet published was undertaken by Jordan, Hart, and Patten.<sup>2</sup> Their observations, which formed the immediate occasion for the present experiments, will be reviewed below.

#### EXPERIMENTAL PART.<sup>3</sup>

The product used in the present study was a solution containing 28.9 per cent of the free acid  $C_2H_8P_2O_9$ , according to analyses by Mr. Hart, who sent it to us for investigation.<sup>4</sup>

**Bacteriological tests.**—It was soon observed that solutions of the acid, made neutral to litmus with sodium hydrate, speedily show the presence of an abundance of micro-organisms, unless antiseptic precautions are taken. This led us to suggest a bacteriological examination of the solutions of the acid itself.<sup>5</sup> Examined in various ways, aerobically and anaerobically, in plate cultures, with peptone, and on agar, the stock solution was always found to be sterile, without aseptic precautions being observed in preserving it. The germicidal properties of the acid solution were investigated as follows: Tubes

<sup>1</sup> GIACOSA: Abstract in *Biochemisches Centralblatt*, 1905, iv, p. 572.

<sup>2</sup> JORDAN, HART, and PATTEN: *American journal of physiology*, 1906, xvi, p. 268.

<sup>3</sup> The writers are indebted to Director JORDAN and Mr. E. B. HART for the opportunity to undertake these studies; and they acknowledge the co-operation of Mr. O. E. CLOSSON in several of the experiments.

<sup>4</sup> Inasmuch as the same sample furnished the material used throughout all our trials, a portion of the unused solution was examined after more than a year to ascertain whether decomposition had taken place with liberation of free phosphoric acid. Owing to the similarity in solubility of the salts of the organic and inorganic acid, the problem is a difficult one. Following Mr. HART's suggestion that if hydrolysis has taken place and phosphoric acid is present, the other product will be inosit, we have removed the preformed phosphoric acid and phytin acid with barium hydrate: the excess of the barium was precipitated with carbon dioxide, and after concentration the filtrate was treated with alcohol and ether for the separation of inosit. Since nothing was obtained by this process, the entire fluid was evaporated to dryness, and the insignificant residue tested for inosit by Scherer's test with negative results. This accords with similar experiences of Mr. HART; it seems unlikely, therefore, that the organic product had been materially altered during the course of our investigation.

<sup>5</sup> The bacteriological tests were conducted by our colleague, Professor RETYGER, and Mr. CLOSSON.

of bouillon medium were prepared so as to include varying proportions of the free acid  $C_2H_8P_2O_9$ . These tubes, as well as control tubes without any of the acid, were inoculated with *B. coli communis* or *B. subtilis*. The results are given below:

*B. coli communis.*

$\frac{1}{6}$  mol.  $C_2H_8P_2O_9$ , sterile.  
 $\frac{1}{25}$  mol.  $C_2H_8P_2O_9$ , slight growth.  
 Acid-free control, abundant growth.

*B. subtilis.*

$\frac{1}{15}$  mol.  $C_2H_8P_2O_9$ , sterile.  
 $\frac{1}{25}$  mol.  $C_2H_8P_2O_9$ , slight growth.  
 Acid-free control, abundant growth.

Again to 10 c.c. portions of nutrient peptone,  $\frac{1}{10}$  c.c.,  $\frac{1}{2}$  c.c., and 1 c.c. of the stock solution (28.9 per cent  $C_2H_8P_2O_9$ ) were added in duplicate, and the tubes then inoculated with *B. coli communis*. The trials were made in both aerobic and anaerobic culture. The tubes containing  $\frac{1}{10}$  c.c. alone showed a doubtful trace of bacterial growth.

**Injection trials. Subcutaneous injections.** — 1. A rabbit weighing 2.3 kilos received a subcutaneous injection of 21 c.c. of a solution containing 219 mgm. of the phospho-organic acid neutralized with sodium hydrate. *i. e.*, 92.8 mgm. per kilo, without showing unusual symptoms of any sort.

2. A dog weighing 7 kilos (see metabolism trial below) received repeated subcutaneous injections of the acid neutralized with sodium hydrate. The doses ranged from  $1\frac{1}{2}$  to 4 gm. (calculated as the acid) dissolved in 20 to 30 c.c. of water. Aside from local irritation produced by the largest dose, no toxic symptoms were noted.

**Intraperitoneal injections.** — 3. A rabbit weighing 1.38 kilos received an intraperitoneal injection of 10 c.c. of a solution containing 289 mgm. of the acid (210 mgm. per kilo) as the sodium salt. One and one-half hours later a second injection (19 c.c.) of a similar neutral solution containing 400 mgm. acid per kilo was given. No untoward effects whatever were noted.

4. The cat previously used in Experiment 6, below, five days later received an intraperitoneal injection (38 c.c.) containing 570 mgm. of the acid (as sodium salt) per kilo. Aside from slight nausea, no toxic symptoms were noted.

5. The only untoward symptoms observed after intraperitoneal injection were seen in the case of a puppy weighing 1.1 kilos which received 18 c.c. of a solution containing 474 mgm. of the acid (as sodium salt) per kilo. The animal was attacked with vomiting and diarrhœa, but soon recovered. On the next day similar, though less intense, transitory symptoms followed the injection of 606 mgm. of the acid (as sodium salt) per kilo.

*Intravenous injections.* — 6. During ether anaesthesia 58 c.c. of a solution containing 1.68 gm. of the acid (as a sodium salt) were slowly injected in the course of half an hour into the jugular vein of a cat weighing 2.3 kilos. Dose = 718 mgm. per kilo. No permanent ill effects followed.

The apparently innocuous effects of relatively large doses of the sodium salt of Patten and Hart's acid led us to study the effects of the acids and its salts directly on the blood. Injections were made into the facial vein in dogs, during A. C. E. anaesthesia, blood pressure being recorded from the carotid by a mercury manometer and blood samples removed from the femoral artery to study the clotting-time.

7. A dog weighing 8.5 kilos received a rapid injection of 10 c.c. of the stock solution of the *free acid* diluted to contain 5.8 per cent  $C_2H_8P_2O_9$ , in twenty-four seconds. Dose = 68 mgm. per kilo. The blood pressure immediately fell, and the animal died at once. The abdominal blood vessels were greatly distended.

8. A dog weighing 6 kilos received a series of injections of solutions containing either the sodium salt or the free acid. The intervals were sufficient to allow the return of blood pressure to the normal. Anaesthesia with A. C. E. mixture. The injections were made quite rapidly.

	Substance used.	Dose per kilo.		Volume injected.	Blood pressure.	Clotting-time (normal, 4 min.).
		mgm.	c.c.			min.
I	Sodium salt	1.7	5		No effect.	..
II	" "	8.7	20		Very slight transitory fall.	..
III	" "	8.7	5		" " "	3
IV	" "	26.0	15		" " "	..
V	" "	52.0	30		" " "	4
VI	Free acid	29.0	30		Slight transitory fall.	..
VII	" "	96.5	10		Permanent fall, followed by death in a short time.	..

*Metabolism trials.* — The preceding experiments have indicated the non-toxicity of large doses of the sodium salt of anhydro-oxymethylene-diphosphoric acid when administered parenterally. In view of the claims already advanced regarding the peculiarly stimulating action of phytin on nitrogenous metabolism, and its rôle in metab-

olism of phosphorus, we have conducted a series of trials with the wheat bran acid on a bitch. The animal was maintained on a constant diet consisting of

	Nitrogen.	P <sub>2</sub> O <sub>5</sub> .	Calories (estimated).
	gm.	gm.	gm.
Hashed meat, 72 gm. . . . .	2.53	0.27	90
Cracker meal, 76 gm. . . . .	1.31	0.74	300
Lard, 14 gm. . . . .	none	none	130
Total . . . . .	3.84	1.01	520

The urine was collected in daily periods by the use of a catheter. Nitrogen analyses were made by the Kjeldahl-Gunning method. Phosphorus was determined by Neumann's moist combustion method<sup>1</sup> in the food and fæces; and in the urine, by titration with uranium solution after numerous comparisons of the two methods had shown a close agreement. The fæces were marked off by the use of lamp black and analyzed in corresponding periods.

A study of the analytical data summarized in the following table fails to reveal any constant effect upon the metabolism of nitrogen produced by large single doses of the sodium salt administered per os or subcutaneously. Two experiments in which sodium phosphate (HNa<sub>2</sub>PO<sub>4</sub>) was injected subcutaneously are introduced for comparison. The excellent utilization of nitrogen is in no way altered by the compounds studied. The nitrogen balance is favorable throughout except in those periods in which the subcutaneous injections were made. Here, however, the greater disadvantage lies with the inorganic phosphate. The phosphorus balance is likewise favorable, and shows a considerable storage throughout the entire period, independently of the phosphorized compounds used. They failed therefore to exert any marked influence upon these features of metabolism under the conditions of experiment observed. What specific effects, if any, might be elicited by long-continued feeding cannot be foretold from such data. Upon this point we refer to the very comprehensive

<sup>1</sup> NEUMANN: *Zeitschrift für physiologische Chemie*, 1902, xxxvii, p. 115; 1904, xliii, p. 32.





Dec. 1	6.8	230	1.012	"	3.30	0.47	41	14.5	0.93	0.18	11.5	10.8	+0.7	3.03	1.65	+1.38	Control period. { Subcutaneous injection of sodium salt <sup>2</sup> = 0.93 gm. P <sub>2</sub> O <sub>5</sub> . }
2	6.8	250	1.010	"	2.46	0.49	}	14	0.84	0.25	11.5	11.5	0	3.96	2.68	+1.28	
3	6.8	305	1.012	"	4.14	0.51											
4	6.8	330	1.020	"	4.29	1.47											
5	6.8	250	1.013	"	3.15	0.51	55	17	0.97	0.27	11.5	10.4	+1.1	3.03	1.64	+1.39	Control period. { Subcutaneous injection of sodium phosphate <sup>3</sup> = 0.59 gm. P <sub>2</sub> O <sub>5</sub> . }
6	6.8	280	1.012	"	3.15	0.45											
7	7.0	260	1.011	"	3.24	0.43											
8	7.0	240	1.011	"	3.03	0.44	45	18	1.10	0.34	11.5	12.3	-0.8	3.62	2.72	+0.90	
9	7.0	280	1.012	"	3.15	0.50											
10	7.0	315	1.020	"	4.56	1.41											
11	7.0	250	1.012	"	3.39	0.47	67	14.5	0.93	0.07	11.5	10.4	+1.1	3.03	1.53	+1.50	Control period. { Subcutaneous injection of sodium phosphate <sup>3</sup> = 0.33 gm. P <sub>2</sub> O <sub>5</sub> . }
12	7.0	280	1.011	"	3.21	0.50											
13	7.0	240	1.012	"	2.61	0.45											
14	7.0	310	1.012	"	3.38	0.54	51	14	0.90	0.31	11.5	11.5	0	3.36	2.29	+1.07	
15	7.0	270	1.012	"	3.48	0.47											
16	7.0	320	1.016	"	3.96	1.01											
17	7.0	280	1.013	"	3.27	0.50	39	16	0.98	0.12	11.5	11.7	-0.2	3.96	2.29	+1.67	Control period. { Subcutaneous injection of sodium salt <sup>2</sup> = 0.93 gm. P <sub>2</sub> O <sub>5</sub> . }
18	7.0	230	1.010	"	3.42	0.47											
19	7.0	260	1.022	"	3.78	1.39											
20	7.0	290	1.012	"	3.39	0.41	66	16	0.98	0.12	11.5	11.7	-0.2	3.96	2.29	+1.67	
21	7.0	300	1.014	"	3.51	0.37											

<sup>1</sup> No account is here taken of the hair and other epithelial products continually lost. A quantitative estimation of the amounts of N lost in this way was made during several periods. Inasmuch as it was quite constant (amounting to about 0.4 gram per period), the slight error thereby introduced has been neglected in the table of balance calculations.

<sup>2</sup> By this is meant the solution of the organic acid C<sub>2</sub>H<sub>18</sub>P<sub>2</sub>O<sub>9</sub> neutralized with NaOH.

<sup>3</sup> The salt used was 11Na<sub>2</sub>PO<sub>4</sub>.

investigation of Jordan, Hart, and Patten<sup>1</sup> on cattle at the New York Agricultural Experiment Station. Their plan consisted in feeding the same animal rations differing greatly in the amount of phosphorus which they supply. The ration low in phosphorus was prepared by removing the phytin from wheat bran, untreated whole wheat bran being used in the companion experiments. It is impossible to review in detail the numerous interesting observations. They noted that the amount of outgoing phosphorus rose and fell with the quantity supplied in the food, though within narrower limits. When the phosphorus supply was abundant, there was a storage of this element. No relation appeared to exist between nitrogen excretion and phosphorus elimination. With these observations our own experience is in substantial accord.

Another point, however, calls for more extended reference. Jordan, Hart, and Patten found that through katabolic changes the phosphorus of the phytin and that of the unused digested nucleoproteids was reduced to *inorganic* combinations, and was excreted chiefly in the feces, though to a small extent in the urine. The *organic* phosphorus bodies of the egesta were little affected, if at all, by the proportions of phosphorus compounds in the food, the rise and fall in the outgoing amounts occurring almost entirely with the inorganic salts found in the excreta. Our own experience with the sodium salt of  $C_2H_4P_2O_6$  corresponds with the preceding in indicating a practically complete conversion of the phosphorus of the organic compound into inorganic phosphate. At any rate we have concluded that it was excreted in this form because of the agreement between the figures obtained by direct titration of the urine and those furnished by the Neumann method. Jordan, Hart, and Patten came to a similar conclusion because they were entirely unable to separate phytin or other organic forms of phosphorus from the urine. Scofone<sup>2</sup> and Giacosa<sup>3</sup> report the same outcome. Our experiments on the dog differ notably, however, in one respect from those obtained with cows. *The phosphorus was eliminated almost entirely by way of the kidneys.* The response in the urine to the increased ingestion of phosphorus compounds was speedy, rarely being prolonged beyond the day of

<sup>1</sup> JORDAN, HART, and PATTEN: American journal of physiology, 1906, xvi, p. 268.

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

<sup>3</sup> GIACOSA: *Loc. cit.*

administration, *i. e.*, no marked "lag" in the urinary excretion of phosphorus was noted.<sup>1</sup>

The path of elimination for phosphorus compounds has been the subject of considerable controversy. It has been noted, particu-

SUMMARY OF EXPERIMENTS ON A RABBIT.

Day.	Composition of urine.		Remarks.
	Nitrogen.	P <sub>2</sub> O <sub>5</sub> .	
	gm.	gm.	
1	0.80	0.35	
2	0.67	0.28	
3	0.87	0.34	
4	0.79	0.50	Neutral solution C <sub>2</sub> H <sub>5</sub> P <sub>2</sub> O <sub>9</sub> = 0.37 gm. P <sub>2</sub> O <sub>5</sub> given through stomach tube.
5	0.66	0.38	
6	0.65	0.44	
7	0.91	0.43	
8	0.98	0.70	Ditto = 0.37 gm. P <sub>2</sub> O <sub>5</sub> .
9	0.81	0.66	
10	0.89	0.47	
11	0.76	0.78	Ditto = 0.37 gm. P <sub>2</sub> O <sub>5</sub> given subcutaneously.
12	0.64	0.45	
13	0.72	0.48	
14	0.72	0.74	Solution of Na <sub>2</sub> HPO <sub>4</sub> = 0.33 gm. P <sub>2</sub> O <sub>5</sub> given subcutaneously.
15	0.49	0.44	

larly in herbivora, that the intestine may play a preponderating rôle in this respect. The discussion of this question must be reserved for a subsequent paper. It will suffice to point out here that in the present experiment the gut has not co-operated noticeably in the removal of the excess of phosphorus — in contrast with the observations on cows. We have also studied the path of elimination in an herbivorous animal, — the rabbit, — after introduction of solutions of the neutralized wheat brand compound. The animal was fed 300 gm.

<sup>1</sup> On the "lag" in phosphorus excretion *cf.* SHERMAN and HAWK. American journal of physiology, 1900, iv, p. 25; HAWK: *Ibid.*, 1903, x, p. 115; HAWK and CHAMBERLAIN: 1903, x, p. 269.

of carrots, and the urine expressed at a fixed hour daily, by pressure on the bladder.

It is evident from these data that in rabbits also the phosphorus of the phospho-organic compound may be speedily eliminated in the urine, precisely as occurs when sodium phosphate is administered. The place of introduction did not alter this.

**Laxative effects of  $C_2H_4P_2O_9$ .** *Experiments.* — In a rabbit a dose of 1.3 gm.  $C_2H_4P_2O_9$  per kilo, administered as a sodium salt through a stomach sound, failed to produce any purgative effect whatever. With dogs the results were variable. A bitch weighing  $8\frac{1}{2}$  kilos was kept under observation in a metabolism cage and fed upon bread and meat twice daily. This diet afforded fairly well-formed faeces. At intervals solutions of the acid (as the sodium salt) were given per os, and frequent observations were made on the quantity and character of the stools. Protocols follow :

- May 16-20. Normal diet. Well-formed faeces daily.
- May 21. 5.8 gm.  $C_2H_4P_2O_9$  fed in two doses. *Diarrhoeal stool.*
- May 22. Ditto, single dose. *Diarrhoeal stool.*
- May 23. Normal diet. No faeces.
- May 24. 2.9 gm.  $C_2H_4P_2O_9$  fed. *Diarrhoeal stool.*
- May 25. Normal diet. *Slight diarrhoeal stool.*
- May 26-29. Normal diet. The faeces regain their normal consistency.

In order to compare the effect of sodium phosphate, to the formation of which this laxative action might be attributed at first glance, solutions of the inorganic phosphate were administered per os. The equivalent doses are : 8.7 gm.  $HNa_2PO_4 \cdot 12 aq.$  = 0.755 gm. P = 2.9 gm.  $C_2H_4P_2O_9$ .

- May 30. About 4 gm. sodium phosphate fed. Moderately soft stool (?)
- May 31. Normal diet. Faeces normal.
- June 1. 8.7 gm. sodium phosphate fed. *Laxative effect very soon.*
- June 2. 9 gm. NaCl given in solution. Vomiting. No purgative effect.
- June 3. Normal diet. No faeces.
- June 4-6.  $C_2H_4P_2O_9$  given as above, but in small portions, at intervals. No purgative effects.
- June 7. 2.9 gm.  $C_2H_4P_2O_9$  given in a single dose. No purgative effect.

Another dog of 19 kilos body-weight, similarly fed, was given 5.5 gm.  $C_2H_4P_2O_9$  (as the sodium salt) without effect. An additional dose of 7.2 gm. on the next day provoked a diarrhoeal discharge. Two days later 17 gm.  $HNa_2PO_4$  (equivalent in phosphorus content to about 5.8 gm.  $C_2H_4P_2O_9$ ) were similarly administered in solution without any pronounced purgative effect within the following ten hours. Later soft faeces were discharged.

In the metabolism experiments reported above, with the doses there

employed no laxative effects were noted except on one day (November 18), when the largest single dose of the phytin acid was fed.

From their experiments on cattle, Jordan, Hart, and Patten conclude that phytin exerts a distinct laxative effect, because its withdrawal from the ration usually was attended with marked constipation. Our experience with the sodium salt of  $C_2H_8P_2O_9$ , in experiments on the dog and rabbit is not so uniformly in accord with this. Several distinctive features should, however, be borne in mind. We have already shown above that the path of excretion for the metabolic products of  $C_2H_8P_2O_9$  does not directly involve the intestine in all animals, a fact which may have a bearing on the production of purgative reactions.<sup>1</sup> Again, the salt fed by us differed from the natural phytin in the character of the bases present; and, finally, our trials were made with single doses and are perhaps not strictly comparable with prolonged feeding trials. It should be noted that investigators are by no means agreed regarding the effects of the saline purgatives. Conditions arise in which pendular movements of the intestine may be increased without purgation, the two phenomena remaining essentially distinct.<sup>2</sup>

It might be objected that the easily digested ration employed by us offers relatively small food residues and is therefore poorly adapted for such experiments. In view of the statement that phytin is not decomposed by alimentary enzymes, one must hesitate to ascribe the supposed laxative effect to soluble phosphate developed in the intestine. With so large a number of complicating factors further experiments in this direction seem unpromising. Perhaps trials with phytin itself will furnish more convincing evidence.

#### SUMMARY.

A solution of anhydro-oxymethylene-diphosphoric acid, the acid radical of phytin, prepared from wheat bran, appears to be quite stable. When present in sufficient concentration, the acid inhibits the growth of bacteria. The salts of the acid are not noticeably bactericidal.

Comparatively large doses of the phospho-organic acid, used as the sodium salt, can be introduced into animals either per os, subcu-

<sup>1</sup> On this point *cf.* MENDEL and THACHER: *American journal of physiology*, 1904, xi, p. 15; MENDEL and SICHER: *Ibid.*, 1906, xvi, p. 151.

<sup>2</sup> *Cf.* AUER: *Proceedings of the society for experimental biology and medicine*, *American medicine*, 1906, p. 33.

taneously, interaperitoneally, or intravenously, without unfavorable effects. The free acid is more toxic.

No marked or immediate characteristic effects of the sodium salt upon general health or nitrogenous metabolism have been observed. The compound is readily absorbed and speedily transformed within the organism. Its phosphorus reappears in the excreta as inorganic phosphates. No constant relation between the metabolism of nitrogen and of phosphorus was observed. In these details our experience with the dog corresponds with the observations of Jordan, Hart, and Patten after feeding phytin to cattle. Our results differ in showing that in both the dog and rabbit the excess of phosphorus was almost entirely eliminated through the kidneys rather than in the faeces. This may have an important bearing on the possibility of producing laxative effects with phytin.

In our experimental animals purgative action could not be constantly provoked. Very large doses were frequently effective. No permanent generalizations can be drawn from the observations made on this point.

THE RELATION OF IONS TO CONTRACTILE PROCESSES. — I. THE ACTION OF SALT SOLUTIONS ON THE CILIATED EPITHELIUM OF MYTILUS EDULIS.

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I. INTRODUCTION.

THE following paper describes the results of part of a comparative investigation on the relation of ions to contractile processes in animal tissues. The present study was begun in the summer of 1904 at Woods Hole with the aid of a grant from the Carnegie Institution, and has been conducted chiefly at the Marine Biological Laboratory, partly at the Zoölogical Station in Naples. I have thought it desirable to study the phenomena of ciliary movement in some detail, since this appears to be the most primitive, as it is the most widespread, form of specialized contractile activity; the fundamental processes concerned in any form of contractility with a fibrillar basis are therefore likely to be found in this tissue in a relatively simple and unmodified form.

The present study, apart from its special relation to contractility, has brought to light many facts bearing on the more general problems of the relation of the electrolytes in cells and tissues to physiological processes. These facts tend strongly to confirm the view that the physiological action of the salts is due chiefly, if not entirely, to the ions resulting from their dissociation, and that anions and cations have in general opposite action on the tissue, this action consisting largely in effecting changes in the aggregation-state of the colloids of the cell.<sup>1</sup> The contractile activity itself is in all likelihood due to changes of this kind.<sup>2</sup> Other physiologically important pro-

<sup>1</sup> Cf. GREELEY's paper on the protoplasm of *Paramœcium* and the action of electrolytes in changing its physical structure. *Biological bulletin*, 1904, vii, p. 117.

<sup>2</sup> Cf. my paper on the relation of coagulative changes to contractility in the Ctenophore swimming plate. *This journal*, 1906, xvi, p. 117.

cesses, as alterations in the water-content of the protoplasm and the related phenomena of absorption, changes in the permeability of the cell membranes, etc., appear to be similarly conditioned. My results also indicate that the injurious action of pure solutions of many salts (as sodium salts) depends largely on their producing alterations in the colloids of the tissue, due to the preponderating action of one or other of the oppositely charged ions of the salt. The so-called "antitoxic" action, by which this injurious action is diminished or removed by the addition of certain other salts to the pure solution, is due apparently to an approximate equalization of the opposite actions of anion and cation by the introduction of physiologically active ions of a sign opposite to that of the prepotent ions in the toxic solution. An approximately balanced condition of the opposite ion-actions is thus secured; hence the restoration of favorable conditions, or the "antitoxic" action. Considerable space is thus devoted below to a consideration of the changes due to direct action of the ions on the colloids of the cell; the phenomena of swelling and of coagulation resulting from such action are seen to have a definite influence on the physiological activity of the cell, and antitoxic action is shown to consist largely in the counteraction of these changes in the colloids. The differences between the physiological activities of the various ions, and the relation of these differences to the physico-chemical characteristics of the ions — migration-velocity, sign and number of charges, detachability of charge (decomposition tension) — are also considered at some length.

## II. METHOD OF EXPERIMENTATION.

The ciliated epithelium of the gill filaments of *Mytilus edulis*, the common mussel of the North Atlantic coast, was the tissue used in most of the following experiments. The specimens of *Mytilus* were in almost every instance collected on the day of the experiment, since the animals undergo a somewhat rapid deterioration if kept in the running water of the laboratory. The gill lamellæ are removed with scissors, cutting close to the base of attachment; the component gill filaments may then be easily separated from one another by a pair of needles. Each gill lamella is composed of a large number of long filaments with opposed flat faces kept in loose connection by tufts of short interlocking cilia (filibranchiate gill structure); there is thus no fusion of adjacent filaments as in the higher mollusca, and the



filaments are easily separable from one another without injury. Such isolated filaments are usually about a centimetre in length, and from one-third to one-half millimetre in average breadth across the opposed faces. They broaden slightly at their tips; the extremity of each exhibits a bay or indentation, so that along the free border of the lamella formed by the numerous closely applied filaments runs a well-defined groove with rounded borders.

The outer surface of each filament is covered by a columnar ciliated epithelium. The cilia are almost uniform in length along the outer surface of each lamella, except at the margin of the above described groove, where they are longer than elsewhere, and in the interior of the groove, where they are several times shorter than over the general surface. The direction of the effective beat is from above downward (dorso-ventrally) along both surfaces of each lamella, and from behind forward (*i. e.*, toward the mouth) along the margins and in the interior of the groove.

Filaments thus isolated may be kept in sea water, in which they remain active and unaltered for an indefinite length of time. Microscopic examination for the purposes of the following investigation was made in watch glasses under a magnification of fifty to seventy-five diameters. In experimenting with a series of solutions my usual procedure has been as follows: a large number of filaments are first of all isolated and kept in watch glasses with sea water. Several filaments are then transferred by forceps to a clean dry watch glass, the filament being grasped at one extremity and removed from the sea water in such a manner as to be freed of all but a very thin layer of adhering sea water. The previously prepared solution (*e. g.*,  $\frac{1}{2}$  NaCl, etc.) is then added to the watch glass in measured quantity (usually 10 c.c.), and the time of addition is noted. Examination may then take place; or the procedure may be immediately repeated with the next member of the series of solutions, and so on. If the filaments are transferred to the successive members of a series of solutions at regular intervals (*e. g.*, of one minute), and the subsequent examinations take place at corresponding intervals, all the solutions are examined at equal intervals of time after the addition of the filaments. This has been the usual practice with series containing a large number of solutions. In order to minimize error arising from variations in the tissue, I have made use in every such series of filaments taken from a single animal.

## III. ACTION OF PURE SOLUTIONS OF VARIOUS SALTS.

Several preliminary determinations were made with pure  $\frac{5}{8}$  *m* solutions of the alkali chlorides LiCl, NaCl, NH<sub>4</sub>Cl, and KCl on the gill filaments of three other species of bivalve mollusk: *Pecten irradians*, *Venus mercenaria*, and *Mya arenaria*. In all these animals the relative favorability of the four salts was the same as with *Mytilus*, and seems indeed, so far as my observations extend, typical and characteristic for the cilia of marine organisms in general. In pure  $\frac{5}{8}$  *m* LiCl and NaCl movement ceases either instantly or within a few minutes (sooner in  $\frac{5}{8}$  *m* LiCl); in  $\frac{5}{8}$  *m* NH<sub>4</sub>Cl movement is decidedly more active and lasts much longer, — at least half an hour, — while  $\frac{5}{8}$  *m* KCl shows the favorable action so typical of solutions of K-salts, movement lasting for several hours. The same order of increasing favorability — Li, Na, NH<sub>4</sub>, and K — is seen in *Mytilus*. Since the four genera are from widely different and representative orders, the presumption is that this condition holds true at least for the generality of marine mollusca, if not of marine animals. Experiments with pure  $\frac{5}{8}$  *m* solutions of alkali earth chlorides showed a similar general resemblance to the conditions met with in *Mytilus*. Relatively few experiments were made with the above forms, and the remainder of the following investigation relates exclusively to *Mytilus*, which is especially favorable on account of the structure of its gills and the ease with which the animals may be obtained at all periods of the year.

**Sodium salts.** — The action of pure solutions of the following sodium salts<sup>1</sup> in  $\frac{6}{10}$  *m* and  $\frac{m}{2}$  concentration was tested on the gill filaments of *Mytilus*: NaF, NaCl, NaBr, NaI, NaClO<sub>3</sub>, NaNO<sub>3</sub>, NaNO<sub>2</sub>, NaCNS, NaB<sub>1</sub>O<sub>3</sub>, NaCOOCH<sub>3</sub>, Na<sub>2</sub>SO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, Na<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>, Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, Na<sub>2</sub>HAsO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, Na<sub>4</sub>Fe(CN)<sub>6</sub>. In every instance ciliary movement, which at first often shows considerable activity, becomes rapidly slower, and as a rule is found to have entirely ceased after fifteen minutes; the great majority of cilia are arrested within a much shorter period. Occasionally filaments have been seen in which a few cilia have continued movement in  $\frac{m}{2}$  NaCl for so long as thirty minutes; this is exceptional, the rule being rapid arrest. The more powerfully acting salts (NaI, NaBrO<sub>3</sub>,

<sup>1</sup> The salts used in the investigation were in nearly all instances Kahlbaum's purest preparations, and the concentrations were standardized in the case of deliquescent salts like CaCl<sub>2</sub>, MgCl<sub>2</sub>, etc.

NaCNS) produce a partial liquefaction of the ciliary substance similar to that seen in *Arenicola* larvæ in pure  $\frac{m}{2}$  NaCl;<sup>1</sup> the cilia of *Mytilus*, however, undergo this change less readily than those of *Arenicola*.

Thus pure solutions of *all* sodium salts produce a rapid arrest of movement. The different salts act with unequal degrees of rapidity; the short duration of the movement makes it difficult to range the salts (or their anions) in the order of their toxicity; this can be done better with salts of ammonium or of potassium in whose solutions movement continues far longer. Nevertheless it is readily observed that in solutions of sodium salts whose anions have markedly toxic properties (as NaF, NaCNS, NaBrO<sub>3</sub>, Na<sub>2</sub>HAsO<sub>4</sub>, Na<sub>2</sub>SO<sub>3</sub>, etc.: see below) movement is arrested distinctly more rapidly than in solutions of NaCl, NaNO<sub>3</sub>, Na<sub>2</sub> tartrate, where the anions are relatively non-toxic.

**Potassium salts.**—In pure solutions of almost all potassium salts movement is from the first remarkably active and energetic, exhibiting a peculiar rapid vibratory quality absent in other solutions; vigorous activity may continue for many hours in solutions of salts with non-toxic anions. The following table summarizes a number of observations made with solutions of various potassium salts; the times given represent the maximum observed duration of movement in  $\frac{m}{2}$  solution.

TABLE I.

$\frac{m}{2}$ salt.	Maximum observed duration of movement in each experiment.
KF . . . . .	0 m.; 4 m.; 1 m.
KCl . . . . .	14 h.; 24 h.; 19 h.; 17 h.
KBr . . . . .	4 h. 25 m.; 6 h. + <sup>2</sup>
KI . . . . .	ca. 40 m.; 14 m.; 2 h. 40 m.; 1 h. 52 m.
KNO <sub>3</sub> . . . . .	4 h. 20 m.; 5 h. 45 m.
KClO <sub>3</sub> . . . . .	26 h.; 4 h. 40 m.
KCOOCH <sub>3</sub> . . . . .	21 h.; 17 h.
KCNS . . . . .	3 m.; 5 m.; 2 m.
K <sub>2</sub> SO <sub>4</sub> . . . . .	4 h. 18 m.; 5 h. 45 m. ±.
K <sub>2</sub> C <sub>2</sub> O <sub>4</sub> . . . . .	2 h. 18 m.; 54 m.; 2 h. 40 m.; 2 h. 44 m.
K <sub>2</sub> C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> . . . . .	4 h. 16 m.; 5 h. 44 m. +.
K <sub>2</sub> HPO <sub>4</sub> . . . . .	9 m.; 18 m.
K <sub>2</sub> HAsO <sub>4</sub> . . . . .	2 m.; 2 m.; 7 m.
K <sub>3</sub> C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> . . . . .	4 h. 10 m.; 5 h. 45 m.
K <sub>4</sub> Fe(CN) <sub>6</sub> . . . . .	1 h. 53 m.; 30 m. +; 2 h. 44 m.

<sup>1</sup> This journal, 1904, x, p. 410.

<sup>2</sup> The + sign indicates that the movement was sufficiently active at the last observation to continue for a considerable period longer. — *i. e.*, probably some hours if the previous duration of movement had been for several hours.

It will be seen that in all but the solutions with the more toxic anions ( $\text{KF}$ ,  $\text{KI}$ ,  $\text{KCNS}$ ,  $\text{K}_2\text{HAsO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{K}_2\text{C}_2\text{O}_4$ ,  $\text{K}_4\text{Fe}(\text{CN})_6$ ) movement continues for many hours. Even in  $\frac{m}{2}$   $\text{KI}$ ,  $\text{K}_2\text{C}_2\text{O}_4$ , and  $\text{K}_4\text{Fe}(\text{CN})_6$ , salts of considerable toxicity, movement may last for two or three hours,—far longer than in the least injurious solutions of sodium salts. The contrast with the sodium series is striking throughout; the potassium ion appears for some unascertained reason to be strongly favorable to ciliary movement; this has been observed in numerous other instances.<sup>1</sup> The explanation of this striking contrast between the salts of two such closely related metals is unknown. Rubidium and caesium bear a marked resemblance to potassium in their relation to this form of protoplasmic activity. Lithium, on the other hand, resembles sodium. It seems probable that the favorable action of potassium, rubidium, caesium, and ammonium is correlated with their relatively high ionic velocity.

Ammonium salts occupy an intermediate position in their relation to ciliary movement; movement is vigorous at first and lasts for a considerable length of time. The following table gives the observed maximum duration of movement in solutions of a number of ammonium salts:—

TABLE II.

Salt.	Maximum observed duration of movement.
$\text{NH}_4\text{Cl}$ . . . . .	60 m. +; 45 m.
$\text{NH}_4\text{Br}$ . . . . .	21 m. +; 3 h. 45 m.
$\text{NH}_4\text{NO}_3$ . . . . .	2 h.; 3 h. 45 m.
$(\text{NH}_4)_2\text{SO}_4$ . . . . .	5 h.; 3 h.
$(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$ . . . . .	5 h.; 6 h.; 4 h. 30 m.
$(\text{NH}_4)_2\text{HPO}_4$ . . . . .	3 m.; < 9 m.
$(\text{NH}_4)_3\text{C}_6\text{H}_5\text{O}_7$ . . . . .	5 m.; 9 m. +.

Since the potassium ion has such remarkable action in sustaining ciliary movement, we may regard the above varying results with solutions of the pure salts as furnishing a certain indication of the relative toxicities of the various anions in relation to this form of protoplasmic activity. The following then appears: fluoride is rapidly destructive. Chloride, bromide, and iodide show increase of toxicity

<sup>1</sup> Cf. my paper on *Arenicola*: *Loc. cit.*: also This journal, 1902, vii, p. 25; LOEB: This journal, 1900, iii, pp. 327, 383.  $\text{KCl}$  also sustains ciliary movement in *Metridium*, where it also induces reversal of stroke;  $\text{NaCl}$  appears more favorable in this form than in the animals I have studied. Cf. PARKER: This journal, 1905, xiii, p. 1.

with increase of molecular weight, or with decrease of decomposition tension of the anion. Acetate and chlorate are similar to chloride, and arrest movement only gradually. Nitrate is like bromide in being somewhat less favorable than chloride. Sulphocyanate is rapidly destructive of ciliary movement. The above are monovalent anions, and their relative toxicities are probably fairly accurately represented as above, since their concentrations in the above solutions are almost alike. Sodium sulphate in dissociating yields partly the bivalent  $\text{SO}_4$ , and partly the monovalent  $\text{NaSO}_4$  anions; tartrate undergoes an analogous dissociation. Both these salts resemble the nitrate and bromide in their general action. Oxalate is more toxic than either. Citrate is in general more toxic than tartrate; this is seen also with sodium salts, and appears more clearly in the experiments below on antitoxic action (p. 104). Phosphate and arsenate are both more toxic than the citrate; the presence of trivalent anions in these last three solutions may account for a portion of the toxicity, though this consideration fails to explain the more favorable action of the citrate. It is somewhat surprising to find that movement, which at first is active and apparently normal, may continue for so long as two or three hours in the solutions of ferrocyanide. This fact, and also the relatively slow action of potassium oxalate solutions, illustrate in a striking manner the specific favorability of potassium salts (inferentially of the K-ion) toward ciliary movement.

A further index of the relative toxicity of the various anions is seen in the varied susceptibility of the different sodium salts to the antagonizing or antitoxic action of salts of bivalent metals, especially of magnesium. Later a further comparison of relative toxicities will be made with these latter facts as a basis. The results of these two independent methods of determination agree very closely (see p. 107). A further peculiarity of potassium salts is the fact that cilia will continue activity for long periods of time in strongly hypertonic solutions of the pure salts. Thus in  $\frac{1}{10}^2 m$  solutions of the following salts, KCl, KBr,  $\text{KNO}_3$ ,  $\text{KCOOCH}_3$ , movement lasted for more than an hour in each instance, and in  $m$ -solutions of these salts and also of KI,  $\text{K}_2\text{C}_2\text{O}_4$  and  $\text{K}_2\text{C}_4\text{H}_4\text{O}_6$ , active movement lasted for even longer periods, — in the case of the less toxic salts for several hours.

Rubidium and cæsium chlorides show a decided similarity to potassium chloride in this respect as well as in their general action. The following solutions were tested: —

TABLE III.  
RUBIDIUM CHLORIDE.

1.  $m$  RbCl, 3.34, Aug. 17, 1904. Trembling movements of cilia begin to appear at 3.41. At 4.03 movement is active and energetic; has almost ceased at 6.27 (3 h.).
2.  $\frac{5}{10} m$  RbCl, 3.35. Active movement at 3.42; continues longer than in Solution 1 cilia are fairly active at 6.28 (3 h.).
3.  $\frac{6}{10} m$  RbCl, 3.36. Active movement; more favorable than Solution 2; active at 6.29 (3 h. +).<sup>1</sup>
4.  $\frac{1}{10} m$  RbCl, 3.37. Like Solution 3, but rather less favorable; swelling and detachment of cells are more marked; movement is fairly active at 6.30 (3 h. +).
5.  $\frac{8}{10} m$  RbCl, 3.38. Activity is less than in Solution 4, and swelling is more marked; cilia have almost ceased at 6.31 (3 h.).

CÆSIUM CHLORIDE.

1.  $m$  CsCl, 3.48, Aug. 17. Movement is well marked at 3.55, though slower than in  $m$  RbCl. At 4.16 only a little slow movement remains; has ceased by 5.02 (28 m.).
2.  $\frac{8}{10} m$  CsCl, 3.49. Like Solution 1; movement is more active (28 m. +).
3.  $\frac{7}{10} m$  CsCl, 3.50. Movement is active at 3.57; almost ceased at 5.05; ceased at 6.24 (1 h. 8 m.).<sup>2</sup>
4.  $\frac{4}{10} m$  CsCl, 3.51. Fairly active movement, ceasing somewhat sooner than in Solution 3 (ca. 1 h.).
5.  $\frac{3}{10} m$  CsCl, 3.52. At 3.59 movement is less active than in Solution 4, and cells show considerable swelling; almost ceased at 4.20 (28 m.).

In  $\frac{2}{10} m$  and  $\frac{1}{10} m$  solutions of RbCl and CsCl the cells rapidly swell and disintegrate, and movement ceases in a few minutes.

The action of RbCl seems thus closely similar to that of KCl. CsCl in the solution employed, made from a supposedly pure preparation of Merck, appeared less favorable than RbCl, though otherwise similar. Potassium, rubidium, and cæsium, according to the results just cited, are thus decreasingly favorable to ciliary movement in the order named; all three, however, differ markedly from sodium and lithium, whose salts in pure solution have almost no power of sustaining ciliary movement.

The difference between K, NH<sub>4</sub>, Rb, Cs, on the one hand, and Na and Li on the other shows an interesting relation to their respective ionic velocities. The absolute velocities of the K and NH<sub>4</sub> ions with a potential gradient of one volt per centimetre are

<sup>1</sup> In  $\frac{5}{10} m$  RbCl in another series of experiments movement lasted *ca.* seven hours; in a third series for more than twelve hours.

<sup>2</sup> In another experiment with  $\frac{5}{10} m$  CsCl and filaments from another animal movement lasted for more than two and one-half hours.

respectively .00067 and .00066, of Na and Li .000451 and .000347 centimetres per second. Rb and Cs, according to Boltwood's and Bredig's determinations, have velocities almost identical with those of K and  $\text{NH}_4$ .<sup>1</sup> These four cations have thus velocities about one and a half times greater than that of Na; the greater inertness of Li as compared with Na shows a similar relation to lower ionic velocity. The favorability of K,  $\text{NH}_4$ , Rb, and Cs is thus apparently correlated with a high ionic velocity. Their ready penetration into the contractile tissue, as evidenced by the action of the above hypertonic solutions, is possibly also to be explained on this ground.

**Salts of the alkali earth metals.**—In pure solutions of the alkali earth chlorides within a certain range of concentration ciliary movement may continue for very considerable periods. Calcium, barium, and magnesium chlorides in concentrations ranging from  $\frac{6}{100}$  m to  $\frac{3}{100}$  m permit movement to continue for many hours. Strontium chloride differs somewhat curiously and unaccountably from calcium chloride in that its pure neutral solutions quickly arrest movement. This action, however, is readily counteracted by the addition of small

TABLE IV.

*Mytilus edulis.*

$\frac{7}{100}$ m $\text{CaCl}_2$ .	Movement ceases at once.
$\frac{6}{100}$ m $\text{CaCl}_2$ .	6 h. 38 m.; 7 h. 25 m.; 18 h. 14 m.; 5 h. 17 m. + <sup>2</sup> ; 4 h. 48 m.; 4 h. 43 m. 18 m.; 8 h. 46 m.
$\frac{5}{100}$ m $\text{CaCl}_2$ .	30 h. 26 m.; 28 h.; 23 h. 25 m.; 6 h. 27 m. + (no movement at next observation, 24 h. later); 25 h. 22 m.; 19 h. 50 m.; 19 h. 22 m.
$\frac{4}{100}$ m $\text{CaCl}_2$ .	30 h. 26 m.; 33 h. 34 m.
$\frac{7}{100}$ m $\text{MgCl}_2$ .	40 m.; 2 h. 37 m.
$\frac{6}{100}$ m $\text{MgCl}_2$ .	3 h. 25 m.; 2 h.; 4 h.; 5 h. 22 m.; 3 h.+; 5 h. 19 m.+; 8 h. 49 m.
$\frac{5}{100}$ m $\text{MgCl}_2$ .	2 h.+; 4 h. 44 m.; 5 h. 14 m.; 5 h. 40 m.+; 8 h. 50 m.
$\frac{4}{100}$ m $\text{MgCl}_2$ .	8 h. 50 m.; 4 h. 44 m.+.
$\text{SrCl}_2$ .	(Movement always ceases within 30 m.)
$\frac{7}{100}$ m $\text{SrCl}_2$ .	Ceased instantly in two cases observed.
$\frac{6}{100}$ m $\text{SrCl}_2$ .	0 m.; 8 m.; 22 m.; 12 m.; 5 m.
$\frac{5}{100}$ m $\text{SrCl}_2$ .	0 m.; 29 m.; 16 m.; 25 m.; 20 m.
$\frac{4}{100}$ m $\text{SrCl}_2$ .	20 m. (one observation).
$\frac{7}{100}$ m $\text{BaCl}_2$ .	7 h. 39 m.; 1 h. 3 m.
$\frac{6}{100}$ m $\text{BaCl}_2$ .	7 h. 39 m.; 2 h. 4 m.; 4 h. 59 m.; 4 h. 45 m.
$\frac{5}{100}$ m $\text{BaCl}_2$ .	7 h. 39 m.; 5 h. 13 m.; 32 m.; 1 h. 2 m.; 30 h. 25 m.; 19 h. 38 m.
$\frac{4}{100}$ m $\text{BaCl}_2$ .	7 h. 39 m.+; 13 h. 56 m.

<sup>1</sup> Cf. KOHLRAUSCH'S tables.

<sup>2</sup> The + sign indicates that movement at the last observation was sufficiently active to continue from one to several hours longer.

quantities of alkali, or of certain other salts with powerfully acting anions (KCN, NaBrO<sub>3</sub>, Na<sub>2</sub>HAsO<sub>4</sub>; see below, Tables XXI to XXIII).

A large number of observations have been made at various times during 1904 and 1905, both at Woods Hole and Naples, with pure solutions of alkali earth chlorides; the foregoing table summarizes the results of these; after each solution is given in hours and minutes the maximum duration of movement observed in the different instances.

In general the chlorides of these metals, with the exception of strontium, resemble those of potassium, rubidium, and cesium in exhibiting a strongly favorable action toward ciliary movement in pure isotonic solution. They differ in the respect that even moderately hypertonic solutions ( $\frac{7}{10} m$  or  $\frac{8}{10} m$ ) rapidly bring movement to a standstill, — an apparent indication that their ions penetrate the tissue with less readiness than those of the above alkali metals. In solutions of the same or slightly lower osmotic pressure than the sea water calcium chloride sustains movement in general longer than magnesium chloride, and this salt than barium chloride.

I found the cilia of *Mytilus galloprovincialis* in Naples more resistant to the action of these solutions than those of *Mytilus edulis*. In one experiment movement lasted in the three solutions  $\frac{6}{10}$ ,  $\frac{5}{10}$ , and  $\frac{4}{10}$  CaCl<sub>2</sub> for sixty-nine, ninety-three, and more than one hundred hours respectively; for the corresponding MgCl<sub>2</sub> solutions the times were twenty-seven, forty-five, and ninety-three hours.

The cells swell and gradually detach themselves from the filaments in solutions of magnesium, calcium, and barium chlorides; the rapidity of the action decreases in the order just given. In pure strontium chloride solutions no swelling occurs; on the contrary, the filaments undergo gradual coagulation. Swelling is more gradual in the above solutions than in those of the alkali salts.

The alkali earth cations exhibit, on the other hand, a decided contrast to those of the alkali metals in exercising a strongly marked antitoxic action when added in small quantity to pure solutions of sodium salts (see below, p. 100). Since they are closely related chemically, and their ions agree in having high decomposition tensions, this general similarity in their physiological action is in accordance with expectation. The exceptional behavior of strontium chloride in pure solution, however, requires explanation. In its antitoxic effectiveness in solutions of sodium salts this salt resembles calcium chloride; yet its pure isotonic solution destroys ciliary move-



ment in a few minutes, and slowly coagulates the gill filament. The other metal of the group, beryllium, shows similar peculiarities in having a strongly marked antitoxic action in low concentration, while the pure  $\frac{m}{2}$  solution of its chloride is rapidly destructive of ciliary movement and coagulates the gill filaments (see p. 101).

**Action of pure solutions of heavy metal salts.**—Experiments with pure solutions of various heavy metal salts have also been tried. With the exception of manganese they all arrest movement rapidly, and cause a breakdown of the cilia due probably to coagulation of the ciliary substance. All salts of trivalent metals ( $\text{AlCl}_3$ ,  $\text{Al}_2(\text{SO}_4)_3$ ,  $\text{Cr}_2(\text{SO}_4)_3$ ,  $\text{FeCl}_3$ ) are instantly destructive in isotonic or slightly hypotonic solutions, and coagulate the filaments. In solutions of many bivalent metal salts some movement may be possible at first; this movement is of a peculiar quick vibratory character; in most cases it ceases within a few minutes. In solutions of manganese chloride, and to a less degree of nickel and cobalt chlorides, it may continue for a considerable length of time.

In pure isotonic solutions of beryllium chloride, of the alkali earth group, movement is brought to rest within a few minutes, as with the heavy metals. Beryllium, however, as will be seen below, possesses strong antitoxic action. In six trials with  $\frac{m}{2}$   $\text{MnCl}_2$ , at different times and with different animals, the maximum observed durations of movement were as follows: sixty-four, forty, and seventy minutes; four hours; three hours and ten minutes; four hours and forty-nine minutes; movement is active and energetic at first, as in solutions of magnesium or calcium chloride.  $\frac{m}{2}$   $\text{CoCl}_2$  and  $\text{NiCl}_2$  also permit movement to continue for some little time; in one instance a trace of movement was observed in a  $\frac{m}{2}$   $\text{NiCl}_2$  solution after thirty-two minutes; as a rule all movement ceases in these solutions within ten minutes. In  $\frac{m}{2}$   $\text{CdCl}_2$  and  $\frac{m}{2}$   $\text{ZnSO}_4$  active vibratory movements occur at first, but cease within two to three minutes;  $\text{BeCl}_2$  acts similarly; while no movement could be seen in solutions of  $\text{Cu}$ ,  $\text{Pb}$ ,  $\text{UO}_2$ , and  $\text{Hg}$  salts.

Manganese thus acts similarly to the alkali earth metals, especially magnesium, as seen in its effectiveness as an antitoxic agent with  $\text{Na}$  salts; a further resemblance consists in the fact that in its solutions the ciliated cells gradually swell and are detached, while in all other solutions of heavy metal salts the filament soon becomes white and opaque as a result of coagulation. These peculiarities of manganese are probably to be brought into relation with the high decomposition

tension of its ion (0.798 volts in N-solution); in this respect it greatly exceeds the other heavy metals, and resembles the more favorable bivalent metals, Ca, Ba, Mg, the decomposition tension of whose ions is, however, considerably greater.

#### IV. ANTITOXIC ACTION OF SALTS OF BIVALENT METALS.

Salts of the majority of bivalent metals have a more or less pronounced antitoxic action when added to pure solutions of sodium salts. The degree of this action shows wide variation with different metals. The most effective are the alkali earth metals, which, as seen above, are also the least poisonous in pure solution. Certain of the heavy metals, as manganese, ferrous iron, and to a less degree cobalt and nickel, also exhibit well-marked action; zinc, cadmium, and lead are less effective; while the more poisonous metals, copper, uranyl, and mercury, show no evidence of antitoxic action, at least in the case of this particular tissue.

The following table (V) summarizes the results of two independent

TABLE V.

	In control ( $\frac{6}{10} m$ NaCl) all trace of movement had ceased after 18 m. (Series A) and 10 m. (Series B).
1. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ CaCl <sub>2</sub> .	Movement is active at first; ceases within 39 m. in Series A and 48 m., Series B.
2. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ SrCl <sub>2</sub> .	Active at first; ceases sooner than in Solution 1: 18 m. (A) and 18 m. (B).
3. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ BaCl <sub>2</sub> .	Active at first; ceases within 39 m. (A) and 60 m. (B).
4. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ MgCl <sub>2</sub> .	Active movement, lasting decidedly longer than in above solutions: 2 h. 49 m. (A), and 3 h. 21 m. (B).
5. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ BeCl <sub>2</sub> .	Active and prolonged movement, lasting (A) 4 h. 31 m. and (B) 5 h. 1 m., and in a third determination on another animal, 5 h. 20 m.
6. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ ZnSO <sub>4</sub> .	(A) Fairly active movement after 18 m.; has ceased after 40 m.: (B) fairly active after 19 m.; ceased within 35 m. (A) 18 m. +; (B) 19 m. +.
7. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ CdCl <sub>2</sub> .	(A) A little movement after 31 m.; has ceased within the hour: (B) a little movement after 19 m. (A: 31 m.; B: 19 m.).
8. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ Cd(NO <sub>3</sub> ) <sub>2</sub> .	Essentially like Solution 7 (A, 64 m.; B, 19 m.).
9. $\frac{6}{10} m$ NaCl + $\frac{m}{1000}$ FeCl <sub>2</sub> .	Fairly active after 18 m. (only one determination, 18 m.).
10. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ NiCl <sub>2</sub> .	Active at first: lasts (A) 21 m.; (B) 53.5 m.
11. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ CoCl <sub>2</sub> .	Active at first: A, 64 m.; B, 52.5 m.
12. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ CuCl <sub>2</sub> .	Movement not prolonged; $\frac{m}{400}$ CuSO <sub>4</sub> , same result.
13. $\frac{6}{10} m$ NaCl + $\frac{m}{400}$ PbCl <sub>2</sub> .	Fairly active at first; movement slightly prolonged: ceases (A) in less than 18 m.; (B) in about 20 m.
14. $\frac{6}{10} m$ NaCl + $\frac{m}{1000}$ UO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub> .	Movement not prolonged.

series of determinations (A and B) with salts of various bivalent metals. The salts were added so as to be present in the solution ( $\frac{6}{10}$  m NaCl) in  $\frac{m}{400}$  concentration.

Another series of determinations, chiefly with heavy metal salts, was made at four different concentrations with the results embodied in the following table (VI). The salts were added to  $\frac{6}{10}$  m NaCl in the concentrations indicated.

TABLE VI.

Salt added.	$\frac{m}{25}$ .	$\frac{m}{50}$ .	$\frac{m}{100}$ .	$\frac{m}{200}$ .
1. MgCl <sub>2</sub>	Active movement lasting longer than 16 h. 27 m. (16 h. 27 m.).	16 h. 27 m. +.	16 h. 27 m. +.	16 h. 27 m. +.
2. BeCl <sub>2</sub>	Movement is slow from first; almost ceased after 1 h. 31 m. (1 h. 31 m.).	Movement as in $\frac{m}{25}$ .	More favorable than $\frac{m}{50}$ ; a little movement after 3 h. 40 m. (3 h. 40 m.).	More favorable than $\frac{m}{100}$ ; movement lasts ca. 7 h. 38 m. (7 h. 38 m.).
3. MnCl <sub>2</sub>	Vigorous movement at first; almost ceased aft. 2 h. 26 m. (2 h. 26 m.).	Like $\frac{m}{25}$ ; (2 h. 26 m.).	More favorable than $\frac{m}{50}$ ; (3 h. 40 m.).	Less favorable than $\frac{m}{100}$ ; almost ceased after 1 h. 30 m. (1 h. 30 m.).
4. ZnSO <sub>4</sub>	A little movement after 19 m.; ceased after 40 m. (19 m.).	Fairly active after 19 m.; ceased after 40 m. (19 m. +).	Like $\frac{m}{50}$ ; < 40 m. (19 m. +).	Like $\frac{m}{100}$ ; < 40 m. (19 m. +).
5. CdCl <sub>2</sub>	Movement ceases within 17 m. (17 m.).	A little movement after 17 m. (17 m.).	A little movement after 17 m. (17 m.).	Ceases within 17 m. (< 17 m.).
6. NiCl <sub>2</sub>	Active at first; a little movement after 57 m.; in another experiment movement after 59 m. (57 m.; 59 m.).	A little movement after 19 m.; ceases in < 40 m. (19 m.).	Like $\frac{m}{50}$ (19 m. +).	Fairly active after 19 m. (19 m. +).
7. CoCl <sub>2</sub>	More favorable than NiCl <sub>2</sub> ; a little movement after 2 h. 6 m.; in another trial 1 h. 28 m. (2 h. 6 m.; 1 h. 28 m.).	A little movement after 1 h. 50 m. (1 h. 50 m.).	A little movement after 1 h. 29 m.; ceased by 1 h. 50 m. (1 h. 29 m.).	Less favorable than $\frac{m}{100}$ ; ceased after 57 m. (39 m. +).
8. CuCl <sub>2</sub>	Movement not prolonged.	Movement not prolonged.	Movement not prolonged.	Movement not prolonged.
9. PVPbCl <sub>2</sub>	Movement lasts less than 19 m.	Movement lasts less than 19 m.	Less than 19 m.	A little movement after 19 m.; ceased by 35 m. (19 m. +).

A separate set of determinations was made with ferrous sulphate, using a freshly prepared solution to avoid so far as possible the presence of Fe<sup>+++</sup> ions. The results may be summarized as follows: —

TABLE VII.

1.	$\frac{m}{2}$ NaCl + $\frac{m}{25}$ FeSO <sub>4</sub> .	Vigorous movement at first; almost ceased after 14 m. (14 m.).
2.	$\frac{m}{2}$ NaCl + $\frac{m}{50}$ FeSO <sub>4</sub> .	Active movement; a little after 14 m. (14 m. +).
3.	$\frac{m}{2}$ NaCl + $\frac{m}{100}$ FeSO <sub>4</sub> .	Vigorous movement; a little after 1 h. 20 m. (1 h. 20 m.).
4.	$\frac{m}{2}$ NaCl + $\frac{m}{200}$ FeSO <sub>4</sub> .	Vigorous movement; remains fairly active after 1 h. 21 m. (1 h. 21 m.).
5.	$\frac{m}{2}$ NaCl + $\frac{m}{400}$ FeSO <sub>4</sub> .	A little movement after 1 h. 25 m. (1 h. 25 m.).
6.	$\frac{m}{2}$ NaCl + $\frac{m}{800}$ FeSO <sub>4</sub> .	Fair movement after 1 h. 25 m. (1 h. 25 m.).

In general the results of the above determinations agree somewhat closely with those obtained with the larvæ of *Arenicola*:<sup>1</sup> Cu and UO<sub>2</sub> give no evidence of antitoxic action,<sup>2</sup> while of the others the degree of antitoxic efficiency seems partly correlated with the decomposition tension of the cation, those in which this value is relatively high being the least poisonous and the most efficient as antitoxic agents. Thus Mn is the most favorable of the heavy metals;<sup>3</sup> then comes Fe<sup>m</sup>; then Co and Ni, of which Co is distinctly the more favorable; then Zn and Cd, which here again are more poisonous than corresponds to their position in the scale of decomposition tensions; Pb has relatively slight action.

Of the alkali earth cations it is to be noted that in low concentrations ( $\frac{m}{200}$  and less) Be and Mg have most effect in prolonging movement in pure NaCl solutions; *i. e.*, their antitoxic efficiency in low concentrations is greater than that of Ca, Ba, or Sr in the same concentrations. In higher concentrations the reverse may be true; and, as seen above, in pure solutions of  $\frac{6}{10}$  m to  $\frac{4}{10}$  m concentration CaCl<sub>2</sub> sustains movement longer than MgCl<sub>2</sub>. Sr appears similar to Ca in its antitoxic action in low concentrations, while Ba is on the whole more favorable than either<sup>4</sup> and approaches Mg, though without being so favorable. The following table, XII, summarizes the results of a series of experiments in which Ca, Ba, and Sr are used with  $\frac{m}{2}$  NaCl throughout a wide range of concentration. SrCl<sub>2</sub> is here most effective in low concentrations ( $\frac{m}{40}$ ); at higher concentration its specific toxicity obscures its antitoxic action. This is not the case with the other two salts.

<sup>1</sup> R. LILLIE: This journal, 1904, vii, p. 419.

<sup>2</sup> On the *Ctenophore* swimming plate UO<sub>2</sub>, Hg, and Ag have well-marked antitoxic action; Cu, on the contrary, is ineffective.

<sup>3</sup> Mn and Fe<sup>m</sup> are also decidedly more favorable than the other heavy metals in the case of the *Ctenophore* swimming plate.

<sup>4</sup> Compare the conditions in *Arenicola*, *Loc. cit.*, p. 428.

TABLE VIII.

Composition of mixture.	A. $\text{CaCl}_2$ .	B. $\text{SrCl}_2$ .	C. $\text{BaCl}_2$ .
1. 95 v. $\frac{10}{2}$ NaCl, 5 v. $\frac{10}{2}$ bivalent chloride.	Active at first; almost ceased after 7 h. 33 m. (7 h. 33 m.).	Active at first; a little movement after 19 h. 20 m. (19 h. 20 m.).	Active after 1 h. 30 m.; ceased by 7 h. 30 m. (1 h. 30 m. +).
2. 90 v. $\frac{10}{2}$ NaCl, 10 v. $\frac{10}{2}$ $\text{CaCl}_2$ , etc.	More favorable than 1 A; fairly active after 7 h. 33 m. (7 h. 33 m.).	Movement fairly active after 7 h. 33 m.; ceased by 19 h. 20 m. (7 h. 33 m. +).	Almost ceased by 7 h. 31 m. (7 h. 31 m.).
3. 80 v. $\frac{10}{2}$ NaCl, 20 v. $\frac{10}{2}$ $\text{CaCl}_2$ , etc.	Considerable activity after 7 h. 33 m. (7 h. 33 m. +).	Like 2 B (7 h. 33 m. +).	A little movement after 7 h. 31 m. (7 h. 31 m.).
4. 70 v. $\frac{10}{2}$ NaCl, 30 v. $\frac{10}{2}$ $\text{CaCl}_2$ , etc.	Like 3 A (7 h. 33 m. +).	A little movement after 7 h. 33 m. (7 h. 33 m.).	Like 3 C (7 h. 31 m.).
5. 60 v. NaCl, 40 v. $\text{CaCl}_2$ , etc.	Like 4 A (7 h. 33 m. +).	Almost ceased by 7 h. 33 m. (7 h. 33 m.).	Like 4 C (7 h. 31 m.).
6. 50 v. NaCl, 50 v. $\text{CaCl}_2$ , etc.	Like 5 A (7 h. 33 m. +).	Movement has ceased by 1 h. 30 m. (1 h. 30 m. +).	Fairly active after 7 h. 32 m. (7 h. 32 m. +).
7. 40 v. NaCl, 60 v. $\text{CaCl}_2$ , etc.	Like 6 A (7 h. 33 m. +).	Largely ceased after 30 m. (30 m.).	A little movement after 19 h. 36 m. (19 h. 36 m.).
8. 30 v. NaCl, 70 v. $\text{CaCl}_2$ , etc.	A little movement after 19 h. 20 m. (19 h. 20 m.).	Like 7 B (30 m.).	Fair movement after 19 h. 36 m. (19 h. 36 m.).
9. 20 v. NaCl, 80 v. $\text{CaCl}_2$ , etc.	Like 8 A (19 h. 20 m.).	Almost ceased by 30 m. (30 m.).	A little movement after 30 h. 48 m. (30 h. 48 m.).
10. 10 v. NaCl, 90 v. $\text{CaCl}_2$ , etc.	Fairly active after 19 h. 20 m. (19 h. 20 m.).	No movement after 30 m. (< 30 m.).	More favorable than 9 C; a little movement after 42 h. 24 m. (42 h. 24 m.).
11. Pure $\frac{10}{2}$ $\text{CaCl}_2$ , etc.	A little movement after 19 h. 20 m.; less favorable than 10 A (19 h. 20 m.).	No movement after 30 m. (< 30 m.).	Less favorable than 10; a little movement after 19 h. 38 m. (19 h. 38 m.).

There are difficulties in giving exact comparisons of the antitoxic capabilities of the various cations, since at one concentration a certain cation may be far more effective than another, while at higher concentrations the relations may be reversed.  $\text{AlCl}_3$ , for instance, is effective in concentrations at which  $\text{CaCl}_2$  has no perceptible action;<sup>1</sup> yet when present in favorable proportions Ca is far more effective than Al. So also in comparing Mg and Be on the one hand with Ca on the other:  $\text{BeCl}_2$  almost instantly arrests movement when present in the concentrations at which Ca acts most favorably and Mg is then less favorable than Ca. The relations thus appear complex. We may

<sup>1</sup> See Table XIX, p. 132.

perhaps regard capability of antagonizing the pure sodium salt as one property, and the specific toxicity of the salt itself as another, to a certain degree independent of the first. Hence many salts which in moderate concentrations are instantly destructive of ciliary activity act favorably at low concentrations. The case of beryllium and of the favorable heavy and trivalent metals exemplifies this (see Table XV). Evidently a salt, in order to exhibit well-marked antitoxic action, must be capable of so acting at concentrations in which its directly toxic action is relatively slight; otherwise the latter may prevent the antitoxic action from becoming evident.

On the whole it seems best, in comparing the antitoxic powers of the bivalent cations, to take as basis of comparison the optimum for each cation, *i. e.*, to compare their relative efficiencies in those concentrations at which each acts most favorably. If we regard as criterion the greatest antitoxic action of which the cation is capable at any concentration, the order of relative effectiveness in the case of Be and the above heavy metals runs somewhat as follows: Be, Mn, Fe<sup>II</sup>, Co, Ni, Cd, Zn, Pb, Cu, UO<sub>2</sub>, Hg. A more exact estimate is not possible on the basis of the above observations. The order, it will be noted, is essentially that of increasing toxicity; *i. e.*, the more toxic a metal is the less likely are its salts to exhibit well-marked antitoxic action. The order is also in general that of decreasing decomposition tensions.

**Relation of the anion of the sodium salt to the possibility and extent of antitoxic action.**—The nature of the cation is thus of prime importance in the antitoxic action of any salt. When to a solution of a given sodium salt is added a quantity of another sodium salt with a different anion, in general no antitoxic effect is seen;<sup>1</sup> *i. e.*, anions do not counteract the toxicity of the pure sodium salt. Anions and cations thus exhibit opposite actions in this relation. It is, however, important to note that the different sodium salts exhibit very unequal capabilities of being thus antagonized; in the case of solutions of certain sodium salts (NaF, NaCNS, NaBrO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub>), the addition of a salt with an efficient cation (as Mg), even in the most favorable

<sup>1</sup> The addition of small quantities of alkali may, however, favor ciliary movement for a time, even though it accelerates the disintegration of the tissue. Salts like Na<sub>2</sub>CrO<sub>4</sub> and KMnO<sub>4</sub> seem to offer an exception to the above rule, since in low concentrations they may exhibit a distinct antitoxic action (see below, Table XVI). This is to be regarded as due to the presence in such solutions of Mn- and Cr-ions of high valence, and is really a cation effect (see MATHEWS: This journal, 1904, xi, p. 237).

concentrations, may have but slight or imperceptible action. Or, with other salts ( $\text{NaI}$ ,  $\text{Na}_2\text{SO}_4$ ), the antagonizing cation must be present in much higher concentrations than with  $\text{NaCl}$  in order to produce the same antitoxic effect.

The following determinations were made in order to ascertain the relative capabilities of the various sodium salts of being antagonized by addition of salts of magnesium, the most efficient cation for this purpose.  $\text{MgCl}_2$  (in  $\frac{m}{2}$  solution) was added to  $\frac{m}{2}$  solutions of the salts till present to a concentration of  $\frac{m}{2.5}$  (in general an approximately optimum concentration for antitoxic action with this salt).<sup>1</sup> The determinations were repeated on another animal in the case of the more toxic salts (Series B in Table IX).

TABLE IX.

1.  $\frac{m}{2}$   $\text{NaF}$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$  (slight precipitate of  $\text{MgF}_2$ ). Movement ceases soon. No movement after 10 m. Result negative.
2.  $\frac{m}{2}$   $\text{NaCl}$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Vigorous and prolonged movement; still active after 35 h.; ceased by 48 h. (35 h. +).
3.  $\frac{m}{2}$   $\text{NaBr}$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Vigorous movement, considerable activity remains after 48 h. (48 h.).
4.  $\frac{m}{2}$   $\text{NaI}$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Vigorous movement at first; cells swell and detach somewhat rapidly. Movement is active after 3 h. 57 m.; has ceased by 6 h. (4 h. +).
5.  $\frac{m}{2}$   $\text{NaNO}_3$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Vigorous movement as in  $\text{NaCl}$ ; almost ceased after 17 h. 53 m. (18 h.).
6.  $\frac{m}{2}$   $\text{NaClO}_3$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Vigorous movement; a little movement after 48 h. 3 m. (48 h.).
7.  $\frac{m}{2}$   $\text{NaBrO}_3$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . (A) active movement at first; ceases relatively soon; almost ceased after 2 h. 1 m. (B) active at first; almost ceased by 50 m. (A: 2 h.; B: 50 m.).
8.  $\frac{m}{2}$   $\text{NaCOOCH}_3$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Active after 8 h. 27 m.; has ceased by 22 h. (8 h. 27 m. +).
9.  $\text{NaCNS}$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . (A) movement has almost ceased after 40 m. (in control — pure  $\frac{m}{2}$   $\text{NaCNS}$  — has entirely ceased in less than 10 m.). (B) movement is less vigorous than in solution 7 B; a little remains after 1 h. 9 m. (A: 40 m.; B: 1 h. 9 m.).
10.  $\frac{m}{2}$   $\text{Na}_2\text{SO}_4$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Movement is still active after 6 h. 42 m.; has ceased after 21 h. (6 h. 42 m. +).
11.  $\frac{m}{2}$   $\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Vigorous movement at first; is still active after 3 h. 48 m.; has ceased after 8 h. (3 h. 48 m. +).
12.  $\frac{m}{2}$   $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  +  $\frac{m}{2.5}$   $\text{MgCl}_2$ . Movement lasts a short time only; is well marked after 10 m.; has ceased after 40 m. (10 m. +).
13.  $\frac{m}{2}$   $\text{Na}_2\text{HPO}_4$  +  $\text{MgCl}_2$  (precipitate of phosphate). (A) a little movement after 50 m.; has ceased by 1 h. 45 m. (in control — pure  $\frac{m}{2}$   $\text{Na}_2\text{HPO}_4$  — has entirely ceased after 6 m.). (B) fairly active at first; has almost ceased after 1 h. 9 m. (A: 50 m. +; B: 1 h. 9 m.).

<sup>1</sup> Cf. Table XI on p. 109, with  $\text{NaCl}$ ,  $\text{NaBr}$ , and  $\text{NaI}$ , +  $\text{MgCl}_2$  in graded proportions.

TABLE IX (continued).

14.  $\frac{1}{2}$   $\text{Na}_4\text{Fe}(\text{CN})_6 + \frac{m}{25}$   $\text{MgCl}_2$ . (A) movement remains fairly active after 1 h. 40 m.; has ceased after 2 h. 15 m. (entirely ceased in control in less than 10 m.). (B) active at first; has almost ceased after 1 h. 9 m. (A: 1 h. 40 m.; B: 1 h. 9 m.).
15.  $\frac{1}{2}$   $\text{Na}_3\text{Fe}(\text{CN})_6 + \frac{m}{25}$   $\text{MgCl}_2$ . Ceases at once.
16.  $\frac{m}{2}$   $\text{Na}_2\text{HAsO}_4 + \text{MgCl}_2$  (arsenate precipitate). (A) considerable movement remains after 40 m.; ceased after 1 h. (in control—pure  $\frac{m}{2}$   $\text{Na}_2\text{HAsO}_4$ —ceases within 5 m.). (B) movement is fairly active after 15 m.; has ceased after 25 m. (A: 40 m.; B: 15 m.-20 m.).
17.  $\frac{m}{2}$   $\text{NaNO}_2 + \frac{m}{25}$   $\text{MgCl}_2$ . (A) active at first; has almost ceased after 1 h. 53 m. (has entirely ceased in control after 20 m.). (B) movement is somewhat slow; has almost ceased after 1 h. 10 m. (A: 1 h. 53 m.; B: 1 h. 10 m.).
18.  $\frac{1}{2}$   $\text{Na}_2\text{S}_2\text{O}_3 + \frac{m}{25}$   $\text{MgCl}_2$ . Vigorous movement; is active after 3 h. 38 m.; has ceased by 8 h. (3 h. 38 m.+).
19.  $\frac{m}{2}$   $\text{Na}_2\text{SO}_3 + \frac{m}{25}$   $\text{MgCl}_2$ . (A) active movement at first; has almost ceased after 30 m. (control has ceased within 5 m.). (B) considerable movement after 25 m.; has ceased after 40 m. (A: 30 m.; B: 25 m.+).

Two other series of determinations on different animals, using the following salts with  $\frac{m}{50}$   $\text{MgCl}_2$ , gave the results summarized in the following table (X):

TABLE X.

1.  $\frac{m}{2}$   $\text{NaF} + \frac{m}{50}$   $\text{MgCl}_2$ . Movement is not perceptibly prolonged.
2.  $\frac{m}{2}$   $\text{NaCl} + \frac{m}{50}$   $\text{MgCl}_2$ . Active long-continued movement (A: 17 h. 22 m.; B: 15 h. 43 m.).
3.  $\frac{m}{2}$   $\text{NaBr} + \frac{m}{50}$   $\text{MgCl}_2$ . Active long-continued movement (A: 4 h. 20 m.+; B: 15 h. 43 m.).
4.  $\frac{m}{2}$   $\text{NaI} + \frac{m}{50}$   $\text{MgCl}_2$ . Movement is active at first; ceases relatively soon (A: 1 h. 15 m.; B: active after 35 m.; has ceased at next examination after 2 h. 38 m. 35 m.+).
5.  $\frac{m}{2}$   $\text{NaNO}_3 + \frac{m}{50}$   $\text{MgCl}_2$ . Active movement in both series after 4 h.; has ceased after 17 h. and 15 h. respectively (A: 4 h. 20 m.+; B: 4 h. 6 m.+).
6.  $\frac{m}{2}$   $\text{NaClO}_3 + \frac{m}{50}$   $\text{MgCl}_2$ . Active movement at first as in  $\text{NaCl}$ . (A) has almost ceased after 5 h. 20 m.; (B) a little movement remains after 15 h. 43 m. (A: 5 h. 20 m.; B: 15 h. 43 m.).
7.  $\frac{m}{2}$   $\text{NaBrO}_3 + \frac{m}{50}$   $\text{MgCl}_2$ . (A) movement ceases relatively soon (< 15 m.) (B) movement feeble and ceases soon; a little movement after 16 m.; ceased after 35 m. (A: < 15 m.; B: 16 m.).
8.  $\frac{m}{2}$   $\text{NaCOOCH}_3 + \frac{m}{50}$   $\text{MgCl}_2$ . (A) Active movement; still remains quite active after 4 h. 20 m. (B) similar; movement lasts longer than in A (A: 4 h. 20 m.+; B: 15 h. 43 m.).
9.  $\frac{m}{2}$   $\text{NaCNS} + \frac{m}{50}$   $\text{MgCl}_2$ . Movement is slow; (A) a little movement remains after 1 h. 15 m.; has ceased by 2 h. 15 m. (B) ceases in < 35 m. (A: 1 h. 15 m.; B: 16 m.+).
10.  $\frac{m}{2}$   $\text{Na}_2\text{SO}_4 + \frac{m}{50}$   $\text{MgCl}_2$ . Active movement at first. (A) has largely ceased after 2 h. 16 m., and completely after 3 h. 20 m. (B) some slow movement remains after 2 h. 35 m. (A: 2 h. 16 m.; B: 2 h. 35 m.).



TABLE X (continued).

11.  $\frac{1}{2}$   $\text{Na}_2\text{C}_4\text{H}_4\text{O}_6 + \frac{m}{50}$   $\text{MgCl}_2$ . Active movement at first; has almost ceased after (A) 2 h. 16 m. and (B) 2 h. 35 m.
12.  $\frac{m}{2}$   $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 + \frac{m}{50}$   $\text{MgCl}_2$ . (A) active movement after 17 m.; has largely ceased after 35 m.; no movement remains after 1 h. 35 m. (B) has almost ceased after 16 m. (A: 35 m.; B: 16 m.).
13.  $\frac{1}{2}$   $\text{Na}_2\text{HPO}_4 + \frac{m}{50}$   $\text{MgCl}_2$ . Movement ceases in both series in less than 15 m. (A: 15 < m.; B: < 15 m.).
14.  $\frac{m}{2}$   $\text{Na}_4\text{Fe}(\text{CN})_6 + \frac{m}{50}$   $\text{MgCl}_2$ . Movement ceases in both series in less than 15 m. (A: < 15 m.; B: < 15 m.).

It will be seen that in all the solutions of the last two tables a certain degree of antitoxic action is evident, with the exception of the fluoride and the ferricyanide. The effect is relatively slight with solutions of the citrate, phosphate, arsenate, ferrocyanide, sulphocyanate, nitrite, bromate, and sulphite; somewhat more marked with the iodide, and well marked with the chloride, bromide, nitrate, chlorate, acetate, sulphate, and tartrate. Using as criterion the degree to which  $\text{MgCl}_2$  in the above concentrations can counteract the injurious action of the pure salt solution, we may range the salts in their order of toxicity somewhat as follows: The chloride, bromide, chlorate, nitrate, and acetate among the salts with monovalent anions may be antagonized with almost equal readiness, although nitrate and acetate appear in the above experiments somewhat less favorable than the other three. The iodide is distinctly less favorable than the salts just named; the bromate, nitrite, and sulphocyanate can be antagonized only slightly, and the fluoride not at all. Of salts with bivalent anions, — sulphate, thiosulphate, and tartrate are in general similar to one another, and comparable to nitrate in their action; sulphite is distinctly more toxic, and is not readily antagonized. Of the salts with trivalent and tetravalent anions, all show a relative insusceptibility to the antitoxic influence of the  $\text{MgCl}_2$  in these concentrations; this may be due in the case of the above experiments to the presence of an insufficiency of the antitoxic salt. It is nevertheless somewhat surprising that in solutions of salts like arsenate and ferrocyanide the presence of magnesium chloride markedly diminishes the toxicity, especially in the latter instance. Phosphate is less toxic than arsenate, according to the above determinations. In comparing these results with those observed in pure solutions of potassium salts, it will be seen that the order of toxicity of the anions is practically the same in both series. For the series of monovalent salts, the order of in-

creasing toxicity corresponds closely with that of increasing activity in producing swelling of the ciliated cells. This, however, is not true of salts with plurivalent anions, many of which (arsenate, phosphate, ferrocyanide, citrate) are strongly toxic — *i. e.*, arrest movement rapidly in pure solution, and are not readily antagonized — without producing noteworthy swelling. The behavior of the monovalent salts indicates, however, that at least part of their toxicity is due to their producing a swelling of the cell; prevention of this action by action of various cations has the effect of counteracting the toxicity to a greater or less degree (see below, Section VII). The unequal toxicity of the various anions is thus to be ascribed largely to the inequality of their action in producing physical alterations in the contractile substance.

**Variations in optimum proportion of antitoxic salt with variation in anion of toxic salt.** — Indications are seen in the above tables that the optimum proportion of  $MgCl_2$  varies with the anion of the toxic salt. One might expect that the salts with the more energetically acting anions would require higher proportions of  $MgCl_2$  for counteraction of their toxic action. With a view to determining if this is so, the following series of determinations were made with the four sodium salts, chloride, bromide, iodide (with monovalent anions of increasingly powerful action as shown by the typical swelling action), and sulphate (with typical bivalent anion). (Table XI.)

All the gill filaments used in this series were taken from the same animal. In every solution, with the exception of the pure sodium salts, movement was active, and continued for several hours.

The difference between iodide and sulphate on the one hand, and chloride and bromide on the other, proved well marked, the former two being distinctly less favorable. In the iodide swelling and detachment of cells take place with much greater rapidity than in the other solutions, and the comparatively great toxicity of this salt is, in all probability, largely due to this action.

The differences between the anions Cl, Br, and I are in general as follows: In their power of producing swelling there is a progressive increase with increase of atomic weight, or decrease of decomposition tension. The most favorable concentration of  $MgCl_2$  for antitoxic action was lower with NaCl than with NaBr, and with NaBr than with NaI. With  $Na_2SO_4$  the optimum proportion of  $MgCl_2$  was apparently almost the same as with NaI. In both  $Na_2SO_4$  and NaI duration of movement proved much shorter than

with NaCl or NaBr; this agrees with the results of the previous determinations (cf. also the series with K-salts). In general the fol-

TABLE XI.

Solution.	A. NaCl.	B. NaBr.	C. NaI.	D. Na <sub>2</sub> SO <sub>4</sub> .
1. "Pure" salt	No movement and a little swelling after 2 h.	Many cells rounded and detached after 2 h.	Swelling and detachment after 2 h. > NaBr.	Swelling and detachment after 2 h. ca. as in NaBr.
2. 95 v. salt 5 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Movement almost ceased after 30 h. 52 m. (30 h. 52 m.).	Fairly active movement after 44 h. (44 h.).	Considerable movement after 7 h. 51 m. (7 h. 51 m.).	Fair movement after 7 h. 53 m. (7 h. 53 m.).
3. 90 v. salt 10 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Like 2 A (30 h. 52 m.).	Like 2 B (44 h. 28 m.).	Like 2 C (7 h. 51 m.).	Like 2 D (7 h. 53 m.).
4. 80 v. salt 20 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Like 3 A (30 h. 52 m.).	A little movement after 44 h. 28 m. (44 h. 28 m.).	Considerable movement after 7 h. 51 m. (7 h. 51 m.).	Like 3 D (7 h. 53 m.).
5. 70 v. salt 30 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Ceased after 30 h. Fairly active after 19 h. 45 m. (19 h. 45 m. +).	Like 4 B (44 h. 28 m.).	Active movement after 7 h. 52 m. (7 h. 52 m. +).	Active after 7 h. 53 m. (7 h. 53 m. +).
6. 60 v. salt 40 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Like 5 A (19 h. 45 m. +).	Trace movement after 44 h. 28 m. (44 h. 28 m.).	Active after 7 h. 53 m. (7 h. 53 m. +).	Like 5 D (7 h. 53 m. +).
7. 50 v. salt 50 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Fair movement after 19 h. 45 m.; less favorable than 6 A (19 h. 45 m.).	Less favorable than 6 B.; little movement after 30 h. 57 m. (30 h. 57 m. +).	Like 6 C (7 h. 53 m. +).	Vigorous movement after 7 h. 52 m. (7 h. 52 m. +).
8. 40 v. salt 60 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Almost ceased by 19 h. 45 m.; less favorable than 7 A (19 h. 45 m.).	Trace movement after 30 h. 57 m. (30 h. 57 m.).	Active after 7 h. 53 m. (7 h. 53 m.).	Somewhat less active than 7 D after 7 h. 52 m. (7 h. 52 m. +).
9. 30 v. salt 70 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Ceased by 19 h. 45 m.; fairly active after 7 h. 55 m. (7 h. 55 m. +).	Less favorable than 8 B; fair movement after 20 h. 37 m. (20 h. 37 m.).	Somewhat less active than 8 C after 7 h. 53 m. (7 h. 53 m.).	Fair movement after 7 h. 52 m. (7 h. 52 m.).
10. 20 v. salt 80 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Like 9 A (7 h. 55 m.).	A little movement after 20 h. 37 m. (20 h. 37 m.).	Fair movement after 7 h. 53 m. (7 h. 53 m.).	Fair movement after 7 h. 52 m. (7 h. 52 m.).
11. 10 v. salt 90 v. $\frac{m}{2}$ MgCl <sub>2</sub>	Like 10 A (7 h. 55 m.).	Ceased by 20 h. 37 m.; fairly active after 7 h. 53 m. (7 h. 53 m. +).	Fair movement after 7 h. 53 m. (7 h. 53 m.).	Fair movement after 7 h. 52 m. (7 h. 52 m.).
12. pure $\frac{m}{2}$ MgCl <sub>2</sub>	Active after 5 h. 40 m.; ceased by 7 h. 55 m. (5 h. 40 m.).			

lowing is indicated as a result of this and the foregoing experiments; (1) that the more powerfully acting anions require higher proportions

of the antitoxic cation for favorable action; and (2) that the more toxic anions can be counteracted to a less degree than the less toxic. In experiments on the antitoxic action of the hydrogen ion similar relations appear (see below, Table XIII, p. 114).

**Antitoxic action in relation to salts of other alkali metals.**—Sodium salts are peculiar in that their toxicity, strongly marked in pure solution, is so readily diminished or counteracted by the presence of relatively small quantities of plurivalent metallic salts, especially those of the alkali earths. This peculiarity is shared to a certain degree by lithium salts, as the table given below will illustrate. Potassium and ammonium salts (and presumably those of rubidium and cesium, which resemble potassium), on the other hand, seem practically devoid of it, so far as my observation has extended.<sup>1</sup>

These facts constitute another exemplification of the importance of sodium salts in contractile processes, and of their partial replaceability by lithium salts, though not by salts of the other alkali metals. The rule that this form of antitoxic action is confined to salts of these metals does not necessarily apply to other than contractile processes; thus the toxicity of potassium salts for early stages of developing eggs can be partly counteracted by addition of salts of bivalent metals.<sup>2</sup>

TABLE XII.

1.  $\frac{m}{2}$  LiCl pure. Movement ceases almost instantly.
2. 90 volumes  $\frac{m}{2}$  LiCl + 10 volumes  $\frac{m}{2}$  MgCl<sub>2</sub>. Movement is feeble, but lasts some time; has almost ceased after 1 h. 58 m. (2 h.).
3. 80 v.  $\frac{m}{2}$  LiCl + 20 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Somewhat more favorable than Solution 2; a little movement after 1 h. 58 m. (2 h.).
4. 70 v.  $\frac{m}{2}$  LiCl + 30 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Movement is sluggish, but lasts longer than in Solution 3; a little remains after 4 h. 20 m. (4 h. 20 m.).
5. 60 v.  $\frac{m}{2}$  LiCl + 40 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Like Solution 4 (4 h. 20 m.).
6. 50 v.  $\frac{m}{2}$  LiCl + 50 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Sluggish movement, lasting longer than in Solution 5 (4 h. 20 m. +).
7. 40 v.  $\frac{m}{2}$  LiCl + 60 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Somewhat more favorable than Solution 6 (4 h. 20 m. +).
8. 30 v.  $\frac{m}{2}$  LiCl + 70 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Movement is more active than in Solution 7; lasts longer than 5 h. (5 h. +).
9. 20 v.  $\frac{m}{2}$  LiCl + 80 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Like Solution 8 (5 h. +).
10. 10 v.  $\frac{m}{2}$  LiCl + 90 v.  $\frac{m}{2}$  MgCl<sub>2</sub>. Movement is fairly active (< 5 h.).
11. Pure  $\frac{m}{2}$  MgCl<sub>2</sub>. Movement is much more rapid than in solutions containing Li,—is quick and vibrating (5 h. +).

<sup>1</sup> These statements also hold true of the Ctenophore swimming plate, in which the toxicity of sodium, and, to a less degree, of lithium salts, can be counteracted, *e. g.*, by MgCl<sub>2</sub>; while potassium and ammonium salts show no such susceptibility.

<sup>2</sup> Cf. LOEB: This journal, 1900, iii, p. 383.

For cilia, however, and for the Ctenophore swimming plates which are modified cilia, I have found this relation to hold constant.

Table XII summarizes the results of a series of experiments with mixtures of LiCl and MgCl<sub>2</sub>.

Ciliary movement is sluggish and wavy in solutions containing lithium salts, the Li-ion being evidently less favorable to contractility than the Na-ion. Antitoxic action is typical, however, the addition of relatively small quantities of MgCl<sub>2</sub> prolonging movement greatly; the conditions become increasingly favorable with decrease in the concentration of LiCl and increase of MgCl<sub>2</sub>.

In similar mixtures of NH<sub>4</sub>Cl + MgCl<sub>2</sub>, and KCl + MgCl<sub>2</sub> no such distinct antitoxic action is seen. With NH<sub>4</sub>Cl, the later members of the series (those in which MgCl<sub>2</sub> preponderates) are more favorable than the earlier, — MgCl<sub>2</sub> being specifically more favorable than NH<sub>4</sub>Cl; but there is no typical antagonization of the alkali salt by a relatively small proportion of the bivalent salt. The case is similar with KCl + MgCl<sub>2</sub>: duration of movement is not prolonged over that in pure KCl solutions, whatever the proportions of the salts in the mixture.

A further series of experiments was performed using solutions of several of the more toxic potassium salts with a view to determining if the well-marked toxicity shown by these (in contrast to KCl, KBr, etc.) could be diminished by the presence of magnesium. The following salts were used in  $\frac{m}{2}$  concentration: KF, KCNS, KI, K<sub>2</sub>C<sub>2</sub>O<sub>4</sub>, K<sub>2</sub>HAsO<sub>4</sub>, each salt being used first in pure solution and then after the addition of MgCl<sub>2</sub> to  $\frac{m}{20}$  concentration (9 volumes K, salt + 1 volume  $\frac{m}{2}$  MgCl<sub>2</sub>). It was found that although the addition of the MgCl<sub>2</sub> seemed to effect a slight improvement in the conditions, no decisive antitoxic action appeared in any instance. This result is in striking contrast with that obtained with the corresponding sodium salts. The difference must be attributed to some specific peculiarity of the Na-ion.

#### V. ANTITOXIC ACTION OF MONOVALENT CATIONS, AND ESPECIALLY OF THE HYDROGEN ION, ON SOLUTIONS OF SODIUM SALTS.

The addition of another alkali metal salt, such as ammonium or potassium chloride, to a pure sodium chloride solution has no distinctly favorable action until the former salt attains such high concentration in the mixture that its specific favorability overbalances the

injurious action of the sodium salt. This result is typical; the alkali metal cations are without distinct antitoxic action in the above defined sense. This lack of such action is probably due not so much to the monovalence of these ions as to their high decomposition tension, which, as Mathews' results indicate, seems correlated with relative physiological inactivity. Other monovalent cations, as Ag and the H-ion possess powerful physiological action, and their behavior in the present relation is accordingly different from that of the alkali metals. Silver salts, however, although their presence in low concentration prevents swelling and gives certain other indications of antitoxic action, do not prolong ciliary movement in *Mytilus*; Ag acts similarly to Hg; the specific toxicity of both metals is such that the concentrations necessary for antitoxic action are rapidly fatal to the tissue. Thus, in a series of solutions of  $\text{Na}_2\text{SO}_4$ , to which  $\text{AgNO}_3$  had been added in concentrations ranging from  $\frac{m}{800}$  to  $\frac{m}{204800}$ , there was no effect in prolonging movement in any solution; the filaments were coagulated in concentrations of  $\frac{m}{12800}$  and above; below this concentration there was well-marked effect in preventing swelling, but ciliary activity ceased in all solutions within ten minutes. Silver salts thus proved ineffective in my experiments on the *Mytilus* gill. Silver, however, as well as mercury, has in low concentrations a well-marked antitoxic action on the Ctenophore swimming plate; with this tissue the most favorable concentrations were very low, ranging from  $\frac{m}{50000}$  to  $\frac{m}{300000}$ . Whether or not it is capable of so acting appears thus to depend on the nature of the tissue.

The hydrogen-ion (or dilute acid) has, on the other hand, a very distinct though not strongly pronounced antitoxic action on the cilia of the *Mytilus* gill. This has been determined for a number of sodium salts; the action is always found in low concentrations, ranging from ca  $\frac{m}{3000}$  to  $\frac{m}{25000}$ . Since the action of dilute acid presents certain definite characteristics that appear to throw light on the general nature of the antitoxic action of salts, the results of these experiments will be presented in some detail.

Table XIII tabulates the results of several series of experiments with various monovalent sodium salts (and  $\text{Na}_2\text{SO}_4$ ) in  $\frac{1}{10}^6 m$  and  $\frac{m}{2}$  concentrations. Standardized HCl solution was added to the salt solutions in the proportions represented.<sup>1</sup> The character of the

<sup>1</sup> *I. e.*, the acid is diluted by addition of the solution to the degree represented. The figures thus simply designate the solution, without considering any interaction between the salt and the acid. Of course this last action must be taken into account in interpreting the observed phenomena.

movement, its duration in the solution, and the swelling or anti-swelling (coagulative) action of the latter are the chief data recorded. In the series with each salt there is seen, first, a certain range of HCl concentration in which the movement ceases sooner than in the pure solution and the filament undergoes coagulation; then a range in which movement is decidedly prolonged and in which some swelling, though less than in the pure solution, may occur; and finally a range of concentration in which there is seen little or no difference from the pure solution. The optimum concentration of HCl thus varies in highly characteristic manner for each salt. A relation is seen between the anti-swelling action of the H-ion (or of the acid, disregarding the terms of the ionic theory) and its action in prolonging movement. This relation gives a certain indication of the nature of the antitoxic action; it seems due, in part at least, to the prevention of the swelling or disintegrative action of the pure salt solution.

In Table XIII each vertical column gives the results of a series of determinations with the salt designated. The horizontal columns give the data for different salt solutions to which the same quantity of acid has been added.

According to the determinations in Table XIII the optimum proportion of HCl for NaCl is lower than for NaBr, lying in the former case below  $\frac{m}{12800}$  and in the latter between  $\frac{m}{1600}$  and  $\frac{m}{6400}$ . In order to determine more precisely the most favorable proportion for each of these salts, I made two series of determinations in which the proportion of acid was gradually decreased in the successive members of the series. HCl was added to the  $\frac{6}{10} m$  NaCl and proportions ranging from  $\frac{m}{10000}$  to *ca.*  $\frac{m}{30000}$  (as follows: .0001 *m*, .00009 *m*, .00008 *m*, .00007 *m*, .00006 *m*, .00005 *m*, .00004 *m*, .00003 *m*), and to  $\frac{6}{10} m$  NaBr in eight similarly graded proportions between  $\frac{m}{2500}$  and *ca.*  $\frac{m}{8000}$  (.0004 *m*, .00036 *m*, .00032 *m*, .00028 *m*, .00024 *m*, .0002 *m*, .00016 *m*, .00012 *m*). With  $\frac{6}{10} m$  NaCl .00009 *m* HCl just sufficed to prevent swelling, and the most favorable concentrations were .00006 *m* and .00005 *m*, which check swelling considerably without wholly preventing it; in these solutions movement was vigorous at first and lasted for about fifty minutes. With  $\frac{6}{10} m$  NaBr there was a little swelling even in .0004 *m* HCl ( $\frac{m}{2500}$  as seen in the table prevents it altogether): conditions seemed most favorable in .00028 *m* concentration where some activity remained after one and a half hours; in the succeeding solutions movement lasted for an hour or more. Here also the most favorable proportion was that in which swelling was materially checked

TABLE XIII.

HCl added.	NaCl.	NaBr.	NaI.	NaNO <sub>3</sub> .
Pure salt solution.	Movement rapidly becomes sluggish and ceases within 15 m.	Movement ceased and cells slightly swollen after 21 m. Marked swelling after 40 m.	Cells swollen and detaching after 14 m. No movement.	Well-marked swelling and detachment of cells after 21 m.; no movement.
$\frac{m}{400}$	Filaments coagulated and opaque within 7 m.; no movement seen.	Filaments coagulated white and opaque within 7 m. No movement.	Filaments coagulated white and opaque within 7 m. No movement.	Filaments white and coagulated after 7 m.; no movement.
$\frac{m}{800}$	Similar to HCl.	Coagulation is much more gradual than in NaCl + $\frac{m}{300}$ HCl. No movement.	Coagulation is less marked than in $\frac{m}{300}$ HCl. No movement seen.	White and coagulated after 7 m.; no movement.
$\frac{m}{1600}$	Similar to HCl.	Considerable movement after 7 m.; only slight evidence of coagulation (7 m.).	After 14 m. filaments are unswollen and almost normal in appearance, — slightly whitened. No movement.	No movement after 7 m.; filaments are then slightly coagulated.
$\frac{m}{3200}$	No movement seen; filaments whiten more gradually than in $\frac{m}{1600}$ HCl.	Vigorous movement at first; considerable movement after 65 m.; slight swelling then evident; ceased by 1 h. 40 m. (65 m. +).	Active vibratory movement at first. A little movement after 20 m.; ceased by 45 m.; cells slightly swollen after 45 m. (20 m. +).	Vigorous movement at first; a little movement after 30 m.; almost no swelling (30 m.).
$\frac{m^*}{6400}$	No movement seen; filaments slowly coagulate.	Active at first; fairly active after 21 m.; ceased by 45 m.; swelling is then well-marked (21 m. +).	Active vibratory movement, cells swell more rapidly than in $\frac{m}{3200}$ HCl. A little movement after 65 m.; swelling is then considerable (65 m.).	Active movement at first; swelling is well marked after 30 m. and movement has ceased (7 m. +).
$\frac{m}{12800}$	Vigorous vibratory movement at first; a little movement after 28 m.; ceased by 42 m. Only slight indication of coagulation (28 m.).	Less favorable than $\frac{m}{6400}$ HCl; swelling is more rapid (21 m.).	Swelling and detachment of cells are well marked after 14 m.; a little movement then; ceased by 21 m. (14 m.).	Similar to $\frac{m}{12800}$ HCl, but swelling is more rapid.
$\frac{m}{25600}$	Movement very active at first; a little movement after 66 m. No coagulation (66 m.).			



TABLE XIII (continued).

NaClO <sub>3</sub> .	NaBrO <sub>3</sub> .	NaCNS.	NaCOOCH <sub>3</sub> .	Na <sub>2</sub> SO <sub>4</sub> .
Swelling beginning after 21 m.; no movement.	Movement ceased within 7 m. and cells swelling.	Well marked swelling and detachment of cells after 7 m. No movement.	Movement almost ceased after 14 m.; ceased by 25 m. (14 m.).	Movement has practically ceased after 6 m.
Filaments white and coagulated after 7 m.; no movement.	Cilia are fairly active after 7 m.; no coagulation; movement has ceased by 14 m.; slight swelling by then. Marked swelling later. (7 m. +).	No movement after 7 m.; filaments white and coagulated.	Filaments do not coagulate; active movement after 35 m. (35 m. +).	Ceased after 6 m.; somewhat coagulated after 12 m.
Like $\frac{m}{400}$ HCl; coagulation is rather less rapid; no movement.	No movement is seen. Swelling is more rapid than in $\frac{m}{400}$ HCl.	No movement after 7 m.; filaments then slightly coagulated.	Like $\frac{m}{400}$ HCl. Movement active after 35 m. (35 m. +).	Active vibratory movement after 6 m.; fair movement after 17 m.; has ceased by 23 m. (17 m.).
Active movement after 7 m.; a little movement after 21 m.; no apparent coagulation (21 m.).	No movement. Swelling is well marked after 7 m.	Considerable swelling after 7 m. No movement.	Less favorable than $\frac{m}{500}$ . Movement active at first; almost ceased after 25 m. (25 m.).	Generally similar to $\frac{m}{400}$ HCl. (12 m. +).
Active at first; no coagulation; like $\frac{m}{1000}$ HCl (21 m. +).	Similar to preceding solution.	A little movement remains after 7 m.; considerable swelling (7 m. +).	Less favorable than $\frac{m}{1000}$ ; almost ceased after 14 m. (14 m. +).	Like $\frac{m}{400}$ HCl. (17 m.).
Active at first; somewhat less favorable than $\frac{m}{3200}$ HCl. Swelling is more rapid. (21 m.).	Similar to preceding solution.	No movement after 7 m.; swelling is marked.	Almost ceased after 14 m.; similar to pure solution (14 m.).	Less favorable than $\frac{m}{3200}$ HCl. (6 m. +).
Active at first. Less favorable than $\frac{m}{1400}$ HCl. (7 m. +).	Similar to preceding solution.	Similar to $\frac{m}{8400}$ HCl.	Similar to $\frac{m}{8400}$ HCl.	

without being quite prevented. Complete prevention of swelling usually implies coagulation, which is destructive of activity. In both series (as in that of each salt of the above table) there is a gradation in the rate and degree of swelling, both increasing as the concentration of acid decreases; the most evident action of the acid is thus to prevent or retard this change.

A number of experiments were made with other salts. In the case of  $\frac{m}{2}$   $\text{Na}_2\text{S}_2\text{O}_3$  fairly active movement remained after sixteen minutes, after addition of  $\frac{m}{1600}$  HCl; and a little activity remained in  $\frac{m}{3200}$  HCl. With lower and higher concentrations of acid movement ceased within a much shorter space of time. In a similar series with  $\frac{m}{2}$   $\text{Na}_2\text{SO}_3$  a distinctly favorable effect was seen with  $\frac{m}{800}$  HCl in which considerable movement remained after fifteen minutes; in other concentrations ( $\frac{m}{1600}$  and  $\frac{m}{3200}$ ) a slight antitoxic action was also seen.

In the case of sodium tartrate no distinct action was seen with HCl in the above concentrations. The reason for this is evidently the slight dissociability of tartaric acid, hence the addition of a relatively large quantity of HCl should theoretically be necessary to increase the concentration of H-ions sufficiently for antitoxic action. In another series with the same salt, in which HCl was added in proportions ranging from  $\frac{m}{25}$  to  $\frac{m}{200}$  evidence of antitoxic action was seen at  $\frac{m}{50}$  and  $\frac{m}{100}$ , though the effect was not marked. With  $\frac{m}{2}$  sodium citrate similar conditions were found. In the pure solution movement had ceased within six minutes; upon addition of HCl to  $\frac{m}{25}$  and  $\frac{m}{50}$  movement was active after this interval and remained for seventeen minutes in the first and for more than twelve minutes in the second instance. The antitoxic action of acid thus proved slight with both these salts.

Addition of HCl in larger quantities than those indicated in the table was also tried with the salts  $\text{NaBrO}_3$  and  $\text{NaCOOCH}_3$  which in the above tabulated experiments showed most favorable action with the higher concentrations of acid. In a second series with  $\frac{m}{2}$   $\text{NaBrO}_3$  similar to the above, with HCl in concentrations from  $\frac{m}{50}$  to  $\frac{m}{12800}$ , a distinct action was found with  $\frac{m}{800}$  HCl, in which a little movement remained after thirteen minutes. On addition of more HCl (a third series ranging from  $\frac{m}{200}$  to  $\frac{m}{800}$  HCl) the most favorable action was found at  $\frac{m}{400}$  HCl, in which solution vigorous and energetic movement lasted for several minutes; at  $\frac{m}{200}$  the filaments were coagulated; at  $\frac{m}{300}$  the movement, active after two minutes, had

ceased by nine minutes; there was no coagulation in this last solution and the swelling, although greatly checked, was considerable after four hours. Here again the best concentration was that just sufficient to check swelling without preventing it altogether.

With  $\frac{m}{2}$  NaCOOCH<sub>3</sub> in a second series with HCl ranging from  $\frac{m}{800}$  to  $\frac{m}{12800}$  the most favorable action was again found at  $\frac{m}{800}$  (a little movement after sixty-nine minutes);  $\frac{m}{1600}$  HCl was somewhat less favorable (a little movement after fifty-two minutes) and the others still less so, as in the above series. In another series of three solutions with more HCl movement was not prolonged at  $\frac{m}{100}$  HCl, was prolonged slightly, if at all, at  $\frac{m}{200}$ , but very considerably at  $\frac{m}{400}$ , where it continued for about an hour (sixteen minutes was maximum duration in pure  $\frac{m}{2}$  NaCOOCH<sub>3</sub>). With  $\frac{m}{25}$  and  $\frac{m}{50}$  HCl the filaments are gradually coagulated. The most favorable concentration of HCl in the case of this salt appears thus to range from  $\frac{m}{400}$  to  $\frac{m}{800}$ . This is again a concentration somewhat less than that required to prevent swelling or produce coagulation.

Addition of HCl (from  $\frac{m}{200}$  to  $\frac{m}{6400}$ ) to  $\frac{m}{2}$  solutions of Na<sub>4</sub>Fe(CN)<sub>6</sub>, Na<sub>2</sub>HPO<sub>4</sub>, and Na<sub>2</sub>HAsO<sub>4</sub> failed to produce any evident antitoxic effect. The filaments remained uncoagulated in all these solutions, an effect to be expected when the strongly marked action of the anions and the weak nature of their acids are considered.

The general results of the foregoing experiments on antitoxic action of acid may be conveniently summarized in the following table, in which opposite the formula of each salt is given the approximate quantity of HCl whose addition to the  $\frac{m}{2}$  solution produces the most favorable antitoxic action.

TABLE XIV.

NaCl . . . . .	Best effect at $\frac{m}{8100}$ to $\frac{m}{25800}$ .
NaBr . . . . .	Best effect at $\frac{m}{3200}$ to $\frac{m}{15800}$ .
NaI . . . . .	Best effect at $\frac{m}{3200}$ to $\frac{m}{8100}$ .
NaNO <sub>3</sub> . . . . .	Best effect at $\frac{m}{3200}$ to $\frac{m}{6400}$ .
NaClO <sub>3</sub> . . . . .	Best effect at $\frac{m}{3200}$ to $\frac{m}{400}$ .
NaBrO <sub>3</sub> . . . . .	Slight action at $\frac{m}{100}$ to $\frac{m}{800}$ .
NaCOOCH <sub>3</sub> . . . . .	Most favorable at $\frac{m}{100}$ to $\frac{m}{800}$ .
NaCNS . . . . .	Slight action at $\frac{m}{3200}$ .
Na <sub>2</sub> SO <sub>4</sub> . . . . .	Most favorable at $\frac{m}{800}$ to $\frac{m}{3200}$ .
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> . . . . .	Most favorable at $\frac{m}{1600}$ to $\frac{m}{3200}$ .
Na <sub>2</sub> SO <sub>3</sub> . . . . .	Some favorable action at $\frac{m}{800}$ .
Na <sub>2</sub> C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> . . . . .	Slight action at $\frac{m}{80}$ to $\frac{m}{100}$ .
Na <sub>3</sub> C <sub>6</sub> H <sub>7</sub> O <sub>5</sub> . . . . .	Slight action at $\frac{m}{25}$ to $\frac{m}{50}$ .
Na <sub>2</sub> HPO <sub>4</sub> . . . . .	No effect seen.
Na <sub>2</sub> HAsO <sub>4</sub> . . . . .	No effect seen.
Na <sub>4</sub> Fe(CN) <sub>6</sub> . . . . .	No effect seen.

The addition of a trace of acid is thus seen to retard, in some instances very considerably, the injurious action of pure solutions of most of the above sodium salts. The more toxic of these are only slightly susceptible to this action — as  $\text{NaBrO}_3$ ,  $\text{NaCNS}$  — or apparently altogether insusceptible — as  $\text{Na}_2\text{HPO}_4$ ,  $\text{Na}_2\text{HAsO}_4$  or  $\text{Na}_4\text{Fe}(\text{CN})_6$ . It is important to note the wide variation in the quantity of acid necessary for distinct antitoxic action with the different salts; with salts of strong acids ( $\text{NaCl}$ ,  $\text{NaBr}$ ,  $\text{NaI}$ ,  $\text{NaNO}_3$ ) the optimum action is obtained on addition of comparatively little of the antagonizing acid ranging from  $\frac{m}{3200}$  to  $\frac{m}{25600}$ . On the other hand, with salts of weak acids (acetate, tartrate, bromate, citrate, sulphite) the requisite concentration of H-ions — the effective agency in the action — can only be secured by the addition of larger quantities of  $\text{HCl}$ ; the reason of this is the production of the corresponding acid, which, being slightly dissociable, especially in the presence of a high concentration of its anions from the salt, must be present in relatively large quantity to give the required concentration of H-ions. In the case of salts of strong acids it is the physico-chemical characteristics of the anion that determine the quantity of H-ions necessary to offset the poisonous action. Thus salts of monobasic acids of practically equal strength ( $\text{NaCl}$ ,  $\text{NaBr}$ ,  $\text{NaI}$ ,  $\text{NaNO}_3$ ,  $\text{NaCNS}$ ) show variations in the quantity of acid needed for optimum action, this being least in  $\text{NaCl}$  ( $\frac{m}{6400}$  to  $\frac{m}{25600}$   $\text{HCl}$ ) and greatest in  $\text{NaCNS}$ , where no antitoxic action is seen at concentrations below  $\frac{m}{3200}$   $\text{HCl}$ : the action is slight with this salt at the best. The explanation of this fact is evidently that the physiologically more active anions require the addition of larger quantities of the oppositely acting H-ions for counteraction of their toxicity.  $\text{NaBr}$  and  $\text{NaNO}_3$ , and especially  $\text{NaI}$  and  $\text{NaCNS}$  have much more rapid action than  $\text{NaCl}$  in inducing swelling and disintegration of the ciliated cells. This is probably due to their anions having lower decomposition tensions than  $\text{Cl}$ . It seems probable that increase in valence of the anion may in itself increase its physiological activity; thus in both  $\text{Na}_2\text{SO}_4$  and  $\text{Na}_2\text{S}_2\text{O}_3$ , salts of strong acids, the optimum proportion of  $\text{HCl}$  is relatively high. In any case the antitoxic cation (in this case the H-ion) appears to act by exercising an action of opposite character to that of the anion; hence the greater the physiological activity of the anion of the toxic salt, the more acid must be required for the most effective antitoxic action.

VI. ANTITOXIC ACTION OF PLURIVALENT CATIONS.

In the antitoxic action of trivalent and tetravalent cations (Al, Cr, Fe<sup>III</sup>, Th, Sn<sup>IV</sup>) on solutions of sodium salts similar phenomena are observed. Salts of such metals induce coagulation when present in more than very dilute concentrations. A concentration insufficient to cause coagulation, but sufficient markedly to retard swelling, is here also most favorable for antitoxic action. Other factors appear also to enter, as in the case of the alkali earth cations, since the favorable action is greater than can be secured by mere addition of acid.

TABLE XV.

SALTS ADDED TO  $\frac{m}{2}$  NaCl IN THE PROPORTIONS INDICATED.

Concentration of antitoxic salt.	AlCl <sub>3</sub> .	$\frac{Cr_2(SO_4)_3}{2}$ .	FeCl <sub>3</sub> .
$\frac{m}{800}$	Movement ceases at once; filaments coagulate within an hour.	No movement; filaments coagulate within an hour.	Filaments coagulate soon; no movements.
$\frac{m}{1600}$	Movement ceases soon; gradual coagulation.	Similar to preceding solution.	Similar to $\frac{m}{800}$ FeCl <sub>3</sub> .
$\frac{m}{3200}$	Filaments do not coagulate; show considerable swelling after 22 h.; some movement after 7 h. 35 m. (7 h. 35 m.).	A little movement after 25 m.; only slight swelling after 22 h. (25 m.).	Filaments coagulate within 25 m.; no movement.
$\frac{m}{6400}$	Considerable swelling after 3 h.; a little movement (3 h.).	Swelling is slight after 20 h. quite active movement after 2 h. 53 m. (3 h. +).	Filaments are coagulated after 25 m.
$\frac{m}{12800}$	Considerable swelling after 2 h.; a little movement (2 h.).	Considerable swelling after 20 h.; some cilia remain active after 7 h. 28 m. (7 h. 28 m. +).	Filaments coagulate slowly; are coagulated after 22 h.; no movement seen.
$\frac{m}{25600}$	Similar to $\frac{m}{12800}$ AlCl <sub>3</sub> (2 h.).	Good many cells are detached after 20 h.; a little movement remains after 22 h. (22 h.).	Slight swelling after 22 h.; active movement at first; movement lasts ca. 2 h. (2 h.).
$\frac{m}{51200}$	A little movement after 52 m. (52 m.).	Disintegration of filaments is well advanced after 20 h.; a little movement remains after 7 h. 29 m. (7 h. 29 m.).	Swelling after 22 h. is greater than in $\frac{m}{25600}$ FeCl <sub>3</sub> ; active movement at first; lasts more than 7 h. (7 h. 26 m.).
$\frac{m}{102400}$	Considerable swelling after 52 m.; slight movement (52 m.).	Well-marked disintegration after 20 h.; movement has almost ceased after 2 h. 53 m. (2 h. 53 m.).	After 22 h. disintegration is greater than in $\frac{m}{51200}$ FeCl <sub>3</sub> ; considerable movement remains after 2 h. 50 m. (2 h. 50 m.).

Of the above three trivalent cations  $\text{Fe}^{+++}$  exceeds Cr in coagulative activity and Cr exceeds Al; the most favorable concentration for antitoxic action is thus higher with Al than with Cr, and with Cr than with  $\text{Fe}^{+++}$ . The high antitoxic effectiveness in such low concentrations is partly a function of the trivalence; yet the difference between the individual cations proves that another factor, or set of factors, is important. Of these the chief is in all likelihood the decomposition tension, which, according to Ostwald and Wilsmore, is comparatively high for Al. I have at present no exact data for Cr and Fe; the physiological indication is that Cr has a lower decomposition voltage than Al, and  $\text{Fe}^{+++}$  than Cr. The value for  $\text{Fe}^{+++}$ , according to Le Blanc, lies about 0.28 volt lower than  $\text{Fe}^{++}$ , which is +0.063, *i. e., ca.* -0.217, a value like that of the H-ion.<sup>1</sup>

In a similar series with  $\frac{m}{2}$   $\text{Na}_2\text{SO}_4$  less favorable action was obtained, and the optimum concentrations of the antagonizing cation were in all cases higher than with  $\frac{m}{2}$  NaCl. These were with Al:  $\frac{m}{1600}$  (movement lasted 51 m.); Cr:  $\frac{m}{800}$  to  $\frac{m}{1600}$  (movement lasted 5 h. 20 m. and 51 m. + respectively);  $\text{Fe}^{+++}$ :  $\frac{m}{12800}$  (movement for 50 m.). The prevention of swelling also required a higher concentration of the antagonizing cation than in the case of NaCl. These differences are apparently correlated with the more energetic activity of the bivalent  $\text{SO}_4$ -ion, which requires for counteraction higher concentrations of the opposed cation. This is another instance of a rule which has already been exemplified in the experiments on the antitoxic action of acid.

With salts of the tetravalent metals thorium and tin (in stannic compounds) similar results were gained, though a less favorable antitoxic action was found.  $\text{Th}(\text{NO}_3)_4$  and  $\text{SnCl}_4$  were added to solutions of NaCl and  $\text{Na}_2\text{SO}_4$  in the same graded proportions as in the above tabulated series.  $\text{Th}(\text{NO}_3)_4$  exhibited a favorable action in  $\frac{1}{10}$  m solutions of NaCl at concentrations of  $\frac{m}{12800}$  to  $\frac{m}{102400}$ , in all of which proportions movement lasted for rather more than an hour. With  $\text{SnCl}_4$  a similar action was found in concentrations of  $\frac{m}{25600}$  to  $\frac{m}{102400}$ , movement lasting from thirty to forty-five minutes in these solutions. With  $\text{Na}_2\text{SO}_4$  as the toxic salt, it was not possible to prolong movement to the same degree, and the results were less decisive. With  $\frac{m}{2}$   $\text{Na}_2\text{SO}_4$  and  $\text{SnCl}_4$  in concentrations of  $\frac{m}{6400}$  and  $\frac{m}{12800}$ , movement lasted for thirty-four and fifty-three minutes respectively, — an undoubted antitoxic action. It is again to be noted that the optimum

<sup>1</sup> Cf. LE BLANC: Elektrochemie, 3t Aufl., 1903, p. 225.

concentration is greater than with NaCl. We also see here again the characteristic effectiveness of plurivalent cations in extremely low dilutions. Th and Sn<sup>IV</sup> resemble Fe<sup>III</sup> rather than Cr or Al, their most favorable action being manifested in lower dilution than with the last two cations. It should be added that their action in preventing swelling shows the same peculiarities as that of the trivalent cations.

**Antitoxic action of chromates and permanganates.** — The coagulative action of chromates, permanganates, tungstates, and other similarly constituted salts, especially in acid solution, is evidently dependent on the anion part of the molecule. There is little doubt, however, that these salts in solution undergo a somewhat complex dissociation, and give rise to the plurivalent cations Mn, Cr, W; there is good reason to ascribe the typical coagulative and oxidative action to the presence of these ions.<sup>1</sup> According to the conception of antitoxic

TABLE XVI.  
SOLUTION: PURE  $\frac{1}{10}$  M NaCl.

Concentration of added antitoxic salt.	K <sub>2</sub> CrO <sub>4</sub> .	KMnO <sub>4</sub> .
1. Pure $\frac{1}{10}$ NaCl	Movement has almost ceased after 13 m.; cells are beginning to detach after 23 m.; movement has then practically ceased (23 m.).	
$\frac{m}{10000}$	A little movement after 24 m.; no coagulation; swelling is more gradual than in pure solution (27 m.).	A trace of movement after 15 m.; filaments are discolored and unswollen after 20 h. (15 m.).
$\frac{m}{25000}$	A little movement after 34 m.; swelling is more rapid than in $\frac{m}{10000}$ K <sub>2</sub> CrO <sub>4</sub> (37 m.).	Active movement at first; has ceased by 15 h.; filaments are almost unswollen after 20 h. (ca. 15 m.).
$\frac{m}{40000}$	A little movement after 1 h. 27 m.; considerable swelling (1 h. 27 m.).	Movement active at first; a little remains after 44 m.; swelling is considerable after 20 h., though less than in control (47 m.).
$\frac{m}{50000}$	Active movement at first, a little movement remains after 2 h. 52 m. (2 h. 52 m.).	A little movement after 33 m.; swelling as in preceding solution (33 m.).
$\frac{m}{100000}$	A little movement remains after 49 m. (49 m.).	A little movement after 45 m.; swelling considerable after 20 h. (45 m.).
$\frac{m}{250000}$	A little movement remains after 35 m. (35 m.).	A little movement after 33 m. (33 m.).

<sup>1</sup> Cf. MATHEWS: This journal, 1904, xi, p. 237.

action advocated above, such salts, when added to solutions of Na-salts, should exhibit antitoxic action in extremely low dilution. I have tested this in the case of potassium permanganate and potassium chromate with the results indicated in the foregoing table.

The action is more distinct with  $K_2CrO_4$ , which has a weaker coagulative action in the dilutions employed. Both salts show definite action at dilutions of  $\frac{1}{160000}$ , or even  $\frac{1}{320000}$ . We may hold that the presence of powerfully acting cations is indicated by these experiments, which may accordingly be regarded as confirming the view that the plurivalent Cr- and Mn-ions (hypothetically hexa- and heptavalent respectively) are among the products of dissociation. Ions of such high valence should be expected to exhibit a most powerful physiological action; their active oxidizing properties are no doubt dependent on this peculiarity, which almost necessarily involves a very low decomposition voltage.

## VII. SWELLING ACTION OF ANIONS.

The following records are given to indicate the comparative activity of the various anions in promoting absorption of water in solutions of their sodium salts; afterwards the minimum concentration of acid needed to prevent or markedly to retard this action is given. It will be seen, as already mentioned, that in general the concentration of acid at which antitoxic action is most favorable coincides approximately with that needed to produce a marked retardation of the swelling action yet without producing coagulation.

Swelling phenomena are conspicuous in the action of pure isotonic solutions of sodium salts; salts of other alkali metals exhibit the same action, as do also, to a less degree, solutions of calcium, magnesium, and barium chlorides among the alkali earth salts. In the above sodium salts the rapidity of this action varies greatly in the different solutions. The visible effect is a rounding and eventually a detachment of the ciliated cells. Thus the epithelium of a gill filament that has lain for some time (*e. g.*, one hour) in (*e. g.*)  $\frac{m}{2}$  a NaBr solution is found to be largely transformed into a mass of rounded, partially detached, and loosely cohering cells. Later the cells undergo still further swelling, which in course of time leads to complete disintegration. These effects are produced still more rapidly in solutions of NaI, while NaCl is less active than either.

The nature of the phenomenon and its variation in different salts



is best indicated in the accompanying table, which is summarized from the results of a large number of independent observations. It thus appears that the degree of swelling action is constant and specific for each salt, and is dependent on the peculiarities of its anion. In the series with K-salts the relative activities of the various salts in producing swelling proved practically identical with those given here for Na-salts.

TABLE XVII.

1.  $\frac{m}{2}$  NaCl . . . Usually the cells are sufficiently swollen within an hour to render the contour of the filament uneven from the projecting cells, a few of which may have detached themselves by then; this rounding and detachment are, as a rule, not distinct until several hours later. Swelling and detachment are well marked after 20 to 24 h.
2.  $\frac{m}{2}$  NaBr . . . Swelling is decidedly more rapid than with NaCl, and numerous cells are rounded and detached after an hour; in 5 or 6 h. the filament takes the appearance of a relatively voluminous mass of detached cells. Disintegration is very complete after 24 h.
3.  $\frac{m}{2}$  NaI . . . Swelling is decidedly more rapid than with NaBr, rounding and detachment being conspicuous within 30 m. or less; within 2 or 3 h. the epithelium is a voluminous mass of partly disintegrated swollen detached cells. After 7 or 8 h. the filament has the appearance of a swollen and almost structureless mass of disintegrated cells.
4.  $\frac{m}{2}$  NaNO<sub>3</sub> . . . Swelling is well marked after 30 m.; the action is more rapid than with NaCl, and less so than with NaBr; rounding and detachment of cells take place as in NaBr, but more gradually.
5.  $\frac{m}{2}$  NaClO<sub>3</sub> . . . Is very similar to NaNO<sub>3</sub> in its action, — intermediate between NaCl and NaBr.
6.  $\frac{m}{2}$  NaBrO<sub>3</sub> . . . Swelling is very rapid; marked swelling and detachment and partial disintegration have taken place within 15 m.; disintegration is more rapid than with  $\frac{m}{2}$  NaI.
7.  $\frac{m}{2}$  NaCNS . . . Marked swelling and detachment of cells after 15 m.; swelling is very rapid; after 5 h. filaments are voluminous masses of swollen, detached, and disintegrated cells. Like NaBrO<sub>3</sub> in its action.
8.  $\frac{m}{2}$  NaNO<sub>2</sub> . . . Apparently similar to NaBr in its action; rather more active than NaNO<sub>3</sub> (2 observations).
9. NaCOOCH<sub>3</sub> . . . Exhibits relatively slight swelling activity; is decidedly less active than NaCl, — swelling is not conspicuous for some hours. After 20 h. there is considerable rounding and detachment, though always less than with NaCl.
10.  $\frac{m}{2}$  Na<sub>2</sub>SO<sub>4</sub> . . . Swelling action is in general similar to that of NaBr, though less energetic. Disintegration is marked after 20 h.
11.  $\frac{m}{2}$  Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> . . . Action is in general like that of Na<sub>2</sub>SO<sub>4</sub>.
12.  $\frac{m}{2}$  Na<sub>2</sub>SO<sub>8</sub> . . . Swelling action appears relatively weak, — less than that of Na<sub>2</sub>SO<sub>4</sub> (2 observations).

TABLE XVII (continued).

13.  $\frac{m}{2}$   $\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$ . Very slight swelling action (less than  $\text{NaCOOCH}_3$ ); after 20 h. filament as a rule almost smooth with few or no detached cells.
14.  $\frac{m}{2}$   $\text{Na}_3$  citrate. Almost no swelling occurs, the contour of the filament remaining smooth after 24 h. or more.
15.  $\text{Na}_2\text{HPO}_4$ . Similar to citrate; little or no swelling action.
16.  $\text{Na}_2\text{HAsO}_4$ . Similar to  $\text{Na}_2\text{HPO}_4$ .
17.  $\text{Na}_3\text{Fe}(\text{CN})_6$ . Practically no swelling after 24 h. or more.
18.  $\text{Na}_4\text{Fe}(\text{CN})_6$ . Practically no swelling action.

The following table gives records of a number of experiments with various chiefly monovalent sodium salts in which the concentration of acid just necessary to prevent swelling was approximately determined. Higher concentrations produce coagulation (cf. Table XII). It will be seen that acid of half this concentration has well-marked, and in some instances the optimum, antitoxic action.

TABLE XVIII.

1.  $\frac{m}{2}$   $\text{NaCl}$ . . .  $\frac{m}{6100}$   $\text{HCl}$  prevents swelling and slowly coagulates the filaments. In  $\frac{m}{12400}$   $\text{HCl}$  swelling is just prevented; movement lasts 28 m. After 19 h. filament retains smooth contour. Cells are unswollen; filaments are slightly whitish. In  $\frac{m}{25000}$   $\text{HCl}$  cells swell, though gradually; movement lasts 66 m.; after 19 h. there is considerable swelling. (In the series on p. 113 the most favorable concentration for movement was *ca.*  $\frac{m}{100000}$   $\text{HCl}$  ( $\frac{m}{100000}$ ), where swelling was slight after 17 h. In  $\frac{m}{11111}$   $\text{HCl}$  swelling was entirely prevented.)
2.  $\frac{m}{2}$   $\text{NaBr}$ . . .  $\frac{m}{1000}$   $\text{HCl}$  coagulates slowly.  $\frac{m}{10000}$   $\text{HCl}$  almost prevents swelling, filaments remaining translucent and almost smooth in contour after 19 h. Movement lasts for more than 7 m. and less than 21 m. In  $\frac{m}{32000}$   $\text{HCl}$  swelling is gradual; is considerable after 19 h. Movement lasts 65 m.
3.  $\frac{m}{2}$   $\text{NaI}$ . . .  $\frac{m}{800}$   $\text{HCl}$  coagulates slowly;  $\frac{m}{10000}$  seems almost the neutral point, filaments are unswollen after 18 h.; at  $\frac{m}{3200}$  there is slight swelling after 19 h.; movement is favored ( $> 20$  m.).  $\frac{m}{400}$   $\text{HCl}$  is most favorable; swelling, though much retarded, is more rapid than in  $\frac{m}{3200}$ ; cells are much swollen after 19 h. Movement lasts more than 65 m.
4.  $\frac{m}{2}$   $\text{NaNO}_3$ . . .  $\frac{m}{10000}$   $\text{HCl}$  slowly coagulates filaments which are white, opaque, and unswollen after 18 h.  $\frac{m}{32000}$   $\text{HCl}$  almost prevents swelling; a little swelling is evident after 18 h. Movement lasts more than 30 m. in this solution. In  $\frac{m}{11000}$   $\text{HCl}$  swelling is more rapid; movement is favored, but less so than in  $\frac{m}{3200}$   $\text{HCl}$ . In  $\frac{m}{125000}$  swelling is still more rapid.
5.  $\frac{m}{2}$   $\text{NaClO}_3$ . . .  $\frac{1m}{800}$   $\text{HCl}$  coagulates filaments within 7 m.  $\frac{m}{10000}$   $\text{HCl}$  does not coagulate, but retards swelling greatly; latter is fairly well marked after 18 h. Movement lasts 21 m. In  $\frac{m}{3200}$  and  $\frac{m}{400}$   $\text{HCl}$  conditions are more favorable for movement, though swelling is more rapid.

TABLE XVIII (continued).

6.  $\frac{m}{2}$   $\text{NaBiO}_3$  . . .  $\frac{m}{200}$  HCl coagulates gradually;  $\frac{m}{300}$  HCl almost prevents swelling; filaments are unswollen after 1 h., though considerably swollen after 4 h.; in  $\frac{m}{100}$  HCl swelling is more rapid, though greatly retarded as compared with the pure solution; this concentration seems most favorable for movement which remains fairly active after 7 m.
7.  $\frac{m}{2}$   $\text{NaCNS}$  . . .  $\frac{m}{800}$  HCl coagulates slowly; in  $\frac{m}{1000}$  HCl filaments swell gradually: in  $\frac{m}{3200}$  with fair rapidity; movement is a little prolonged at this concentration. Swelling in the pure solution is very rapid. (A second trial gave the same result;  $\frac{m}{800}$  HCl prevents swelling; in  $\frac{m}{1000}$  HCl gradual swelling occurs.)
8.  $\frac{m}{2}$   $\text{NaCOOCH}_3$   $\frac{m}{20}$  HCl gradually coagulates; at  $\frac{m}{100}$  HCl filaments swell gradually, movement, however, was not prolonged in this series; at  $\frac{m}{200}$  and  $\frac{m}{400}$  movement is prolonged, especially at latter concentration (35 m. +).  $\frac{m}{800}$  HCl also prolongs movement (35 m. +).
9.  $\frac{m}{2}$   $\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$   $\frac{m}{25}$  HCl was not sufficient to coagulate filaments. So also with citrate; there was slight antitoxic action at  $\frac{m}{50}$  and  $\frac{m}{100}$  HCl.
10.  $\frac{m}{2}$   $\text{Na}_2\text{SO}_4$  . . .  $\frac{m}{100}$  coagulates gradually;  $\frac{m}{800}$  almost prevents swelling — a few cells are detached after 22 h.; in  $\frac{m}{1000}$  there is slow swelling. Movement is most favored at  $\frac{m}{800}$  and  $\frac{m}{1000}$  (ca. 17 m.). In  $\frac{m}{2}$   $\text{Na}_2\text{S}_2\text{O}_3$   $\frac{m}{800}$  HCl also practically prevents swelling without coagulating; in  $\frac{m}{1000}$  swelling is considerable after 18 h.; this is the most favorable concentration for movement (see Table XIII).

Thus the general result appears that a concentration of acid sufficient markedly to retard swelling, but insufficient to produce coagulation of the cell-proteids, is in almost all instances that which has most effect in prolonging movement. The "antitoxic" action is in fact to be regarded as largely due to the retarding influence which the H-ions exercise on the disintegrating or liquefactive tendency of the anion of the toxic salt. Thus we see that the more powerful the action of these anions, the higher the concentration of H-ions necessary to counteract their action, — *i. e.*, the more acid must be added to produce the most favorable antitoxic effect. This is seen most clearly in the series of salts with monovalent anions. The order of increasing effectiveness in producing swelling — which is to be regarded as indicating the relative activity of the anions (these being present in  $\frac{m}{2}$  solutions in practically the same concentration, since the dissociation of such salts is almost equal) — is substantially as follows:  $\text{COOCH}_3$ , Cl,  $\text{NO}_3$ ,  $\text{ClO}_3$ , Br, I, CNS, and  $\text{BrO}_3$ ; I,  $\text{BrO}_3$ , and CNS are closely similar in their behavior.  $\text{COOCH}_3$  is less active, and  $\text{NO}_3$  and  $\text{ClO}_3$  are somewhat more active than Cl. Accordingly, in order to neutralize the swelling tendency, or to procure distinct antitoxic action, Cl requires least acid,  $\text{ClO}_3$  and  $\text{NO}_3$  somewhat more,

Br somewhat more than these, I apparently about the same as Br (perhaps a little more), and CNS rather more than I. The quantity of HCl added practically represents in the case of the majority of these salts the concentration of H-ions in the solution, since the corresponding acids are all strong except  $\text{HBrO}_3$  and  $\text{HCOOCH}_3$ . In the case of  $\text{NaBrO}_3$  and  $\text{NaCOOCH}_3$  the resultant acid dissociates slightly, and hence more HCl must be added to produce a sufficient concentration of H-ions. This is seen also with salts of weak organic acids, as tartrate and citrate. With salts of acids of approximately equal strength the degree of physiological activity exhibited by the anion is what determines the proportion of acid or of other salt (*e. g.*,  $\text{MgCl}_2$ ; see above, p. 108) needed for antitoxic action.

Antitoxic action with acid is found in solutions of sodium salts and possibly also in those of lithium, though I have as yet few data on the behavior of Li-salts. Conditions are different in those of potassium: thus it was found that addition of alkali or acid to pure  $\frac{m}{2}$  KCl failed, in any of the concentrations employed, to increase the favorability of the solution. This result agrees with that mentioned above for experiments with mixtures of magnesium and potassium salts.

**Nature of antitoxic action with other salts.** — Is the antitoxic action of other cations also due to their counteracting the swelling action of the anions? It seems doubtful that the favorable action can be due to his factor alone, since otherwise it would be difficult to explain why the H-ion, which in appropriate concentrations may completely prevent swelling without causing coagulation, has only a limited antitoxic action. It is possible that acid may be injurious on account of its checking respiration or otherwise interfering with the metabolic processes concerned in the production of the energy required for the movement. But in all likelihood there exist (*e. g.*) a pure  $\frac{m}{2}$  NaCl solution several independently injurious conditions, of which the disruptive tendency due to swelling is only one. If this can be checked, the result is favorable, as we have seen; yet the totality of conditions may remain injurious to the tissue and so unfavorable to movement. The heavy metal and the trivalent and tetravalent cations appear also to owe a large part of their antitoxic efficiency to their action in retarding swelling (see Table XIX), and the mechanism of their action is probably in large part similar to that of the H-ion. In the concentrations in which these salts have the most effective antitoxic action, swelling and detachment of cells are greatly retarded (as compared with the pure  $\frac{m}{2}$  NaCl), although

not entirely prevented; and a concentration sufficient to coagulate the filaments is always too high for antitoxic action. The same relation holds in part as with the H-ion; this was especially evident with the trivalent and tetravalent metals and the salts of Cd, Zn, Ni, Co, Pb, Mn in the experiments of Tables V, VI, and XV above.

In the case of the alkali earth metals and magnesium, whose salts are by far the most effective in antitoxic action, the characteristically high degree of favorability must be due largely to some other factor or factors, since even pure  $\frac{m}{2}$  solutions of the chlorides induce swelling, though more slowly than the salts of the alkali metals. In favorable mixtures of  $\frac{m}{2}$  NaCl and an alkali earth chloride (of Be, Mg, Ca, Ba, or Sr) swelling is greatly retarded and detachment of cells is gradual, the filament retaining smooth contour and normal appearance for at least several hours, while the cilia remain vigorously active. Nevertheless the mere prevention of swelling is probably a subsidiary factor here; for even in favorable mixtures of  $\frac{m}{2}$  NaCl and  $\frac{m}{2}$  MgCl<sub>2</sub> numerous cells become rounded and detached in the course of a few hours; notwithstanding this, many of these cells exhibit active ciliary movement while floating freely in the solution. Apparently some other peculiarity of action is responsible for the high antitoxic efficiency of these cations. What this is, is at present difficult to say: the ciliary substance is in some manner enabled to preserve a state of physical consistency (of possibly special permeability or non-permeability to certain ions) favorable to contraction.

In the series of Tables IX and X (various  $\frac{m}{2}$  Na-salts with  $\frac{m}{2.5}$  and  $\frac{m}{5.0}$  MgCl<sub>2</sub>) swelling was greatly retarded in all the solutions containing MgCl<sub>2</sub> relatively to the pure solution; yet the characteristic differences in the swelling action of the various anions appeared as before. Numerical comparisons are hardly possible with data of this kind; but the solutions admit of a classification on the basis of their unequal swelling action somewhat as follows: (1) those in which swelling was either absent or slight, only a few cells at most being detached after twenty-four hours; this class includes all of the salts with plurivalent anions, even Na<sub>2</sub>SO<sub>4</sub> — tartrate, sulphite, thiosulphate, citrate, phosphate, arsenate, ferrocyanide, ferricyanide — acetate and chloride; (2) salts in whose solutions a considerable but not large number of cells are detached in the same time, — nitrate, chlorate, bromide; and (3) salts which produce marked swelling so that disintegration is rapid and a voluminous mass of detached cells is formed,

— iodide, sulphocyanate, bromate; of these bromate seems most effective. The order of effectiveness in producing swelling is thus practically the same as that observed in pure solutions of potassium and sodium salts with various anions (*cf.* Table XXI, p. 136), although the process itself is greatly retarded by the action of the antitoxic salt.

The general order of effectiveness of anions in producing swelling of the living cells, as deduced from the above and the earlier series of experiments, is essentially as follows (order of increasing action): fluoride, the above inactive plurivalent anions,<sup>1</sup> tartrate, acetate, chloride, nitrate, chlorate, sulphate, bromide, iodide, sulphocyanate, bromate. It is noteworthy that this order corresponds closely with that given by Hofmeister and by Pauli for the relative effectiveness of anions in furthering the swelling of gelatine plates,<sup>2</sup> in raising the melting-point of gelatine solutions,<sup>3</sup> in anti-precipitating action (in solutions of alkali salts) on proteid solutions,<sup>4</sup> and in furthering proteid precipitation in solutions of alkaline earth and heavy metal salts<sup>5</sup> (*i. e.*, when the precipitating salt is such as to give the colloid particles a positive charge<sup>6</sup>). Pauli gives the following series as indicating the order of activity of the anions in these relations: fluoride < sulphate < phosphate < citrate < tartrate < acetate < chloride < nitrate < chlorate < bromide < iodide < sulphocyanate. Bromate, according to my determinations, comes after sulphocyanate. In the case of the salts with monovalent anions the above order of toxicity corresponds closely with the order of activity in producing swelling of the ciliated cells; this fact indicates that the toxic effect of these salts is largely dependent on this latter action. The case of the salts with trivalent

<sup>1</sup> The relative inactivity of the salts with polybasic acid is possibly due largely to the presence of relatively few plurivalent anions in  $\frac{m}{2}$  solution, dissociation being chiefly into (*e. g.*)  $\text{Na}^+$  and  $\text{Na}_2\text{C}_6\text{H}_5\text{O}_7^-$ ,  $\text{Na}^+$  and  $\text{NaHPO}_4$ , etc., these anions being in all likelihood relatively inactive, or having difficulty in penetrating the cell.

<sup>2</sup> HOFMEISTER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1890, xxvii, p. 395; and *Ibid.*, 1891, xxviii, p. 210.

<sup>3</sup> PAULI and RONA: *Beiträge zur chemischen Physiologie und Pathologie*, 1902, ii, p. 1.

<sup>4</sup> PAULI: *Ibid.*, 1902, iii, p. 225.

<sup>5</sup> PAULI: *Ibid.*, 1903, v, p. 27; and 1905, vi, p. 233. See also POSTERNAK, who obtained a similar order of effectiveness of anions in the precipitation of positive proteid hydrosols: *Annales de l'Institut Pasteur*, xv, 1901, p. 85.

<sup>6</sup> *Cf.* BILLITZER: *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Klasse*, 1904, cxiii, p. 1201.

anions shows, however, that toxicity may be independent of swelling action. The reason why increase of valence as such should interfere with swelling is not evident; yet it can scarcely be a coincidence that in the above series all of the plurivalent anions are relatively inactive in producing this change. In my own determinations the sulphate had a well-marked swelling action similar to that of bromide. In other respects, however, the correspondence with Pauli's series is a close one, especially as regards the relative activities of the series of monovalent anions.

### VIII. ANTITOXIC ACTION OF ANIONS.

We have seen above that in the case of salt solutions whose toxicity is due to the preponderating action of the anion the addition of salts with active cations retards the poisonous action, — or, as we have expressed it throughout (after J. Loeb), has an antitoxic action. Many salts are destructive to physiological processes for a precisely opposite reason; in their solutions the action of the cation preponderates, and the total action is accordingly coagulative. This is true of solutions of heavy metal salts and salts of trivalent and tetravalent metals. The alkali earth metals appear, in their action on the ciliated cell, almost intermediate between the above two classes; the action of their chlorides is apparently partly coagulative and partly liquefactive, depending on the nature of the colloids concerned.

The question now to be considered is: can the toxic action of pure solutions of these salts be diminished by the action of ions that check or retard coagulative changes? The alkali earth chlorides offer the best opportunity for testing this question. With  $\frac{m}{2}$  solutions of  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{BaCl}_2$  we should expect that addition of a little acid would check the slight swelling action and so prove favorable. On the other hand, addition of alkali or of salts with strongly acting anions ( $\text{BrO}_3$ ,  $\text{CNS}$ ,  $\text{CN}$ , etc.) should retard whatever coagulated action there is, and should therefore prevent the slight coagulative action of  $\frac{m}{2}$   $\text{SrCl}_2$ , and so lessen the injurious action of this solution. The following experiments were designed to test these possibilities.

In the case of solutions of calcium and apparently also of magnesium salts the results are somewhat equivocal, since both acid and alkali may have a favorable action in proper concentrations. With  $\frac{m}{3}$   $\text{SrCl}_2$ , on the other hand, a distinct antitoxic influence is exercised

by alkali and by a considerable number of salts with powerful anions, while acid is quite devoid of such action. The results with this salt are thus in accordance with the theory that the present form of antitoxic action depends on an approximate equalization between the opposed actions of the two sets of oppositely charged ions in the tissue.

In pure  $\frac{m}{2}$  solutions of both  $MgCl_2$  and  $CaCl_2$  ciliary movement may last for several hours; the same is true of  $BaCl_2$ ; while in  $\frac{m}{2}$   $SrCl_2$  movement ceases within a few minutes after immersion in the solution. The swelling action is also characteristic. In solutions of the first three salts the cells swell gradually, and after a few hours considerable numbers are found to be rounded and detached. This action is more rapid in  $MgCl_2$  than in  $CaCl_2$ , and in  $CaCl_2$  than in  $BaCl_2$ ; in the latter salt there is usually relatively slight detachment of cells after six hours; while in the former two the action is well marked after this interval. On the other hand, in  $\frac{m}{2}$  and even in  $\frac{1}{10} m$  and  $\frac{3}{10} m$   $SrCl_2$ , the cells remain permanently unswollen, and the filaments retain their smooth contour; after a few hours they are found to be perceptibly whitened and to exhibit a certain opacity indicative of a partial coagulation; the prevailing change is thus evidently in a direction the reverse of that caused by the first three salts. In all these solutions swelling is much less rapid than in solutions of salts of the alkali metals.

In determining the conditions of antitoxic action in solutions of these salts the following was observed in general: in a number of experiments with solutions of  $CaCl_2$  a trace of acid was found to prolong movement somewhat (*e.g.*, from *ca.* 6 h. in pure  $\frac{m}{2}$   $CaCl_2$  to *ca.* 34 h. in  $\frac{m}{2}$   $CaCl_2 + \frac{m}{3200}$   $HCl$ ) apparently by checking the swelling action; but in order to produce distinct and well-marked antitoxic action the addition of salts with strongly acting anions was required. In  $\frac{m}{2}$   $BaCl_2$  acid had apparently no antitoxic action in any concentration; the same was true of  $\frac{m}{2}$   $SrCl_2$  where swelling does not occur. In solutions of the latter salt addition of certain salts with powerful anions prevents the coagulation and induces swelling; if the proportions are favorable, the antitoxic action is then very decided. In my experiments with  $\frac{m}{2}$   $MgCl_2$  the antitoxic action was not so well marked; no distinct results appeared with acid; but with alkali and certain salts ( $NaBr$ ,  $KCN$ ) definite antitoxic action was observed, apparently due to the anions.

In the explanation of why slight addition of acid should favor



ciliary activity in  $\text{CaCl}_2$  solutions, it must be assumed that the presence of H-ions in low concentrations hinders some destructive action of the pure solution. Since H-ions do in fact hinder the swelling of the cells — an effect evidently due to their possessing an anti-swelling or coagulative action — it seems probable that their favorability in this instance is due to some such action. The structures concerned in ciliary movement consist in all likelihood partly of colloids that are caused to swell by  $\text{CaCl}_2$  solutions; prevention of this action would be favorable to preservation of the contractile structures and hence to continuation of movement. On the other hand, the fact that salts with active anions have a more pronounced antitoxic action seems to indicate that the chief colloid structures concerned in the contractile activity undergo a change of the reverse kind in  $\frac{m}{2} \text{CaCl}_2$ ; this is on the assumption that the favorable action of the anions depends largely on their checking such coagulative changes, — which seems clearly indicated in the experiments with  $\frac{m}{2} \text{SrCl}_2$  below. In my studies on the Ctenophore swimming plate I have found that the fibrillar substance of the plate remains white and unswollen in  $\frac{m}{2} \text{CaCl}_2$ , indicating a preponderantly coagulative action. In view of the resemblance between this tissue and cilia it seems probable that here also this salt has a slowly coagulative action on the colloids directly concerned in contractile activity (perhaps on those forming the substance of the cilia themselves), although its action on other cell structures may be, on the whole, of the reverse character — hence the gradual swelling of the entire cell. If this surmise is true, anions act favorably by checking coagulation of the ciliary substance in  $\frac{m}{2} \text{CaCl}_2$ .

**Antitoxic action of anions on solutions of alkali earth chlorides.** — Addition of alkali to  $\frac{m}{2} \text{CaCl}_2$  has a distinctly favorable action through a certain range of concentrations. In one series of experiments, in which sodium hydroxide was added to  $\frac{m}{2} \text{CaCl}_2$  in a series of graded concentrations ranging from  $\frac{m}{1600}$  to  $\frac{m}{12800}$ , movement was markedly prolonged in the last three concentrations ( $\text{NaOH}$  to  $\frac{m}{3200}$ ,  $\frac{m}{6400}$  and  $\frac{m}{12800}$ ). In  $\frac{m}{1600} \text{NaOH}$  swelling and detachment of cells were greatly accelerated; after one and a half hours many cells were rounding and detaching, and movement had almost entirely ceased after six hours and thirty minutes. Swelling was progressively less rapid as the concentration of alkali decreased, although in all cases it was decidedly accelerated relatively to the control,  $\frac{m}{2} \text{CaCl}_2$ . Movement remained in  $\frac{m}{3200}$  and  $\frac{m}{6400} \text{NaOH}$  after eighteen hours and

forty-seven minutes; in  $\frac{m}{12800}$  NaOH conditions were less favorable, though movement was distinctly more active than in the control after six hours and twenty-seven minutes. In this last solution (neutral  $\frac{m}{2}$  CaCl<sub>2</sub>) movement was fairly active after six hours and twenty-five minutes; it had ceased and disintegration was well advanced after eighteen hours. Then the hydroxyl ion, while promoting swelling, also favors activity in  $\frac{m}{2}$  CaCl<sub>2</sub>.

We have now to determine whether the presence of other definite anions in the solution has a similar effect. This was tested as follows: the solution of the sodium salt whose anion was to be tested was added to the  $\frac{m}{2}$  CaCl<sub>2</sub> solution in graded concentrations as above. In most instances the sodium-salt solution was added in insufficient quantity to reduce the concentration of CaCl<sub>2</sub> more than very slightly. That the Na-ion is not the effective agent is shown by the fact that adding sodium chloride in equivalent concentrations does not produce the effect observed.

In the following (Table XIX) the results of two series of experiments with sulphate, tartrate, and citrate are given. Salts with bivalent and trivalent anions were used with a view to obtaining indications of the possible importance of valence in the antitoxic process.

TABLE XIX.

- The results of two independent series are incorporated in this table. In series A sulphate and tartrate were used in the two concentrations  $\frac{m}{20}$  and  $\frac{m}{40}$ ; in series B the concentrations for these salts were  $\frac{m}{40}$ ,  $\frac{m}{80}$ , and  $\frac{m}{160}$ . In both series citrate was used in concentrations  $\frac{m}{40}$ ,  $\frac{m}{80}$ , and  $\frac{m}{160}$ .
1. Pure  $\frac{6}{10} m$  CaCl<sub>2</sub>. A: almost ceased by 4 h. 43 m. B: almost ceased by 4 h. 48 m. (Ca. 5 h.).
  2. 9 vol.  $\frac{6}{10} m$  CaCl<sub>2</sub> + 1 vol.  $\frac{m}{2}$  Na<sub>2</sub>SO<sub>4</sub> ( $\frac{m}{20}$ ). A (slight precipitate CaSO<sub>4</sub>): active, long-continued movement; a little movement after 43 h. 50 m. (43 h. 50 m.).
  3. 19 vol.  $\frac{6}{10} m$  CaCl<sub>2</sub> + 1 vol.  $\frac{m}{2}$  Na<sub>2</sub>SO<sub>4</sub> ( $\frac{m}{40}$ ). A: action similar to Solution 2; a little movement after 43 h. 50 m. B: a little movement after 41 h. 14 m. (A: 43 h. 50 m.; B: 41 h. 14 m.).
  4.  $\frac{6}{10} m$  CaCl<sub>2</sub> + Na<sub>2</sub>SO<sub>4</sub> to  $\frac{m}{80}$ . B: rather less favorable than Solution 3; trace of movement after 41 h. 14 m. (41 h. 14 m.).
  5.  $\frac{6}{10} m$  CaCl<sub>2</sub> + Na<sub>2</sub>SO<sub>4</sub> to  $\frac{m}{160}$ . B: less favorable than Solution 4; fairly active after 19 h. (19 h.).
  6. 9 vol.  $\frac{6}{10} m$  CaCl<sub>2</sub> + 1 vol.  $\frac{m}{2}$  Na<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> ( $\frac{m}{20}$ ). A (some Ca tartrate crystallizes out): active long-continued movement as in Solution 2; active after 43 h. 50 m. (43 h. 50 m.).
  7. 19 vol.  $\frac{6}{10} m$  CaCl<sub>2</sub> + 1 vol.  $\frac{m}{2}$  Na<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> ( $\frac{m}{40}$ ) (a little Ca-tartrate crystallizes out). A: action like Solution 6; long-continued movement; a little remains after 65 h. 43 m. B: a little movement after 41 h. 10 m. (A: 65 h. 43 m.; B: 41 h. 10 m.).

TABLE XIX (continued).

8.  $\frac{6}{10}$  *m* CaCl<sub>2</sub> + Na<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> to  $\frac{m}{80}$ : B: like Solution 7, though apparently less favorable; a little movement after 41 h. 10 m. (10 h. 41 m.).
9.  $\frac{6}{10}$  *m* CaCl<sub>2</sub> + Na<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> to  $\frac{m}{180}$ : B: less favorable than Solution 8; fairly active after 19 h. (19 h. +).
10. 9 vol.  $\frac{6}{10}$  *m* CaCl<sub>2</sub> + 1 vol.  $\frac{m}{2}$  Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>. A. (a little precipitate of Ca-citrate) a little movement after 4 h. 55 m.; ceased by 18 h. (4 h. 55 m.).
11. 19 vol.  $\frac{6}{10}$  *m* CaCl<sub>2</sub> + 1 vol.  $\frac{m}{2}$  Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> ( $\frac{m}{40}$ ). A: active movement at first; a little movement after 24 h. B: a little movement after 41 h. 13 m. (A: 24 h.; B: 41 h. 13 m.).
12.  $\frac{6}{10}$  *m* CaCl<sub>2</sub> + Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> to  $\frac{m}{80}$ . A: more favorable than Solution 11: a little movement after 43 h. 51 m. B: a little movement after 41 h. 14 m. (A: 43 h. 51 m.; B: 41 h. 14 m.).
13.  $\frac{6}{10}$  *m* CaCl<sub>2</sub> + Na<sub>3</sub>C<sub>6</sub>H<sub>7</sub>O<sub>6</sub> to  $\frac{m}{100}$ . A: a little movement after 43 h. 51 m. B: a little movement after 41 h. 14 m. (A: 43 h. 51 m.; B: 41 h. 14 m.).

In a third similar series with  $\frac{m}{2}$  CaCl<sub>2</sub> even more favorable results appeared. In presence of  $\frac{m}{40}$  Na<sub>2</sub>SO<sub>4</sub> a little movement was found to remain after eighty-six hours; and movement lasted for the same period in solutions containing tartrate and citrate in the proportions  $\frac{m}{20}$ ,  $\frac{m}{40}$ , and  $\frac{m}{80}$ ; in  $\frac{m}{160}$  citrate solution some movement persisted after sixty hours and forty-two minutes.

The above salts thus exhibit well-marked antitoxic effectiveness in CaCl<sub>2</sub> solutions. The indications are that the citrate, which yields a certain number of trivalent anions in its solutions, is more effective in low concentrations than the tartrate or sulphate. Increase of valence seems generally to impart physiological effectiveness to an ion, whether this be due to the presence of a greater number of charges within the volume occupied by the ion, or to an incidental decrease in its electrical stability resulting from increase in number of charges. Valence, however, does not seem a factor of so great importance in the case of the anions as of the cations.

On reference to the table on page 103 (Table VIII), it will be seen that mixtures of nine volumes  $\frac{m}{2}$  CaCl<sub>2</sub> and one volume  $\frac{m}{2}$  NaCl are more favorable than the pure  $\frac{m}{2}$  CaCl<sub>2</sub>. The same is seen in corresponding mixtures of  $\frac{m}{2}$  BaCl<sub>2</sub>, and MgCl<sub>2</sub> (Tables VIII, XI) with  $\frac{m}{2}$  NaCl. In this solution the concentration of Cl-ions is only slightly different from that in pure  $\frac{m}{2}$  CaCl<sub>2</sub>, while that of the Ca-ions is reduced by almost 10%; the effective action of the anions is thus increased, since Na is a less active cation than Ca which it replaces. The addition of NaCl thus appears to have an antitoxic effect which some might consider due to the Na. But that the effect is in reality chiefly due to

the increased anion action is indicated by the following series of experiments, in which it is shown that the bromide has a much more effective action than the chloride under the same conditions.

TABLE XX.

1.  $\frac{6}{10}$  m CaCl<sub>2</sub>. Trace of movement after 18 h. (18 h.).
2. 9 v.  $\frac{6}{10}$  m CaCl<sub>2</sub> + 1 v.  $\frac{6}{10}$  m NaCl. After 18 h. movement is considerably more active than in Solution 1. Movement has practically ceased by 42 h. (42 h.).
3. 9 v.  $\frac{6}{10}$  m CaCl<sub>2</sub> + 1 v.  $\frac{6}{10}$  m NaBr. Action is distinctly more favorable than in Solution 2; a little movement remains after 66 h. In a repetition of this experiment a little movement remained after 86 h. 56 m. (66 h.; 87 h.).

This marked degree of antitoxic effectiveness is to be ascribed to the presence of the Br-ion which, as seen above, is more energetic in its action (as seen by its greater effect in producing swelling, etc.) than the Cl-ion. The action of the Ca is thus counteracted to a greater degree. The same is seen in the experiments with SrCl<sub>2</sub> and BaCl<sub>2</sub> below. The greater antitoxic effectiveness of the bromide in this relation is probably to be ascribed to the lower decomposition voltage of the Br-ion, since it is similar to the Cl-ion with respect both to valence and ionic velocity.

The presence of small quantities of potassium cyanide is very effective with  $\frac{m}{2}$  SrCl<sub>2</sub>, as will be seen below. In the case of  $\frac{m}{3}$  CaCl<sub>2</sub>, the cyanide produces less decidedly favorable results. Yet in low concentrations it certainly prolongs movement; thus in a series of solutions of  $\frac{6}{10}$  m CaCl<sub>2</sub> containing KCN in the concentrations  $\frac{m}{4000}$ ,  $\frac{m}{6000}$ , and  $\frac{m}{16000}$ , movement was found to last distinctly longer than in the pure solution. Whether this action is due chiefly to the CN-ion (which on account of the slight dissociability of the hydrolytically formed HCN is present in such solutions in very low concentrations), or to the OH-ions (which are present in numbers almost equal to those of the K-ions), or to the undissociated HCN molecules, is difficult to say with any certainty. The increased alkalinity, no doubt, plays a rôle, since an equivalent addition of an alkali hydroxide has a similar effect; yet, as will be seen more clearly in the case of  $\frac{m}{2}$  SrCl<sub>2</sub>, cyanides have a more powerful antitoxic action than hydrates in very low concentration, so that some rôle must be ascribed to either the HCN molecules or the CN-ions in the solution (see the experiments with  $\frac{m}{2}$  SrCl<sub>2</sub> below).

With  $\frac{m}{2}$  MgCl<sub>2</sub> the addition of a little alkali is also favorable, although little can be added on account of the insolubility of magnesium hydrate. A distinct increase in the activity of movement and in the

rate of detachment of cells is seen, and the movement, in all of the four experiments tried, lasted decidedly longer than in the pure solution. The addition of small quantities of sulphate, tartrate, and citrate as above had, however, no such decided effect as with  $\frac{m}{2}$   $\text{CaCl}_2$ . In two series of experiments with varying mixtures of  $\frac{m}{2}$   $\text{MgCl}_2$  and  $\frac{m}{2}$   $\text{MgSO}_4$  (1 vol.  $\text{MgCl}_2$  + 9 vols.  $\text{MgSO}_4$ , 2 vols.  $\text{MgCl}_2$  + 8 vols.  $\text{MgSO}_4$ , etc.) the most favorable solutions contained approximately equal volumes of the two salt solutions; the difference from the pure solution of either salt was not very decided. In two experiments with mixtures of 9 volumes  $\frac{1}{10}$   $m$   $\text{MgCl}_2$  + 1 volume  $\frac{1}{5}$   $m$   $\text{NaBr}$  movement was considerably prolonged,—in one case a little movement remained after 66 hours. KCN in the concentrations  $\frac{m}{4000}$ ,  $\frac{m}{8000}$ ,  $\frac{m}{16000}$  also prolonged movement considerably. In general, however, the action of salts with active anions in prolonging movement was less striking than with  $\frac{m}{2}$   $\text{CaCl}_2$  or  $\text{SrCl}_2$ . It may be that in  $\frac{m}{2}$   $\text{MgCl}_2$  solutions there is a closer approach to an equality of action between anion and cation than in  $\text{CaCl}_2$  solutions; hence antitoxic action is in general less marked.

With  $\frac{m}{2}$   $\text{BaCl}_2$  no evidence of antitoxic action was seen with acid. On the other hand, addition of alkali to  $\frac{1}{10}$   $m$   $\text{BaCl}_2$  proved favorable; in each of the four concentrations of  $\text{NaOH}$ ,  $\frac{m}{1600}$ ,  $\frac{m}{3200}$ ,  $\frac{m}{6400}$ , and  $\frac{m}{12800}$ , movement was decidedly more active and prolonged than in the pure solution, lasting for more than 18 hours in each instance; while in the control all activity had ceased in less than 4 hours. The typical increase in swelling and detachment of cells is also seen in these solutions, to a degree proportional to the concentration of alkali. Of salts with active anions the bromide, cyanide, and citrate proved effective; in one instance movement continued for over 90 hours in a mixture of 9 volumes  $\frac{1}{10}$   $m$   $\text{BaCl}_2$  and 1 volume  $\frac{m}{2}$   $\text{NaBr}$ . The addition of a trace of cyanide enabled movement to continue for 18 hours, and of citrate for 30 hours; no distinct action was obtained with tartrate. The almost complete insolubility of the sulphate precludes its use in experiments with barium salts.

The action of anions in counteracting a toxicity due to predominance of cation action appears most clearly in solutions of strontium chloride. Acid has no antitoxic action, merely accelerating the coagulation which we have seen to be the effect of pure neutral solutions of this salt; while alkali has a most striking and decided influence. The following series illustrates this action; standard  $\text{KOH}$  solution was added to the pure  $\frac{m}{2}$   $\text{SrCl}_2$  in the concentrations named. The

action of each solution in promoting movement and in producing swelling of the cells is briefly indicated.

TABLE XXI.

1. Pure  $\frac{m}{2}$  SrCl<sub>2</sub> . . . Slight movement after 10 m.; ceased by 20 m. No swelling of filaments; filaments are white, opaque, and smooth in contour after 20 h. ( $<20$  m.).
2.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{4000}$  KOH . Movement is active and cells are swelling after 18 m.; after 42 m. movement has almost ceased and filament already shows marked swelling and disintegration (42 m.).
3.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{8000}$  KOH . Active long-continued movement; considerable swelling after 1 h. 20 m.; a trace of movement remains after 19 h. 24 m.; swelling and disintegration are then marked (19 h. 24 m.).
4.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{16000}$  KOH . Active movement as in Solution 3; swelling is more gradual; after 20 h. disintegration is well marked, but less so than in Solution 3 (19 h. 24 m. +).
5.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{32000}$  KOH . Active movement; swelling is more gradual than in Solution 4; after 20 h. conditions are similar to Solution 4 (19 h. 37 m. +).
6.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{40000}$  KOH . Movement has almost ceased by 19 h. 37 m.; swelling is relatively slight (19 h. 37 m.).
7.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{128000}$  KOH. Active movement at first; is greatly slowed after 2 h. 30 m.; has almost ceased by 6 h. 30 m. After 20 h. filaments are even-contoured (no swelling) and slightly whitish (6 h. 30 m.).
8.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{256000}$  KOH. Quite active movement at first; ceases within 1 h. 15 m.; filaments after 20 h. are opaque and white as in neutral solutions ( $<1$  h. 15 m.).

The antitoxic action is thus clearly a most pronounced one, and runs closely parallel to the anti-coagulative action of the alkali. In another series of four solutions (KOH from  $\frac{m}{10000}$  to  $\frac{m}{80000}$ ) the most favorable action was seen at  $\frac{m}{40000}$  (cilia still active after twenty-four hours and nineteen minutes; at  $\frac{m}{80000}$  there was only slight swelling and movement was considerably slowed after three hours and forty-five minutes. In the above series  $\frac{m}{16000}$  and  $\frac{m}{32000}$  KOH appear most favorable; in  $\frac{m}{40000}$  KOH the swelling action is too energetic, and induces rapid disintegration. In a third series of four solutions of  $\frac{m}{2}$  SrCl<sub>2</sub> with sodium hydrate as antagonist, the action was similarly favorable in the concentrations  $\frac{m}{16000}$ ,  $\frac{m}{32000}$ ,  $\frac{m}{64000}$ ; at  $\frac{m}{128000}$  movement was only slightly prolonged. Apparently a concentration of alkali lying between  $\frac{m}{80000}$  and  $\frac{m}{128000}$  is the least that will completely prevent the coagulative action of the Sr-ions and so produce a marked counteraction of the toxicity of the pure solution.

Solutions of potassium cyanide have an equal or even more pronounced antitoxic action. The following table summarizes the results of one series of experiments with this salt:

TABLE XXII.

1.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{400}$  KCN. Movement has almost ceased after 42 m.; swelling is then well marked (42 m.).
2.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{800}$  KCN. Movement is active and prolonged; swelling is less rapid than in Solution 1; a little movement remains after 19 h. 41 m. (19 h. 41 m.).
3.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{1600}$  KCN. More favorable than Solution 2; a little movement remains after 43 h. 24 m.; swelling is less marked than in Solution 2 (43 h. 24 m.).
4.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{3200}$  KCN. Movement as in Solution 3; after 20 hours filaments remain clear and translucent in appearance; slight swelling (43 h. 24 m.).
5.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{6400}$  KCN. Apparently more favorable than Solution 4; filaments remain translucent; somewhat swollen after 20 h. (43 h. 24 m. +).
6.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{12800}$  KCN. Active at first; after 2 h. 16 m. there remains relatively little movement; after 20 h. filaments are smooth and unswollen and almost opaque, though less so than in pure  $\frac{m}{2}$  SrCl<sub>2</sub> (2 h. 16 m. +).
7.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{25600}$  KCN. Fairly active after 1 h. 16 m.; filaments are opaque and white after 20 h., like pure  $\frac{m}{2}$  SrCl<sub>2</sub> (1 h. 16 m. +).
8.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{51200}$  KCN. Like Solution 7, though less active (1 h. 16 m.).
9.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{102400}$  KCN. A little movement after 42 h.; coagulated after 20 h. as in pure  $\frac{m}{2}$  SrCl<sub>2</sub> (42 m. +).

The optimum concentration of KCN seems here  $\frac{m}{4000}$  to  $\frac{m}{8000}$ . In another experiment movement lasted for more than eighteen hours in  $\frac{m}{4000}$  KCN. In another series of determinations with  $\frac{m}{2}$  SrCl<sub>2</sub> the maximum observed duration of movement in the different solutions was as follows:  $\frac{m}{2000}$  KCN, five hours and fourteen minutes;  $\frac{m}{4000}$  KCN, forty-two hours and eight minutes;  $\frac{m}{8000}$  KCN, sixty-seven hours and twenty-eight minutes;  $\frac{m}{10000}$  KCN, eighteen hours and twenty-eight minutes. The optimum here corresponds to that observed in the series of Table XXII. A distinct though slight antitoxic action is observed in concentrations of KCN so low as  $\frac{m}{102400}$ .

**Other salts.** — Sodium tartrate added in concentrations of  $\frac{m}{20}$  and  $\frac{m}{40}$  had a distinct though not pronounced action in increasing activity and prolonging movement in  $\frac{m}{2}$  SrCl<sub>2</sub>. The addition of sodium bromide in somewhat considerable quantity also proved effective and to a greater degree. Thus in a mixture of 6 volumes  $\frac{m}{2}$  SrCl<sub>2</sub> and 4 volumes  $\frac{m}{2}$  NaBr movement continued for eighteen hours in one experiment and for two hours and twenty-three minutes + in a second; the filaments showed marked swelling and disintegration after twenty hours. Solutions with 8 volumes  $\frac{m}{2}$  SrCl<sub>2</sub> and 2 volumes  $\frac{m}{2}$  NaBr were less favorable; in each of two experiments movement remained active

after forty minutes, but had ceased after five hours. With NaBr in lower concentrations (9 vols.  $\text{SrCl}_2$  + 1 vol.  $\frac{m}{2}$  NaBr, etc.) no antitoxic action could be seen; in these solutions the filaments coagulate.

Sodium iodide acts similarly to the bromide. In 6 volumes  $\frac{m}{2}$   $\text{SrCl}_2$  and 4 volumes  $\frac{m}{2}$  NaI movement lasted for two hours and twenty-two minutes in one experiment, and in another was still active after thirty-six minutes; coagulation is prevented in this solution. In presence of less NaI conditions proved less favorable, as with NaBr; 9 volumes  $\frac{m}{2}$   $\text{SrCl}_2$  and 1 volume  $\frac{m}{2}$  NaI proved slightly effective. Iodide is thus more active than bromide. Slight indications of antitoxic action were also obtained with solutions of sodium ferro-cyanide; also with sodium sulphocyanate in  $\frac{m}{200}$  and  $\frac{m}{400}$  concentrations.

The most effectual salts for antitoxic action with  $\frac{m}{2}$   $\text{SrCl}_2$  proved in all cases to be those with strongly marked anion action; thus  $\text{NaBrO}_3$  and  $\text{Na}_2\text{HAsO}_4$  were found to exhibit conspicuous antitoxic activity. The following table illustrates:

TABLE XXIII.

1. 9 vol.  $\frac{m}{2}$   $\text{SrCl}_2$  + 1 vol.  $\frac{m}{2}$   $\text{NaBrO}_3$  ( $\frac{m}{2}$ ). Movement remains fairly active after 8 h. 57 m.; filaments are translucent and somewhat swollen (9 h. +).
2.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{46}$   $\text{NaBrO}_3$ . Movement is fairly active after 1 h. 45 m.; has ceased by 2 h. 45 m.; filaments swell, though less than in Solution 2; movement has almost ceased by 1 h. 45 m.; practically no swelling; filaments are opaque and white (partial coagulation) after 20 h. (1 h. 45 m.).
3.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{10}$   $\text{NaBrO}_3$ . Less favorable than Solution 2; movement has almost ceased by 1 h. 45 m.; practically no swelling; filaments are opaque and white (partial coagulation) after 20 h. (1 h. 45 m.).
4.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{100}$   $\text{NaBrO}_3$ . A little movement after 42 m.; coagulation as in Solution 3 (42 m.).
5.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{20}$   $\text{NaBrO}_3$ . Similar to Solution 4 (42 m.).
6.  $\frac{m}{2}$   $\text{SrCl}_2$  9 vols. + 1 vol.  $\frac{m}{4}$   $\text{Na}_2\text{HAsO}_4$  ( $\frac{m}{4}$ ). (Slight precipitate) quick vigorous movement; filaments remain translucent; fairly active movement after 3 h. 45 m. (3 h. 45 m.+).
7.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{20}$   $\text{Na}_2\text{HAsO}_4$ . Quick movement at first; a little movement after 8 h. 54 m.; filaments swell (8 h. 54 m.).
8.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{10}$   $\text{Na}_2\text{HAsO}_4$ . Active movement at first; fairly active movement remains after 21 h. 46 m.; swelling is then well marked (21 h. 46 m.+).
9.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{100}$   $\text{Na}_2\text{HAsO}_4$ . More favorable than Solution 8; active long-continued movement. Fair movement after 32 h. 38 m.; swelling well marked after 20 h. (32 h. 38 m.+).
10.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{20}$   $\text{Na}_2\text{HAsO}_4$ . Vigorous and long-continued movement; more favorable than Solution 9; a little movement after 46 h. 6 m.; well-marked swelling after 20 h. (46 h. 6 m.).
11.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{46}$   $\text{Na}_2\text{HAsO}_4$ . Active movement; ceases sooner than in Solution 10; fairly active after 28 h. 45 m.; well-marked swelling after 20 h. (28 h. 45 m.+).
12.  $\frac{m}{2}$   $\text{SrCl}_2$  +  $\frac{m}{100}$   $\text{Na}_2\text{HAsO}_4$ . A little movement after 28 h. 45 m.; well-marked swelling after 20 h. (28 h. 45 m.).



TABLE XXIII (continued).

13.  $\frac{m}{9}$  SrCl<sub>2</sub> +  $\frac{m}{2800}$  Na<sub>2</sub>HAsO<sub>4</sub>. Active at first; almost ceased after 19 h.; swelling less than in Solution 12 (19 h.).
14.  $\frac{m}{9}$  SrCl<sub>2</sub> +  $\frac{m}{8120}$  Na<sub>2</sub>HAsO<sub>4</sub>. Active at first; almost ceased after 5 h. 6 m.; fair swelling after 20 h. (5 h. 6 m.).
15.  $\frac{m}{9}$  SrCl<sub>2</sub> +  $\frac{m}{10200}$  Na<sub>2</sub>HAsO<sub>4</sub>. Similar to Solution 14; a little movement after 4 h. 20 m.; only slight swelling after 20 h. (4 h. 20 m.).
16.  $\frac{m}{2}$  SrCl<sub>2</sub> +  $\frac{m}{20400}$  Na<sub>2</sub>HAsO<sub>4</sub>. Movement much slower after 32 m.; filaments are unswollen and opaque after 20 h. (32 m. +).
17.  $\frac{m}{9}$  SrCl +  $\frac{m}{40200}$  Na<sub>2</sub>HAsO<sub>4</sub>. Like Solution 16; filaments coagulated after 20 h. (32 m. +).
18.  $\frac{m}{7}$  SrCl<sub>2</sub> +  $\frac{m}{81000}$  Na<sub>2</sub>HAsO<sub>4</sub>. A little movement after 31 m.; filaments coagulated after 20 h. (31 m.).
19.  $\frac{m}{7}$  SrCl<sub>2</sub> +  $\frac{m}{103800}$  Na<sub>2</sub>HAsO<sub>4</sub>. Less movement than in Solution 18; ceased by 30 m.; filaments coagulate (6m. +).

Sodium arsenate thus exhibits remarkable antitoxic activity in solutions of strontium chloride; here again the action runs closely parallel with its power of preventing coagulation; this power is still effective at  $\frac{m}{10000}$  dilution; in lower concentrations the filaments slowly coagulate and movement is prolonged relatively slightly, although some slight action is evident even in  $\frac{m}{80000}$  dilution. Sodium bromate is less effective and requires higher concentrations. The superior effectiveness of the arsenate tends to confirm the view that valence as such is an important factor in determining the physiological effectiveness of an ion. According to the above results the trivalent AsO<sub>4</sub> ion must be regarded as highly effective in this relation.

**Antitoxic action in solutions of other salts of plurivalent metals.**— I have made various attempts to antagonize  $\frac{m}{2}$  solutions of AlCl<sub>3</sub> and Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> by means of the above salts: sodium sulphate, citrate, arsenate, and potassium cyanide. In all solutions the action of the Al-ions was prepotent, as shown by the prompt coagulation of the filaments and the absence of swelling; ciliary movement also failed to be prolonged perceptibly in any instance. The insolubility of aluminium hydroxide precludes the use of alkali for this purpose. Experiments were also made on the effects of addition of alkali and acid to solutions of heavy metal salts. No indication of any antitoxic effect was seen except in the case of manganese chloride, where the addition of a little alkali appreciably accelerated the swelling and seemed to increase the activity of ciliary movement. The action was slight, however; the addition of sulphate and citrate had no evident

action. In brief, of various attempts to antagonize salts of aluminium and the heavy metals none gave decisive results. It would appear that in these solutions the action of the cation preponderates to a degree that renders its counteraction impossible by addition of other salts. We may infer that it is only in solutions where the influence of the ions of one sign preponderates relatively slightly over that of the others that a really decided antitoxic action is possible; the alkali earth salts, on the one hand, and the various relatively non-toxic sodium salts, on the other, exemplify this. In other words, the above form of antitoxic action consists, according to this view, in the counteraction of a relatively slight prepotency on the part of one or the other of the two sets of oppositely charged ions in the tissue; hence its possibility is confined to relatively few salts whose action is approximately a balanced one. In living tissues under normal conditions the balance between the two opposed ion actions must be preserved by some automatic self-regulatory process of adjustment, such as appears in most if not in all cells to maintain the reaction of the protoplasm at an approximate neutrality.

#### SUMMARY AND CONCLUSION.

1. The injurious action of pure solutions of most sodium salts on the ciliated epithelium of *Mytilus* may be counteracted by the addition of salts of certain metals (*e. g.*,  $MgCl_2$ ) to a degree varying with the nature of the anion of the Na-salt. Salts with the more toxic anions ( $NaI$ ,  $NaCNS$ ,  $NaBrO_3$ , etc.) admit of relatively slight counteraction. Anions may thus be ranged in their order of toxicity.
2. This antitoxic action is well marked with Li- and Na-salts; slight or absent in  $NH_4$  and K-salts.
3. Antitoxic action in solutions of Na-salts depends on the cation of the added antitoxic salt; the most effective antitoxic salts are those of bivalent metals whose cations have high decomposition tensions (alkali-earth metals and manganese); heavy metals are less effective; Fe<sup>II</sup>, Co, Ni, Zn, Cd, Pb show diminishing effectiveness in this order; Cu, Hg,  $UO_2$  are without favorable action.
4. Acid (the hydrogen-ion) shows well-defined antitoxic action with a large number of Na-salts; in general the more toxic the anion of the Na-salt the more H-ions (or other antitoxic cations) are needed for optimum antitoxic action.
5. Trivalent and tetravalent cations (Al, Cr, Fe<sup>III</sup>, Th, Sn<sup>IV</sup>) ex-

ercise antitoxic action in much lower dilution than bivalent cations; Al, Cr, and  $Fe^{III}$  show optimum action in increasingly dilute solutions in this order; Th and  $Sn^{IV}$  resemble  $Fe^{III}$  in their optimum dilution relations.

6. The individual anions show a specific activity in producing swelling of cells in isotonic solutions of K- or Na- salts. The order of increasing toxicity of the monovalent anions corresponds with the order of increasing activity in furthering absorption of water. This order is:  $COOCH_3$ , Cl,  $NO_3$ ,  $ClO_3$ , Br, I, CNS,  $BrO_3$ , OH. The plurivalent anions in general show little activity in producing swelling.

7. Antitoxic action with solutions of Na-salts appears to depend largely on the action of the antitoxic cation in retarding the swelling or disintegrative action due to preponderant anion action. The favorable action thus depends largely on the production of an approximate balance between the opposed actions of anion and cation.

8. In solutions of  $SrCl_2$  a relation appears between antitoxic action and the *anticoagulative* action of the antitoxic salt; the action is due to the checking of a preponderant *cation* or coagulative action; salts with active *anions* thus show antitoxic action (NaOH, KCN,  $Na_2HAsO_4$ , NaBr, NaI, NaCNS). Other alkali earth chlorides show similar relations.

9. In general, antitoxic action seems due to an approximate equalization of the opposite actions of anion and cation on the colloids of the tissue. Hence salts whose toxicity is due to predominant anion action require salts with active cations for counteraction and *vice versa*.



## THE ACTION OF ERGOT UPON THE STOMACH AND INTESTINES.

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### INTRODUCTORY.

THE literature on the pharmacology and toxicology of ergot contains many experimental studies, but they relate chiefly to the action of that drug upon blood pressure and upon the contractions of the uterus. Does ergot have a direct action upon the movements of the stomach and intestines, does it affect the motor mechanism of the gastro-intestinal canal, as it undoubtedly affects the uterine motor apparatus? As far as we could ascertain, no experiments have been published which deal especially with this problem. It is true, some text-book writers on pharmacology mention explicitly that ergot irritates the muscle fibres of the intestines, and Cushny (*Pharmacology and Therapeutics*, 3d edition, p. 477) cites Kobert, who "observed that stomach and bowels contract powerfully under cornutin." In the literature which was accessible to us the following is all that Kobert said on the subject:<sup>1</sup> "Stellt man einen derartigen Versuch an einem weiblichen Thiere an, dem man unter den nöthigen Cautelen den Bauch geöffnet hat, so sieht man in dem Stadium, wo es beim nicht schwangern Thiere zu heftigen Bewegungen des Magens und Darms und Entleerung derselben nach oben und unten kommt, ganz entsprechende Bewegungen auch am schwangern und nicht schwangern Uterus auftreten." We note here, in the first place, that the chief object of this statement is the occurrence of the uterine contractions; the violent contractions of the stomach and intestines are mentioned only to indicate the stage of the intoxication. Furthermore the appearance of violent movements of the stomach and intestines is mentioned here as something known, and is noted in connection with the

<sup>1</sup> KOBERT: *Archiv für experimentelle Pathologie und Pharmakologie*, 1884, xviii, p. 336.

occurrence of vomiting and diarrhœa. Now the occurrence of these latter symptoms in intoxications by ergot is indeed a well-known fact. In both forms of epidemic ergotism vomiting and diarrhœa are predominant symptoms. But their occurrence was not ascribed to the production of violent peristalsis by the secale poison, but to the production of hemorrhages and ulcerations in the gastro-intestinal canal. The violent movements of the stomach and intestines which Kobert has observed in conjunction with vomiting and diarrhœa might, therefore, have been only secondary phenomena due to irritating inflammatory processes within the gut, and the question remains open whether ergot can produce gastro-intestinal peristalsis as a primary effect.

We came across this problem while studying the effect of magnesium salts upon the peristalsis of the alimentary canal. The normal peristalsis in the opened-up abdomen of the animal is too insignificant and inconstant a phenomenon to rely upon in an experiment in which its inhibition should be satisfactorily demonstrated. In looking for means by which the normal peristalsis could be reliably intensified we had recourse to ergot, of which we had known by previous personal experience that in intravenous injections it is capable of augmenting gastric peristalsis. However, since the results which we have obtained in this series of experiments were indeed satisfactory, and not finding in the literature a report of experiments made directly upon the subject in question, we decided to carry out a systematic series of experiments on several species of animals in which the action of ergot upon the gastro-intestinal peristalsis was the chief subject of investigation.<sup>1</sup>

During the somewhat belated preparation of this manuscript an article appeared on the Action of Ergot<sup>2</sup> in which, among other points, the effect of ergot upon the movements of the stomach and intestines was also studied and briefly reported. While the results mentioned in that article agree in general with our own observations, there are abundant reasons for our belief that the appearance of that interesting article does not make the report of our results superfluous.

**Method.**—The experiments were made on dogs, cats, and rabbits. As an ergot preparation we have employed exclusively the fluid ex-

<sup>1</sup> A brief report of the results was made at the last meeting of the American Physiological Society, December, 1905. See this journal, xv, p. xxxi.

<sup>2</sup> DALE: Action of ergot, Journal of physiology, 1906, xxxiv, p. 163.

tract (Squibb's) for the following accidental reason. As we stated above, we started using ergot, not to investigate this drug, but to obtain by its aid an increase of the peristaltic movements, and we utilized for this purpose the preparation which we had on hand. After obtaining uniform and positive results with that preparation, we preferred to continue the investigation with a product the effects of which we had already satisfactorily tested. (Dale made his investigations chiefly with "chrysotoxin." He tested also cornutin (Kobert) and sphacelotoxin (Jacobj), and states that there was practically no difference whichever preparation was employed.)

The administration of the ergot was invariably carried out by the intravenous method. In a few rabbits this was done through the marginal ear vein; in all other cases the injection was made through a cannula in the external jugular vein.

In the majority of the experiments the behavior of the stomach and intestines was observed by direct ocular inspection; in a smaller number of experiments the graphic method was employed by introducing rubber balloons into the stomach and finger cots into the intestines.

We have to state, however, that in our opinion the graphic method is a very unsatisfactory procedure in studies upon gastro-intestinal peristalsis, especially in problems of the kind we tried to solve. In the first place, the various sections of the canal never show a uniform behavior, and thus it would frequently happen that, while a large part of the intestines showed various and variable contractions, just that piece of the gut which contained the small inflated balloon remained at rest most of the time; or, what may happen more frequently, the balloon-bearing piece will show lively rhythmical contractions, while the rest of the gut will be sluggish or entirely at rest; the behavior of the piece prepared for graphic purposes cannot be taken as an indicator of the condition and behavior of the balance of the gut. Furthermore the presence of such a foreign body as an inflated balloon in some part of the gut, the incision into that part for the insertion of the balloon, the suturing of this incision, and sometimes the ligation on both sides of the balloon,—all these gross traumatic factors make that segment an extremely pathological sample of the intestine, from the behavior of which no conclusions ought to be drawn as to the actual physiological activity of a normal gut. In our experiments we have therefore relied essentially upon the results we gathered from ocular inspection of the entire unmolested canal, taking the precaution to draw conclusions only from gross unmistakable changes.

Another difficulty we have to contend with in such experiments is the method by which the viscera are made accessible to our inspection or for our

experimentation. We are of the opinion that most investigators underestimate the profound effect which the simple opening of the abdomen exerts upon the activity of the stomach and intestines. What we see, then, even under the best conditions, is only a fraction of what really takes place normally. We shall deal with this question at another time. The effect of the opening is not due to the pain brought on by the incision; it lasts many hours.

The procedure introduced by Van Braam-Houckgeest, namely, the opening of the abdominal canal while the animal is submerged in a bath of physiological salt solution, is now considered as the best physiological method for this purpose. We have our doubts as to the indifference of these baths. The solution in which the intestines of the animal swim, receives all the dirt of the animal's skin, and the feces and urine which the animal passes during the experiment, and certainly does not remain of a physiological indifferent character. Besides, it is now generally admitted that the so-called physiological solution of sodium chloride is to a degree injurious to nerve and muscle tissue. Why should we assume that it is indifferent to the delicate, sensitive motor apparatus of the gastro-intestinal canal? Furthermore the gas-containing intestines have of course a tendency to reach the surface of the liquid. When the quantity of the latter is abundant enough, the intestines rise as high as the mesentery permits; this rising causes a pull upon the mesentery, and with it a stimulation of the inhibitory nerve fibres carried by them to the intestines, which surely is not without effect upon the movements of the intestines. It seems to us that it would be rather profitable to acknowledge that the information derived by any single method does not give the whole truth about the physiology of gastro-intestinal peristalsis, but that, on the other hand, every method is capable of contributing some part of the truth. The bath method contributes one side and the fluoroscopic method another side of the complex gastro-intestinal activity. Furthermore, even the observation of the exposed viscera without any bath is apparently capable of shedding light upon our question. The discovery of the inhibitory function of the splanchnics and other important observations were made by this simple method. We shall mention in this paper some methods of observing the abdominal viscera which we have employed in this and in other researches and which are perhaps new. Without claiming too much for them they too might serve to bring out some new facts less accessible to observation by other procedures.

As to the present research, the absolute exactness of the method is not such an important factor, since the aim was not so much to study the precise behavior of the gastro-intestinal canal under normal conditions as to bring out the effect which ergot might have upon that behavior. As long as the conditions of the observations are the same before as after the administration of ergot, any changes in the behavior following this administration might justly be looked upon as the effect of ergot. However the results which we have obtained were tried and verified by various methods which we shall presently briefly describe.



In one group of animals the abdomen was widely opened, so as to show the stomach and all parts of the intestines. The viscera were kept constantly moist by abundant warm normal saline, and the temperature was regulated by the heat irradiating from an electric lamp kept over the animal at a suitable distance. At frequent intervals the abdomen was closed for ten or fifteen minutes.

In another group of animals the skin, after an incision was made in the middle line, was extensively dissected free from the underlying musculature, and so suspended as to form a deep receptacle. This was filled with warm normal saline. The muscular cover was then opened in the linea alba and retracted. The viscera were thus exposed to full view in a complete saline bath. The liquid had to be often renewed, as a part of it was absorbed into the subcutaneous tissue. Now and then, when the solution became turbid by some admixture of blood, it was removed and replaced by fresh saline. The blood pressure in cases in which it was tested remained constant. Wrapping the animal in cotton, application of hot-water bags, and the heat from an electric lamp, etc., served to keep up the normal temperature of the animal. The method is a satisfactory one. It has probably already been employed by other investigators, but we are not aware that it has been described by any one.

In another group of animals, especially in young rabbits, still a different method of observation proved to be very practicable. The abdominal skin was extensively freed from the underlying musculature, which was left intact. Through the translucent musculature several parts of the viscera can thus be fairly well discerned. After a little practice the behavior of the stomach, the cæcum, the ascending colon, and the mass of small intestines lying on the left side in the angle formed by the stomach and cæcum can be well recognized. A definite increase or decrease in the movements of these parts can be established with safety. The observation is still more facilitated by a destruction of the dorsal and lumbar spinal cord. Several parts of the viscera are frequently plainly outlined through the relaxed abdominal musculature.

We are not aware that this method of observation has been used and described by any one. E. H. Weber has observed through the peritoneum the peristaltic movements of the intestines in a freshly killed rabbit from the abdomen of which all the muscles were quickly removed. Pal described the movements of the intestines in lean curarized dogs, which he observed through the well-shaven but

unopened skin during the suspension of artificial respiration. The mode of observation described above by us might be a useful addition to this line of methods. At any rate, by this method we were enabled to detect the normal movements of the cæcum in the rabbit which cannot be seen after opening the abdomen. We shall deal with that in another paper.

Finally, we might mention that in dogs we were able to study the effect of ergot upon the stomach by the *pyloric sound*, that is, by the sound that is audible (and palpable) when the strongly contracting stomach is pressing air through the pylorus.

All of the animals were completely etherized during the operation, and most of them received a subcutaneous injection of morphin previously.

#### THE EFFECT OF ERGOT UPON THE STOMACH AND INTESTINES OF DOGS.

In all cases except one the cord was not destroyed; neither were the splanchnics cut. In many cases both vagi were cut. After opening the abdomen, stomach and intestines were observed for some time with regard to the occurrence of spontaneous movements of any sort. Also the peripheral end of one vagus (usually the left) was repeatedly stimulated with induction currents for the purpose of studying the effect of such stimulation upon the motor activity of the stomach and intestines.

The "normal" movements of the stomach and intestines. — In most of the dogs we have experimented upon there were more or less pronounced movements of the stomach before any injection of ergot was made. In some animals shallow waves were running sluggishly over the stomach from the fundus toward the pylorus. In others the pylorus was the seat of deep, travelling contractions. In one case in which the dorsal cord was destroyed (from the fifth dorsal vertebra down) the contractions of the antrum as well as of the preantral region were very strong. In two animals no movements were observed in any part of the stomach.

The intestines have shown practically no spontaneous movements in any of the dogs. In the animal with the destroyed cord some movements were present, but they were moderate and distinctly less frequent and less energetic than in the stomach of the same dog. There was a slight temporary motion in one other dog with an intact cord. (All dogs we have experimented upon were in the same stage

of digestion; they were not fed on the day of operation, and stomach and small intestines were fairly empty.)

Stimulations of the vagus were usually of short duration, rarely lasting longer than fifteen seconds. Nearly without exception the stomach responded to a stimulation of the vagus with a definite contraction; in some experiments, however, the contractions, mostly of the antrum, did not set in until immediately after the interruption of the stimulation. The intestines, on the other hand, showed only in one case a response to vagus stimulation; in all the other experiments they remained quiet during as well as immediately after stimulation. Even in the experiment in which the cord was destroyed and in which the intestines have shown some spontaneous movements, stimulation of the vagus brought no increase of the movements. It should be added that as a rule before the injection of ergot no more than two or three stimulations were made.

**After the injection of ergot.** — It may be stated in a general way that, with the exception of one experiment, the spontaneous movements of the stomach were definitely increased after the injection of ergot. Since in the majority of the experiments spontaneous movements of the stomach were present before the injection, the changes effected by ergot were not always of a striking nature. However, even under such circumstances, the increase was often well marked. The following brief protocol of an experiment will illustrate it:

*Experiment 1. Dec. 14, 1905.* — Female dog; 4000. 0.04 morphin; ether . . . 3.57 P. M. began to observe stomach, 3.58 wave passing, 4.02 slight wave, 2 waves in five minutes. 4.04; 3 c.c. ergot injected jugular vein. Until 4.09 11 waves passed over the stomach, of which 7 were marked "fair" and 4 waves marked "good." — 11 waves in five minutes. 4.10; again 2 c.c. ergot injected. From now on about 2 waves ("fair" and "good" ones) passed every minute over the stomach. 4.18 wave starts at antrum and travels toward fundus—antiperistalsis. 4.20 contraction of "transverse band" remains localized, does not travel. . . .

In this experiment before the injection the waves of contraction were weak and infrequent, two in five minutes. After the administration of ergot the waves became considerably more frequent, two per minute, and the contractions were stronger. At a later period the contractions became somewhat abnormal, antiperistaltic waves appeared, and also tetanic contractions.

The increase consisted sometimes only in greater intensity of con-

traction, or in the waves having their starting-point farther and still farther back toward the fundus. When the contractions of the antrum became very strong, as soon as the wave approached the pylorus, a distinct prolonged sound emanated from over the pylorus, the vibrations of which could also be definitely felt with the finger. As the stomach was empty, the sound was produced by the passage of air through the pylorus.

The appearance of such a pyloric sound can be well established by means of auscultation with a stethoscope in the right hypochondriac region in a non-operated dog after the intravenous administration of a sufficient dose of ergot, as the following abbreviated protocol of such an experiment will illustrate.

*Experiment 2. Jan. 31, 1906.*—Medium-sized female dog. Morphin and ether. Cannula in femoral vein, no other operation.

1.20 P. M. Auscultation of abdomen with phonendoscope, no sounds heard.

Until 2.16. 11 c.c. of ergot injected, no sound heard so far.

2.26. A dull rumbling sound heard in the right hypochondriac region, appearing every twelve seconds and lasting about five seconds (metronome). Sounds occur with perfect regularity.

2.32. Abdomen opened, animal in deep anæsthesia from ergot. No contractions of stomach occur.

2.38. 4 c.c. of ergot injected. Immediately after a powerful contraction sweeps over the antrum. As pylorus is reached, a sound is heard by unaided ear, vibrations easily felt with finger.

2.49. No contractions of stomach to be seen. 3 c.c. ergot injected. Waves appear, starting at the preantral region, recurring about every twenty seconds; less strong; no sound heard.

2.56. 2 c.c. ergot injected again. Contractions the same, but less strong.

From 3.05 left peripheral vagus stimulated several times with 120 coil distance. Heart stops. Stomach contracts slowly but powerfully, waves stronger every sixteen seconds.

3.13. 1.5 c.c.  $\frac{1}{10}$  per cent atropin sulph. (= 1.5 mgr.) injected into right femoral vein. 3.17. Very superficial waves passing over stomach.

3.20. L. p. v.<sup>1</sup> stimulated with 120. Heart not stopped, stomach not contracted.

In this experiment the pyloric sounds were brought out by the gradual injection of a comparatively large dose of ergot. The open-

<sup>1</sup> L. p. v. means that left peripheral cut end of the vagus nerve was stimulated with induction currents.

ing of the abdomen removed the contractions as well as the sounds. The addition of ergot brought on once more powerful contractions and sounds, but the further addition of ergot reduced the energy of the contractions and abolished the sounds. (The other observations in the experiments will be discussed later.)

*Experiment 3. Feb. 1, 1906.* — Good-sized dog. Morphine and ether. Canula in right femoral vein.

1.20. Dull "pressing" sounds heard with phonendoscope over the pyloric region.

2.09 and 2.35. 3 c.c. ergot injected each time.

2.50. No sound heard over pyloric region since injection of ergot.

2.53. 3 c.c. ergot again.

3.09. Squirting muffled sounds lasting about one second heard over pyloric area, recurring every thirteen seconds.

3.13. 3 c.c. ergot again. Pyloric sounds much louder and longer now, occur every thirteen seconds; can be heard with unaided ear and can be definitely felt. Sounds recorded on drum.

3.40. Sounds much louder and longer than before.

3.46. Again 3 c.c. ergot injected.

4.10. Sounds same as before, except that they are higher pitched.

4.15. 1 c.c.  $\frac{1}{10}$  per cent atropine sulph. (= 1 mgr.) injected into femoral vein.

4.20. No sounds heard over the pyloric region.

4.30. Wound sewed up and dog removed from table. Animal in deep anaesthesia and perfectly relaxed. Next day apparently perfectly well.

In this experiment there was a dull short pyloric sound which disappeared after the administration of some ergot. Later on, when the dose reached 9 to 12 c.c., the sounds reappeared and became gradually distinctly louder and longer.

The increase of the contractions of the exposed stomach appeared sometimes immediately after the injection, sometimes not until a few injections were given. After further administration of ergot a stage was reached when not only no further increase was produced, but, on the contrary, the result was sometimes a decrease in the energy and frequency of the contractions.

The augmenting effect of ergot upon the motor conditions of the intestines was very evident in every experiment, perhaps especially so on account of the frequent absence of any movements of the intestines before the injection. There was one form or another of motor augmentation in the intestines after every injection of ergot. In

some cases moderate spontaneous movements would soon appear in different parts of the small intestines which subsided again after a while. By repeating the injections the movements would become very active and moderately lasting. In many cases the movements assumed a regular peristaltic character, in others the contractions would be very irregular, and in still others the contractions would have more of a tonic or tetanic character. All flatness of the intestines would disappear, the tubes although empty would look round, stiff, slightly beaded, with hardly any motion. The latter form of augmentation would occur frequently, especially after many injections of ergot were given. In other words, smaller doses of ergot would favor rather the peristaltic movements of the small intestines, larger doses would tend to bring on a tonic or tetanic state.

Ergot had frequently an augmenting effect also upon the movements of the duodenum, but the augmentation ran by no means parallel with that of the jejunum and ileum. (The protocols contain very few notes on the spontaneous movements of the colon.)

The injections of ergot undoubtedly augmented the motor effect of stimulation of the vagus upon the stomach as well as upon the intestines. And here, again, the augmentation was more evident in its effect upon the intestines than in that upon the stomach, and again perhaps, essentially for the reason of the frequent absence of any effect of stimulation of the vagus upon the intestines before the injection of ergot, the sharp contrast made the effect more evident. In the stomach as well as in the intestines the augmented contractions were in most cases of a tonic character and rarely consisted in increased peristalsis. The effect upon the stomach would manifest itself mainly in contractions of the antrum, and only in a few cases was there a moderate tonic contraction of the whole stomach. The antrum would contract mostly during stimulation, but sometimes only immediately after its interruption. The development of the contraction would as a rule be very slow, but reached sometimes extreme degrees, so that the entire antrum looked like a gray thick cord. However, such extreme degrees of contraction of the antrum are liable to occur sometimes without the influence of ergot. It was different, however, with the intestines. In some cases, after a few injections of ergot were given, on stimulation of the vagus the intestines stood up as loops of gray cord; there would be a state of tetanic contraction such as we have never observed occurring in normal conditions of the animal.

In a few cases after the intestines have shown a distinct augmentation in response to the vagus stimulation, further injections of ergot brought out opposite responses to stimulations of the vagus. Either the intestines failed to contract, or the stimulation inhibited an existing spontaneous peristalsis, or, if the gut was previously tonically contracted, the stimulation would bring a distinct relaxation.

Some authors state that stimulation of the vagi exerts no augmenting effect upon the movements of the large intestine. In a few instances, after injection of ergot, stimulations of the vagus brought on definite peristaltic and tonic contractions of the colon.

The stimulus which produced the strong contractions of the intestines had in many cases to be distinctly stronger than the one which completely stopped the heart beat. This fact gave sufficient evidence that the contractions of the intestines were not a secondary effect due to the anæmia resulting from the stoppage of the heart. Another fact in support of this view is the observation that in some instances after injection of a dose of atropin sufficient to abolish the cardiac effect of the vagus, a stimulation of this nerve was still capable of bringing out some definite contractions of the intestines. We have, however, to state that in our experience atropin reduced in all cases the effect of vagus stimulation upon the contractions of stomach and intestines, and in a few instances entirely abolished the effect upon the stomach. This occurred even with such small doses as one milligram of the drug. Even the spontaneous movements were often distinctly diminished.

The following few abbreviated protocols will serve to illustrate some of the statements made above:

*Experiment 4. Dec. 15, 1905.*—Young female dog, 2500. Morphine and ether.

3.15 P. M. Observed for twenty minutes. Pyloric part of the stomach shows active peristalsis, occasional slight movements of the duodenum, no movements of the small intestines. L. p. v. stimulated with 110: strong contraction of pylorus, strong peristalsis of antrum, beginning only after stimulation. No effect upon small intestines.

3.25. 2 c.c. ergot injected. One minute later duodenum and small intestines show definite moderate movements. No change in stomach.

3.28. Again 2 c.c. ergot.

3.29. Small gut much more folded, no other change. L. p. v. 110: strong contraction of pylorus to whitening, duodenum blanched, movements of small gut increased. After stimulation good and strong contractions of antrum and pylorus; good pyloric sounds with each wave.

A few more stimulations show the same effect, except that colon also contracts during stimulation.

3.55. Stimulation has now no more effect upon the small gut.

3.58. 2 c.c. ergot again. At 4.00 and 4.04 stimulation same as before, with strong pyloric sounds; again definite effect upon small intestines and colon.

4.10. Effect of vagus upon small intestines gone again.

In this experiment with each injection of ergot the spontaneous movements of the intestines as well as the vagus influence upon them were at first distinctly augmented, but the augmentation became less some time after every injection.

*Experiment 5. Jan. 9, 1906.*—Female dog. Morphin and much ether.

3.48. Stomach and gut quiet. L. p. v. 120: good contraction of stomach and intestines, but no peristalsis.

3.53. 1 c.c. ergot. 3.55. Slight peristaltic contractions in stomach and intestines; disappeared in a few minutes. L. p. v. 120: no perceptible change.

4.03. 1 c.c. ergot. Stomach and gut now show fair peristalsis.

4.12. L. p. v. 120: powerful contraction of the stomach and gut.

4.18, 4.30, and 4.49. L. p. v. 120: very powerful contractions of small intestines, loops stand up like wire coils.

4.53. 1 c.c. ergot. L. p. v. 120: as before, powerful contractions of stomach, shows some antiperistaltic movements. . . .

The influence of the ergot upon the vagus effect, especially upon the intestines, was in this experiment, as in some others, very striking indeed.

*Experiment 6. Jan. 11, 1906.*—Female dog. Morphin and ether.

4.10. Slight motion of stomach and gut. L. p. v. 120: stomach contracts well, no response of gut. Stimulation repeated 7 times with about same effect.

4.34. 2 c.c. ergot. One minute later small gut shows definite increase in peristalsis.

4.47 and 4.50. L. p. v. 120: good contraction of stomach; gut more active during stimulation, greater tonus.

4.55. 1 c.c. ergot. Small gut definitely more active (peristalsis), stomach quiet.

4.57. L. p. v. 120: good contraction of stomach, movements of gut stopped. After stimulation: waves passing slowly over stomach, gut shows more motion than before stimulation.



5.01. 1 c.c. ergot. 5.03. L. p. v. 120 : as before, very powerful contractions of stomach.

5.15. L. p. v. 120 : stomach contracts, *gut relaxes*.

5.18. 1 c.c. ergot. 5.21. L. p. v. : *gut relaxes during stimulation*.

In the above experiment, then, after repeated injections of ergot stimulation of the vagus caused repeatedly a distinct relaxation of the intestines.

*Experiment 7. Jan. 29, 1906.*—Medium-sized dog. Morphin and ether. By graphic method : rubber balloon in stomach connected with Marey's tambour.

2.05. L. p. v. : definite contraction of stomach.

2.07. Intravenous injection of 2 c.c.  $\frac{1}{10}$  per cent atropin. L. p. v. 120 : stomach no effect ; heart not stopped.

2.08. 1 c.c. ergot. L. p. v. 120 and R. p. v. 120 : stomach no effect ; heart not stopped.

Abdomen opened and viscera examined by ocular observation.

2.20. L. p. v. 120 : gave fair contraction (contractions not transmitted by balloon), no effect upon gut.

2.35. L. p. v. 100 : stomach fair contraction, movements of gut definitely increased, heart not stopped.

2.39. 2 c.c. ergot : good deep waves passing over stomach, pyloric sounds heard at regular intervals, gut moves well.

2.44, 2.49, and 2.52. L. p. v. 100, long stimulations : stomach movements at first less, then gradually improving ; pylorus sounds less frequent during stimulations ; gut moves less during stimulation.

3.00. 2 c.c. ergot. 3.02. L. p. v. 100 : during stimulation gut a little more active, and stomach less ; after stimulation contractions of stomach improved.

3.08. 2 c.c. ergot. 3.09. Stomach and gut definitely less active than before injection. L. p. v. 100 : good antiperistaltic wave sets in at end of stimulation (stomach).

In this experiment a dose of atropin which abolished the cardiac effect of the vagus permitted some action of this nerve upon stomach and intestines. The effect, however, was apparently greatly impaired. In the next experiment the same dose of atropin interfered radically with the vagus effect upon the stomach.

*Experiment 8, Jan. 20.*—Medium-sized female dog. Morphin and ether. cord destroyed below the 4th dorsal vertebra.

1.25. Powerful contractions sweeping over stomach, gut shows slight motion. L. p. v. 150. Powerful contraction of stomach ; gut shows no effect.

1.40. 2 c.c. ergot. 1.42. Gut distinctly more active now, "separated into many rings." Stomach contracts much more than before, and pyloric sounds heard now at regular intervals.

1.48 and 1.50. L. p. v. 150: heart stopped, stomach contracts very powerfully, gut blanched, increased tonic contraction, no increased peristalsis.

1.53. 2 c.c.  $\frac{1}{10}$  per cent atropin (2 mgr.).

1.55. Stomach movements strongly decreased, gut moving as before.

1.58. L. p. v. 150: heart not stopped, no effect upon stomach, gut shows slightly increased contractions, no peristalsis.

1.59. 2 c.c. ergot. 2.03. L. p. v. 150: as before.

2.06. 2 c.c. ergot. 2.09. L. p. v. 150: as before, but gut shows slight increase in peristalsis. Two more stimulations with same result.

2.17. 2 c.c. ergot. No increase in spontaneous movements of gut; stomach perfectly quiet. L. p. v. 150: same as before.

2.24. L. p. v. 100, stimulated twice. Moderate increase of movements of gut. After long stimulation and even during it pylorus contracted, and once some antiperistalsis followed.

2.28. 2 c.c. ergot. 2.32. L. p. v. 100: after a long stimulation pylorus contracted (not stomach) and gut showed fair increase of peristalsis; heart not stopped.

In this experiment, as in others, atropin interfered with the action of the vagus upon the stomach a good deal more than with its action upon the intestines.

It may be mentioned that in dogs ergot worked as an anæsthetic. After the animals received 4 or 5 c.c. of ergot they remained in profound narcosis for the rest of the experiment without any other anæsthetic.

**Experiments on cats.**— Only five experiments were made on cats. The following two abbreviated protocols will illustrate the essential facts of experiments from this series:

*Experiment 9, June 18, 1906.*— Medium-sized cat. Morphine subcutaneous 0.01 and ether. Incision in middle line of abdomen, skin dissected off so as to form a receptacle above the muscular cover. Filled up with warm saline and muscles of abdomen opened. No movements of stomach or gut.

11.40. L. p. v. 120: practically no effect upon gut or stomach.

Until 11.56 L. p. v. 110 stimulated three times: gut no effect, stomach disappears from view during stimulation. Since stimulations began stomach shows spontaneous movements, superficial waves passed toward the pylorus, about 5 in a minute, gradually getting deeper.

12.02. 1 c.c. ergot. From 12.04 small intestines and colon show fair contractions, coils of small gut definitely rounder now than before; contractions in small gut slightly travelling, some slight peristalsis. Stomach, no definite change.

From 12.27 to 12.35. L. p. v. 110, 4 times: gut contracted only once and wave swept down. Gut shows swaying motion between stimulations. Stomach waves now powerful.

12.42. 1 c.c. ergot. 12.45. Contractions of small gut more frequent and stronger than before, movements progress more rapidly and only in one direction. Constrictions of the stomach deeper, 6 to the minute.

12.53. L. p. v. 110: tonic contractions of entire small gut, coils stood up like wire rings, beaded, remained in same state for fifteen or twenty seconds after stimulation. Stomach disappeared from view during stimulation; when it reappeared the travelling contractions seemed to be still deeper.

Until 2.21 ergot was injected 5 times, 1 c.c. each time. The spontaneous contractions of gut lost more and more their travelling character, gut became more tonically contracted, looked beaded. Contractions of stomach gradually became less vigorous. L. p. v. 110, stimulated a dozen times. Character of effect upon intestines remained the same, but diminished in energy. At first the after effect gradually disappeared; later the gut began to relax, while the stimulation still continued. The onset of the tonic contraction during stimulation became less abrupt, and the gradual successive entrance of the different parts of the small intestines (from above downwards) into the contraction became gradually very plain.

2.25. 2 c.c. of  $\frac{1}{10}$  per cent atropin (2 mgrs.) injected.

2.26. L. p. v. 110 and 90: heart not stopped, no effect whatsoever upon gut and stomach.

The augmenting action of ergot upon the spontaneous contractions as well as upon the vagus effect was in this experiment, as far as the gut is concerned, beyond any doubt. There was also a greater vigor of the contractions of the stomach after the first injections of ergot. It is, however, not absolutely proven that it would not have occurred even without the ergot, since the stimulations of the vagus alone seemed to produce some lasting effect upon the spontaneous activity of the stomach.

*Experiment 10, June 19, 1906.*—Cat. (All preparations as in previous experiment, except that Ringer's fluid was used for the bath instead of a solution of sodium chloride.)

12.15. No movements of stomach and intestines. L. p. v. 160: some tonic contractions of entire stomach.

12.20. 1 c.c. ergot. 12.25. Moderate waves pass now over stomach, gradually increase in vigor. 12.27. Slight motions set in also in gut, become pendular. L. p. v. 160: twice, responds once with contraction of stomach, no effect upon gut. 12.39. 1 c.c. ergot. 12.42. Stomach: waves more frequent, deeper and starting farther to the left. Small gut: pendular movements and slight travelling of contractions. 12.51. L. p. v. 160, stimulated ten seconds: contractions of stomach increased; small intestines contract strongly exactly in the manner described in Experiment 9 (the coils standing up like large rings) with same after effect. 12.55. Stomach: waves more frequent, but more superficial; small gut round; moderate swaying movements. 1.00. 1 c.c. ergot. 1.05. Entire stomach more tonically contracted; waves deeper, but less frequent. Small gut round, tonically contracted; infrequent irregular contractions. 1.08. L. p. v. 160: stomach could not be observed; small gut contracts as during last stimulation, but relaxed soon after, and a few seconds later contracted tonically again, showing at the same time some travelling movements.

Until 2.30 three more doses of 1 c.c. ergot were given. The spontaneous movements became less, the tonic contractions remained about the same, gut looked beaded. The course of the response of the small gut to L. p. v. 160 was about the same as in Experiment 9, except that after the relaxation in the after-effect a distinct tonic contraction set in again, lasting ten or fifteen seconds. The spontaneous contractions of the stomach became gradually less, although each injection of ergot had a tendency to strengthen them for a short time. L. p. v. 160 had more and more the tendency to abolish the waves of the stomach, although sometimes a moderate tonic contraction would seem to set in instead. (In this experiment spontaneous movements of the colon would appear sometimes after ergot, and L. p. v. 160 would sometimes bring on a moderate contraction.)

2.35. 1 c.c. of  $\frac{1}{10}$  per cent atropin (1 milligram). L. p. v. 160 and 140: heart not stopped, no effect upon stomach or intestines; no spontaneous movements of stomach and intestines seen any more.

In this experiment also the most striking effect of ergot was its augmentation of the vagus action upon the gut. The effect upon the stomach, although a little more pronounced than in Experiment 9, was also in this experiment not very striking.

The contraction of the stomach in cats, although sometimes at least as vigorous as those observed in dogs, never brought out any "pyloric sounds."

We should also mention that in cats ergot did not seem to aid the general anæsthesia to any degree.

**Action of ergots in rabbits.**— In all the experiments in which the abdomen was opened, there was not a single case in which the stomach before the injection of ergot had shown spontaneous movements. Stimulation of the vagus brought out a moderate contraction of the pyloric part in some cases; in others it had no effect at all. The small intestines have shown some degree of motion before the injection of ergot in the majority of the experiments, and in a few of the intestines were quite active. Stimulation of the vagi had in most cases no effect at all, and in one instance, in which there was a lively spontaneous activity, the stimulation definitely stopped all the movement. In two cases in which stimulation seemed to have some effect upon the intestines, the affected movements were very slight. In every one of the experiments the injection of ergot had an unmistakable augmenting influence upon a good part of the motor phenomena under investigation.

The spontaneous movements of the stomach in rabbits with open abdomen benefited very little from the ergot. There was only one case in which after the injection the stomach showed slight spontaneous movement. A somewhat better effect was exerted by the ergot upon the response of the stomach to the stimulations of the vagus. There were definite contractions of the stomach in cases where before the injection no response at all could be elicited; and in cases in which stimulation of the vagi caused some contraction of the stomach, those contractions were unmistakably augmented. However, ergot showed a more pronounced influence upon the movements of the intestines. Nearly in all cases sooner or later after the injection spontaneous peristaltic movements of the small intestines set in, and if there were some movements present before the injection, those were distinctly increased after it. And here again, as in the dog and the cat, the greatest influence of the ergot was exerted upon the response to vagus stimulation. The first influence was, as a rule, to produce a definite increase of the peristaltic movements. At a later stage, however, or after two or more injections, a vagus stimulation would bring on striking tonic contractions of parts or the whole of the small intestines. The following two abbreviated protocols will illustrate some of our general statements.

*Experiment 11, December 15, 1906.*— Rabbit. Ether.

5.00. No movements of stomach, slight movement of small intestines, colon quiet. 5.05. L. p. v. 130 and 110: no effect upon stomach or intestines. 5.09. 1 c.c. ergot. Movements of lower small gut become

quite active. Stomach quiet, colon shows some motion. 5.12. L. p. v. 110: movements of gut became at once more active. Later stomach showed a constriction at antrum, did not travel to pylorus. No movements of duodenum at any time. 5.18. 0.6 c.c. ergot. Movements of gut become more active. 5.19. L. p. v. 110: same as before, except that stomach showed constriction at antrum and pylorus. 5.22. Lower small gut very lively; stomach and duodenum quiet. 5.25. L. p. v. 110: antrum and pylorus contracted strongly, duodenum blanched, cæcum showed strong wave of contraction; movement of lower small gut not increased. 5.27. L. p. v. 110: stomach contracts strongly as before, gut more active, also colon; no effect upon cæcum. (Among the stimulations at this period there was one at 6.23, which had no augmenting effect upon stomach, and the movements of the stomach became definitely less.)

*Experiment 12.*—Gray rabbit, 1500. Ether. 3.35. Good vermicular movements of small gut (stomach not noted). 3.37. L. p. v. 110: strong tonic constriction of antrum, movement of small gut stopped; after stimulation small gut active again for a short while. 3.43. Gut quiet. L. p. v. 110: no effect on stomach or gut. 3.46. Moderate squirming of small gut. L. p. v. 110: contraction of antrum, small intestines become more quiet. 3.50. Left splanchnic (intact) 110: everything quiet. 3.52. Gut quiet. L. p. v. 110: contraction of antrum, gut remains quiet. 3.55. 1.5 c.c. ergot. Shortly after slight waves passing over stomach, very active pendular movements of small gut, good peristaltic waves of colon. 4.02. L. splanchnic 110: everything quiet. A few seconds after stimulation: movements of small gut begin again. 4.07. L. p. v. 110: very powerful contraction of antrum, small gut more active, movements of colon.

4.15. Respiration poor; artificial respiration started and kept up during rest of experiment. 4.19. Good peristaltic waves of small gut; stomach shows occasional moderate peristaltic waves passing towards pylorus. L. p. v. 110: slow, strong contraction of antrum; marked contraction of duodenum and small gut. ("A patch of about 1 centimetre would contract down to a cord and gradually relax; this swept as a wave down the gut.") Colon contracts slowly after stimulation; this effect continues for some time. 4.25. Small gut shows good movements, stomach and colon quiet. L. p. v. 110: antrum moderately constricted, small gut practically contracted into a cord; after stimulation gut relaxed, and no movements. 4.32. L. p. v. 110: the entire lower small gut contracted into a cord.

In some experiments atropin was given; the spontaneous movements of the gut, as well as the effect of vagus stimulations upon these movements, seemed to be more or less affected by it.

As we have stated above, in rabbits we have studied in a number of experiments the movements of the abdominal viscera through the

intact muscles of the abdomen after removing the skin. On the left side of the abdomen in the angle formed by the stomach and the cæcum, a convolution of small intestines can be fairly well observed. Here we have often seen that after injection of ergot the peristaltic movements were distinctly increased. The tonic contractions of the intestines cannot be recognized by this method.

By this method as well as through the normal skin we could study with profit the movements of the cæcum and stomach in rabbits. We shall deal with it in a separate paper. Here we wish only to state that, when the movements of these organs were studied by the methods mentioned, it was found that ergot increases definitely the spontaneous movements of the rabbit's stomach as well as of the cæcum.

Our experiments on the three species of animals show conclusively that intravenous injection of ergot augments the motor activity of the gastro-intestinal canal. The spontaneous movements of the stomach in dogs were unmistakably increased; they were also apparently increased in cats. There was practically no increase in the spontaneous movements of the rabbit's stomach when it was observed in the opened abdomen, but, as we know from other experiments, ergot increases undoubtedly the movements of the stomach when they are not profoundly affected by the abdominal incision.

The spontaneous movements of the small intestines were undoubtedly augmented by ergot in all the three species of animals. There was also some increase of the movements of the colon, although by far not in so striking a manner as in the small gut.

The motor response of the stomach to stimulation was distinctly augmented by the injection of ergot in all the species. The most striking effect of ergot was observed in the influence of vagus stimulation upon the contractions of the intestines. In nearly each and every case in all three species of animals stimulation of the vagi brought out an unmistakable increase of the peristaltic movements, as well as a remarkable tonic contraction of the entire small gut.

The last results are of special interest. As is well known, there is quite a divergence of opinion with regard to the effect which stimulation of the vagi has upon the movements of the intestines. While most of the earlier writers reported that they had seen a contraction of the intestines (Ed. Weber, Budge, Nasse and others), or sometimes an increase and at other times a decrease of the intestinal move-

ments (Pflueger and others), some of the writers stated that in their experiments on dogs stimulation of the vagi had no effect upon the movements of the intestines (Animus and Legros, Pohl) or that there never were any circular contractions (Courtade and Guyon). Recently, in a series of experiments on dogs, cats, and rabbits, Bunch<sup>1</sup> reported that in twenty-five animals he had not seen in a single instance that stimulation of the vagi caused any contraction of the intestine or any increase of the normal rhythmic movements. There could therefore be some doubt whether the vagi contain motor fibres for the intestines. Bayliss and Starling,<sup>2</sup> however, ascribe such failures to simultaneous inhibitory influences, and in studying the effects of the vagi, they took the precaution to destroy the cord or cut the splanchnic nerves. Furthermore these authors observed a motor effect upon the intestines only after repeated stimulations of the vagus nerve, and even then mostly only in the after-effect of a long stimulation. In our experiments, with very few exceptions, the cord was not destroyed and the splanchnic nerves were intact. Nevertheless, after injection of ergot, soon after the beginning of a vagus stimulation and during its continuation, a motor effect of a very striking character appeared frequently (tetanic contraction of a large part of the small gut). This occurred especially after repeated doses of ergot or after a single large dose. The repetition of the stimulus did not seem to have any influence upon the appearance of this striking phenomenon. We are not aware that this was described by any previous observer.

The augmenting effect of a single injection of ergot would seem to wear off gradually and would disappear at first completely in ten or fifteen minutes. After repeating the injections, the effect would at first grow in extent; the increased activity of the spontaneous movements as well as the increased effectiveness of vagus stimulation would last longer. However, with the further continuation of the injections the augmenting effect would reach a certain limit. Instead of peristaltic contractions the gut would be quiet, but would appear to be round and beaded; in short, it appeared to be tonically contracted. Stimulations of the vagus would at first have a prevailing tendency to cause a tonic contraction, later there would be in most cases no effect at all. In a few cases after repeated injections stimulation of

<sup>1</sup> BUNCH: *Journal of physiology*, 1897, xxii, p. 357. See literature there.

<sup>2</sup> BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 99: 1900-1901, xxvi, pp. 107, 125.



the vagus either stopped the movements or relaxed the tonically contracted gut.

In a general way it may be stated that smaller doses of ergot have a tendency to increase the rhythmic contraction of the gastrointestinal canal, larger doses have a tendency to put it to rest in an aggravated tonic state, and still larger doses might occasionally even favor a relaxation of the gut.

It will be remembered that the same is assumed by a great many writers for the effect of ergot upon the uterus: small doses increase the rhythmic activity of the organ, large ones cause a tetanic contraction.

We should mention that in some of our experiments after injection of ergot, stimulation of the vagus distinctly caused a contraction of the colon. Starling and Bayliss state that they have never seen any effect upon the colon from stimulation of the vagus. The same was stated previously by other authors. Pal, however, asserts strongly that in his observations stimulation of the vagus causes contraction of the colon. While we have certainly seen such an effect occurring after the injection of ergot, the effect is apparently by far not so frequent as in the case of the small intestines.

We have mentioned above that Dale, in his recently published interesting researches upon the action of ergot, has also studied its effect upon the stomach and intestines. His statements are very brief. For the stomach he states "That the injection of fifty milligrams of active chrysotoxin intravenously (cat) caused . . . a slight increase in the tone and rhythm of the stomach." The influence upon the effect of the vagus he does not mention. For the intestines he states that "in the 'stimulant stage' in the dog . . . the pendulum movements of the small intestines showed when fifty milligrams of chrysotoxin were given intravenously, a brief inhibition followed by a permanent increase both of the tone of the muscle and the amplitude of the pendulum movements." "The effects were neither striking nor constant, and in any case difficult to interpret." "It is difficult to determine to what extent these effects are due to the changes of blood pressure in the pithed animal or to the progressive asphyxia in the animal without artificial respiration." The last remark we do not fully understand. At any rate, we never worked with animals "in progressive asphyxia without artificial respiration," and our results seem to us to be rather striking. As to the doses, we do not know how fifty milligrams of chrysotoxin compare with the doses

of fluid extract which we have employed. We wish to call attention to the fact that Dale has studied the small intestines "by the balloon method," that is, with a balloon in the small intestines. This method, however, which gives us graphic curves accessible to analysis, is at best, as we believe, capable only of giving information of the state in which the abnormal injured small piece of gut is, which contains the balloon, but not of the condition of the balance of the gut, which also, as Bayliss and Starling pointed out, is not in a normal state, the distending balloon (and the cut in the intestine, see Cannon<sup>1</sup>) affecting it more or less profoundly. We believe, therefore, that by reason of the difference in the method alone our results are not exactly comparable with those of Dale. At the same time it may be pointed out that in a general way Dale also observed the augmenting influence of ergot upon the movements and tonicity of the stomach and intestines, and also that the effect of the vagus stimulation was increased.

Of great interest in Dale's studies are his observations on the action of ergot upon blood pressure and some other functions. He finds that while medium doses of ergot have a stimulating effect, large doses produced a "paralytic stage," so that for example in this stage adrenalin, instead of causing an increase of blood pressure, causes a fall. Dale assumes that large doses of ergot paralyze the motor nerve endings or their "myoneural junctions," leaving the inhibitory fibres unaffected; hence the relaxing effect. It will be remembered that in a few instances we observed that after large doses of ergot stimulation of the vagus caused a stoppage of the peristaltic movements or a relaxation of the contracted gut. We were inclined also to assume that in these cases the large doses of ergot paralyzed the motor fibres, leaving the inhibitory nerve fibres unaffected; hence the inhibitory effect. Dale, however, restricts his theory to the sympathetic nerves, assuming that the cranial (and sacral) nerve supply is not paralyzed by ergot. Future studies will throw light on this interesting question.

Finally, we have to add a word on our experience with the effects of atropin upon the movements of the stomach and intestines. Nearly all investigators state that atropin paralyzes the effect of the vagus upon the stomach and intestines, and reduces the spontaneous movements of the digestive canal. Our own observations were in

<sup>1</sup> CANNON and MURPHY: *Annals of surgery*, April, 1906.

harmony with these statements. Sometimes only one milligram of atropin was sufficient to completely abolish the effect of the vagus upon the stomach, even with ergot. The effect of the vagus upon the intestines seemed to be a little more resistant to atropin. The spontaneous movements were also distinctly reduced, and here again the movements of the stomach seemed to suffer more than those of the intestines. In some cases atropin seemed to be less effective, and a dose which was sufficient to abolish the cardiac inhibition still permitted a motor effect from the vagus upon the stomach; the effect, however, even in these cases, was greatly reduced.

Many recent investigators have used atropin systematically in all their studies of the effect of the vagus upon stomach and intestines. We believe that in these cases atropin was at least one of the factors why in some of these studies the motor effect of the vagus was missing or greatly reduced. Bayliss and Starling have used (it seems regularly) four milligrams of atropin. It is, perhaps, on that account that these authors, even after the elimination of inhibition, have seen the augmenting effect only after repeated stimulations and only after a long stimulation, and then mostly only in the after-effect, while many of the older investigators had no difficulty in observing the motor effect of the vagus upon the intestines without any further preparation. While atropin eliminates the possibility that the motor effect is only secondary to cardiac inhibition, it affects, too, profoundly the effect itself and therefore greatly obscures the observation. We may state that in one case, in which two milligrams of atropin completely abolished the vagus effect upon the stomach, we found that about one hour later the pylorus commenced to respond moderately to vagus stimulation. We assumed then that it was possibly due to a gradual wearing off of the atropin effect.

#### RÉSUMÉ.

Ergot increased the spontaneous movements of the stomach and intestines and augmented the motor effect of the vagus. Moderate doses increased the frequency and energy of the normal movements; large doses had a tendency to produce more tetanic and less rhythmic effects, — a condition similar to that observed in the uterus. After large doses stimulation of the vagus frequently produced a tetanic contraction of a large part of the small intestines. Very large doses

reduced the motor effect of the vagus, and in a few cases its stimulation produced a relaxation of the gut.

Atropin frequently abolished completely the vagus effect upon the stomach and reduced greatly its effect upon the intestines. It impaired in nearly every case also the spontaneous movements of stomach and intestines even after the injections of ergot.

## EXPERIMENTAL STUDIES ON THE PHYSIOLOGY OF THE MOLLUSCS — THIRD PAPER.

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**I**N the two preceding papers<sup>1</sup> upon this subject we have discussed the digestive mechanisms of the marine gasteropod *Sycotypus canaliculatus*, and, in greater detail, the so-called liver of this animal because of its unique function in the storage of copper and zinc. In this paper we wish to present some additional facts developed in our study of the muscle extractives and the respiratory pigments of the blood and tissues.

### THE MUSCLE EXTRACTIVES.

The most important contractile tissue of *Sycotypus*, in point of size, is the large pedal muscle occupying the open end of the shell and, when extended, projecting some distance beyond. It makes up approximately half of the body mass exclusive of the shell. When retracted, it effectually closes the opening of the shell by the chitin-like operculum attached to its anterior end.

In structure the muscle closely resembles other invertebrate muscles. It consists of an intricate network of radial and circular bundles of fibres, with relatively large interstitial spaces for the gradual circulation of blood. A definite circulation can hardly be detected except in the very centre of the muscle mass, where distinct vessels are found with a surrounding sponge of connective tissue and muscle fibres constituting a blood sinus. These vessels and the spongy sinus into which they empty are supplied by the large pedal artery. A somewhat unique feature of this pedal muscle is the fact that all the main nerve trunks present in it lie embedded in this vascular sponge, each in a distinct tube or vessel, — an arrangement by

<sup>1</sup> MENDEL and BRADLEY : This journal, 1905, xiii, p. 17; 1905, xiv, p. 313.

which respiration and nutrition are assured to these more important tissues.

When the foot is extended, as during locomotion, the amount of blood present in the interstitial spaces and in the central sinus is large; while during contraction, the greater portion of it is expelled by the contracting muscles. Thus extension of the foot, as of the proboscis, is apparently accomplished by the relaxation of the circular and radial muscle fibres and by the turgescence of the organ.

The large size of the pedal muscle makes it a most convenient material for the study of invertebrate muscle extractives. They represent the katabolic changes taking place in a muscle whose normal contractions are extremely slow, and are consequently of especial interest when compared with the extractives from an active, rapidly contracting, invertebrate muscle, such for example as the adductor of *Pecten*, or the muscles of *Octopus* and the allied types of molluscs. A large number of pedal muscles were comminuted and extracted with water. By heating and acidifying slightly with acetic acid, the coagulable proteids were removed and the filtrate then concentrated.

**Glycogen.**—When the filtrate was treated with alcohol to about sixty-six per cent, glycogen was precipitated in large amounts. By washing, redissolving, and reprecipitating several times, a fairly pure preparation of glycogen was obtained, giving the characteristic reaction with iodine and yielding dextrose after inversion.<sup>1</sup> Although no estimations were made to determine the amount of glycogen present in the muscle, it was evident from the quantity obtainable that this carbohydrate is present in the muscle in unusual amounts comparable to the glycogen in the liver of well-fed herbivora or in the active adductor muscle of *Pecten*. The fact that so inactive an organ should be provided with so large a supply of reserve carbohydrate can hardly be explained on the supposition that it is a local reserve for the muscle itself, to insure the presence of nutrient material sufficient for intense or prolonged work. The pedal muscle, as has been stated, is one of limited activity. It seems probable that the muscle serves a further function, like the liver of higher animals, in storing up reserve carbohydrate for the general needs of the organism. This suggestion is supported by the fact that no glycogen could be found in the liver of *Sycotypus*, though dextrose was uniformly present there.

<sup>1</sup> The character of the product was further established by an elementary analysis and determination of the specific rotation of a purified sample, by Mr. R. W. LANGLEY.

The alcoholic filtrates from the glycogen separation were concentrated to a syrup and allowed to stand for several days in the cold. At the end of this time crystals of two distinct types had formed, — long needle-shaped prisms suggestive of taurin, and small white burrs. Filtering through cloth under suction and washing with cold water left a mass of nearly white crystals which could be separated by the greater solubility of the taurin in hot water. The residual burr-shaped crystals were then dissolved in boiling water and recrystallized. The substance was found to be magnesium lactate.

**Lactic acid.** — An analysis of the crystals was made with a view to determining whether the lactic acid present in the muscle tissue was the sarcolactic acid characteristic of vertebrate muscle, or fermentation lactic acid. The method of identification depends upon the fact that the zinc salts of the two acids crystallize with different amounts of water, corresponding to the two formulas:  $Zn(C_3H_5O_3)_2 \cdot 2 H_2O$  for the sarcolactate, and  $Zn(C_3H_5O_3)_2 \cdot 3 H_2O$  for the salt of fermentation lactic acid.

The crystals, purified by repeated recrystallizations from hot water, were dissolved in  $H_2SO_4$ , and the solution extracted with ether. The residue obtained by evaporation of the ether was dissolved in water, and  $ZnCO_3$  added to the solution until the reaction was complete. The excess of  $ZnCO_3$  was filtered off and the zinc lactate solution concentrated to crystallization. The salt obtained, after purification, was dried to constant weight at  $100^\circ$  for analysis. The experimental data which follow indicate that the substance was fermentation lactic acid.

Salt dried at $100^\circ$ . . . . .	= 0.5626 gm.
Salt dried at $110^\circ$ . . . . .	= 0.4608 gm.
Salt dried at $140^\circ$ . . . . .	= 0.4605 gm.
Loss of water . . . . .	= 0.1021 gm.
Water of crystallization . . . . .	= 18.15%
Calculated for $Zn(C_3H_5O_3)_2 \cdot 3 H_2O$	= 18.18%

A determination of the ZnO present in the salt was made as a further check:

ZnO found . . . . .	27.45%
ZnO calculated . . . . .	27.26%

The occurrence of fermentation lactic acid alone and in such considerable amounts in the muscle of *Sycotypus* is unique and characteristic of invertebrate muscle metabolism, — at least of molluscan

muscle. In a recent study of the muscle extractives of Octopus, Henze<sup>1</sup> also found that sarcolactic acid was wanting among the extractives, while fermentation lactic acid was normally present. That this acid cannot come from any possible fermentation of the carbohydrate of the tissue, Henze has shown conclusively; for the Octopus muscle contains no glycogen. In our own experiments the possibility of fermentation was precluded by using only the living tissues, under conditions which excluded bacterial action.

**Taurin.** — The solution of taurin was concentrated, decolorized, and alcohol added to incipient crystallization. Upon cooling, a mass of fine crystals with the characteristic prism form of taurin separated. The characteristic solubilities, the presence of both sulphur and nitrogen, and the high temperature of decomposition gave further proof of the identity of this compound. In samples purified for analysis decomposition was found to begin at 280° to 290° without any previous fusion. This was found to correspond closely to the decomposition figures for taurin obtained from the bile.

Analyses were made to determine the sulphur and nitrogen content of the compound as the final proof of its identity. Nitrogen was estimated by the Kjeldahl-Gunning method, giving 11.37 per cent. Calculated for  $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-SO}_3\text{H}$ , the nitrogen is 11.22 per cent. Sulphur was determined after fusion with sodium hydrate and potassium nitrate, by precipitating it as barium sulphate. The sulphur found constituted 25.89 per cent of the compound, 25.62 per cent being the calculated amount.

The occurrence of taurin in the muscle of invertebrates has been known since 1845, when Karsten<sup>2</sup> isolated a crystalline body from the watery extracts of certain molluscs, and identified it qualitatively with the taurin obtained from the bile. Since that time the list of molluscs known to have taurin as a normal constituent of their muscle extractive has been greatly extended by the work of Kruckenberg<sup>3</sup> until it includes the following: *Doriopsis*, *Turbo*, *Cassidaria*, *Mytilus*, *Ostrea*, *Pinna*, *Arca*, *Spondylus*, *Pectunculus*, *Pecten*, *Murex*, *Octopus*, *Loligo*, and *Sepia*. Recently Agnes Kelly<sup>4</sup> determined the amounts of taurin present in the tissues of *Pecten*

<sup>1</sup> HENZE: *Zeitschrift für physiologische Chemie*, 1904, xliii, p. 477.

<sup>2</sup> KARSTEN: *Nova Acta Academiæ Leopoldino-Carolinæ*, Serie 21, 1 Teil, 1845; VON FÜRTH: *Vergleichende chemische Physiologie*, p. 207.

<sup>3</sup> KRUCKENBERG: *Cf. von Fürth, loc. cit.* p. 437.

<sup>4</sup> KELLY: *Beiträge zur chemischen Physiologie*, 1904, v, p. 377.



opercularis and the common mussel, *Mytilus edulis*, while Mendel<sup>1</sup> and Jaffa in this laboratory isolated and identified the same body in the pedal muscle of *Haliotis*, the common abalone of the Pacific coast. Evidently taurin is a characteristic compound formed in the molluscan muscle katabolism. The finding of it in the invertebrate muscle is of particular physiological interest in view of the close relationship which has recently been shown to exist between taurin, cystin, and the proteids. In many invertebrates taurin is accompanied by glycocholl, as was shown first by Chittenden<sup>2</sup> in his examination of the extractives of the adductor muscle of *Pecten irradians*. We have been unable to isolate any glycocholl from the muscle extracts of *Sycotypus*. If glycocholl is present in this case, it must be in insignificant amounts.

After the removal of the lactates and taurin, the filtrate was treated with a slight excess of lead acetate and the insoluble lead salts filtered off. The excess of lead was removed as the sulphide, and the filtrate concentrated. A second crop of taurin crystals was obtained at this point, but no glycocholl. The purin bodies were precipitated as silver compounds, the precipitate, washed with dilute ammonia, dissolved in hot 5 per cent nitric acid, and the solution slowly cooled.

**Hypoxanthin.**—A precipitate of hypoxanthin silver nitrate separated from the cold acid solution in characteristic microscopic crystals. It was decomposed by hydrogen sulphide to remove the silver, and the hypoxanthin nitrate allowed to crystallize. The compound gave the typical hypoxanthin reactions—negative murexide test, and (after reduction with zinc and hydrochloric acid), a red color with alkali in excess.

**Xanthin.**—The filtrate from the hypoxanthin silver nitrate was made ammoniacal again to precipitate the xanthin silver. The latter was then digested with hydrogen sulphide until the silver was completely separated as silver sulphide. The solution containing the free xanthin was then evaporated to dryness, and the yellow residue gave a typical murexide reaction for xanthin. The bulk of the residue was dissolved in warm dilute potassium hydrate solution and made up to a volume of about 200 c.c. Following Horbaczewski's method, acetic acid was added until the solution was faintly acid, and then alcohol

<sup>1</sup> Cf. MENDEL: Beiträge zur chemischen Physiologie, 1904, v, p. 582.

<sup>2</sup> CHITTENDEN: Annalen der Chemie und Pharmacie, 1875, clxxviii, p. 266; American journal of science, 1875, (3), x, p. 26.

until the volume was about 300 c.c. This dilute, faintly acid, alcoholic solution deposited the xanthin in large perfect burrs of plates in the course of several days. The crystal form and the characteristic solubilities of the xanthin compounds, together with the murexid test, served to identify it.

The muscle of *Sycotypus* has thus been shown to contain the following typical extractive compounds in significant amounts: Glycogen, lactic acid (fermentation), taurin, hypoxanthin, and xanthin. The most important recent contribution to the subject of invertebrate muscle katabolism is the previously mentioned paper by Henze upon the extractives of *Octopus* muscle. He likewise found taurin, fermentation lactic acid and hypoxanthin present; and glycocoll was wanting as in the muscle of *Sycotypus*. The most striking difference between the muscles of these two molluscs is the glycogen content. *Octopus* muscle contains no glycogen, while the tissue of *Sycotypus* is exceptionally rich in it. In the case of *Octopus* the carbohydrate of the muscle is apparently combined in the form of a glycoproteid, as boiling with acid produced a reducing sugar.

#### THE BLOOD.

The blood of *Sycotypus* consists of formed elements and plasma. The formed elements — large amœboid cells with characteristic long pseudopodia — are comparatively few. They are quite typical of molluscan blood in general, and exhibit no characteristics peculiar to this gasteropod. The plasma, on the other hand, is of interest because of the nature of the respiratory proteid which it holds in solution. Incision into the pedal sinus is followed by an abundant flow of the opalescent, nearly colorless blood, squeezed out forcibly by the reflex contraction of the muscle. In contact with the air, the blood changes from a colorless to a deep blue solution. For this reason and from the fact that copper is present in loose combination, we may call its respiratory proteid, provisionally at least, a hæmocyanin. Further study has shown it to differ in several important respects from other hæmocyanins.

In general the hæmocyanins — for there seems to be good evidence that the bloods of various species contain somewhat different forms of this respiratory proteid — are characterized by the presence of copper in proteid combination analogous to the iron of hæmoglobin. They are all colorless in the reduced condition, deep blue in

the oxidized or oxygenated state. As a rule, they coagulate at about 63°. Octopus hæmocyanin can be crystallized from its solutions.

Although it had been well known for some time that certain marine animals have blood of a characteristic blue color, Frédéricq<sup>1</sup> was the first to study these bloods quantitatively. He showed the uniform presence of copper, the evident analogy between the presence of this metal and the iron of hæmoglobin, and the dependence of the compound for its respiratory efficiency upon the metallic combination. He also suggested the name hæmocyanin for the blood proteid of this type. Since then it has been found that the hæmocyanins are of very wide distribution; not only are they common among the marine molluscs, but they are also found in many land types, in the crabs, arthropods, etc.

Owing to the readiness with which it may be secured, cephalopod blood has been the subject of the most careful investigation. Griffiths<sup>2</sup> found its proteids to contain: Nitrogen = 16.27 per cent; copper = 0.33 per cent. The hæmocyanin in this case was purified by precipitation with  $MgSO_4$ , resolution and reprecipitation in alcohol. Henze,<sup>3</sup> after showing the possibility of obtaining hæmocyanin in crystalline form, separated the latter, and obtained analytical figures, nearly identical with those of Griffiths: Nitrogen = 16.08 per cent; copper = 0.30 per cent. In our examination of the blood of *Sycotypus* we obtained copper and nitrogen figures quite different from those of Griffiths and Henze. Instead of the ratio N 16 per cent: Cu 0.35 per cent, we have N 14 per cent: Cu 0.043 per cent. That is, the blood proteids of *Sycotypus* contain less nitrogen than those of Octopus, and only one-tenth of the copper content of the latter. The lack of copper is in part supplemented by the presence of zinc in *Sycotypus* hæmocyanin, as is shown in the table of analyses (see p. 174).

The hæmocyanin of the blood was obtained fresh in each case from the pedal sinus, by precipitation with alcohol, washed with a large amount of the latter, and finally dehydrated with pure alcohol and ether, and dried to constant weight for analysis.

"**Hæmosycotypin.**" — Evidently in the respiratory proteid of *Sycotypus* we have a hæmocyanin-like compound in which zinc as well as copper is normally present. Qualitative tests made at intervals

<sup>1</sup> FRÉDÉRICQ: Comptes rendus de l'académie des sciences, 1878, lxxxvii, p. 996.

<sup>2</sup> GRIFFITHS: *Ibid.*, 1892, cxiv, p. 496.

<sup>3</sup> HENZE: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 370.

covering a period of over two years, on animals dredged from widely separated portions of Long Island Sound, all show the uniform presence of zinc in the blood. The zinc of the liver and the zinc of the blood are presumably interdependent and coexistent in *Sycotypus*. For this reason, and because of certain other differences between this compound and the typical hæmocyanins — such, for example, as the failure to obtain it in crystalline form and its low nitrogen content — we provisionally propose the name of “hæmosycotypin” for this respiratory proteid.

## BLOOD ANALYSIS.

Ash.	Cu in ash.	Cu in tissue.	Zn in ash.	Zn in tissue.	N.
per cent	per cent	per cent	per cent	per cent	per cent
8.83	0.506	0.045	0.332	0.029	14.38
9.12	0.445	0.041	0.284	0.026	14.35
Av. 8.97	0.474	0.043	0.305	0.027	14.37

The more active an animal is in its metabolic processes, the more rapid must be its respiratory exchanges also. As a rule, therefore, those groups of animals which exhibit the most pronounced activity — such as the vertebrates, for example — are provided with the most perfect circulatory mechanisms and the most efficient respiratory pigment — hæmoglobin. Forms of low metabolic activity require less perfect circulatory systems and less efficient oxygen carriers. In the mollusc family, therefore, the greatest variety of respiratory pigments is to be found. The most striking features of these proteids are their characteristic metallic elements; copper is found most frequently, iron less so, manganese and zinc in rare or unique instances. Thus the hæmocyanins are the most common molluscan oxygen carriers, hæmoglobin and hæmorhodin prominent, while pinnaglobin and “hæmosycotypin,” containing respectively manganese and zinc, are only known in rare or isolated instances.

Not only do the respiratory proteids vary in composition and in their characteristic metallic content, but the oxygen-carrying efficiency of bloods with the same metallic element varies. This fact has been suggested in the case of the true hæmocyanins at least. Dhéré<sup>1</sup> pointed out that the efficiency of a hæmocyanin varies with

<sup>1</sup> DHÉRE: Comptes rendus de la société de biologie, 1900, lii, p. 52.

the amount of copper present in it, and that the amount of oxygen absorbed is proportional to the content of copper. Thus *Octopus hæmocyanin* contains ten times the amount of copper present in "hæmosycotypin"; presumably its efficiency in carrying oxygen is proportionately larger; and this difference expresses fairly well the difference in the general activity of the two molluscs, *Octopus* being a highly active, rapidly moving type of mollusc, with highly developed sense organs, while *Sycotypus* typifies the other extreme. Dhéré's analyses of the blood of another mollusc of low metabolic type — *Helix pomatia* — show a similar difference in the constitution of the hæmocyanins. In 100 c.c., *Octopus* blood yielded 18 to 23.5 mgm. of copper; *Helix* blood only 6.5 to 12.5 mgm.; that is, the blood from *Octopus* has a copper content decidedly greater than that of *Helix*.

**Hæmoglobin.** — In many of the gasteropods the pharynx muscles operating the apparatus for comminuting the food are pigmented red. In some the red pigment seems to be associated with the muscle plasma only, and can thus be readily separated from the tissue by washing. In others the pigment is also fixed in the muscle cells themselves; and this fraction is much more difficultly separable. In the case of the pharynx muscles of *Sycotypus* which are thus pigmented, the coloring compound is present both in the plasma and in the muscle cells. The heart muscles are also pigmented, as are the ganglia and main nerve trunks, though the identity of the pigment in the latter is less certainly determined.

From the pharynx muscles a colored solution can be obtained by grinding the tissue in a mortar and extracting with water. As was mentioned in a former paper,<sup>1</sup> this pigment is identical, apparently, with oxyhæmoglobin from vertebrate blood. It gives the characteristic spectral absorption bands, it can be reduced with Stokes's fluid to hæmoglobin, and on shaking with air may be reoxidized to oxyhæmoglobin. It will also give the hæmin crystals.

The heart muscles are much less brilliantly pigmented, and the pigment is much more firmly fixed in the tissue; but it is possible to separate enough of the compound to show the characteristic absorption bands of oxy- and reduced hæmoglobin. The pigment of the nerve trunks and ganglia could not be identified with any great accuracy because of the small amount of material and the difficulty of separating the pigment. What slight evidence was obtained

<sup>1</sup> MENDEL and BRADLEY: *Loc. cit.*

indicates that the nerves and ganglia are also pigmented with hæmoglobin.

What the significance of this pigment in the heart and pharynx muscles is we may conjecture from the functions of these organs. They are the only muscles of *Sycotypus* that are required to perform rhythmic contractions over long periods of time. The heart contracts rhythmically, though slowly, and must continue to do so when the animal's respiration is seriously interfered with. The heart must therefore have a more certain provision for this continued activity than the pedal muscle, for example, would require. The pharynx muscle in the same way is required at certain times to contract rhythmically and rapidly, as when food is being comminuted, so that it too is provided with a special mechanism for tissue respiration. The importance of the hæmoglobin as a respiratory pigment in these organs of exceptional activity in the animal is apparent when the superiority of this pigment over hæmocyanin as an oxygen carrier is recalled. Henze showed that the efficiency of *Octopus* hæmocyanin is about one-fourth that of hæmoglobin, and we may safely assume that the efficiency of "hæmosycotypin" is much less, because of its smaller copper content. It is adapted to the demands of the low metabolism of the animal as a whole, but in those more active organs mentioned where a more rapid respiration is essential, a more efficient oxygen carrier has been provided in the course of the animal's evolution.

#### SUMMARY.

The pedal muscle of *Sycotypus canaliculatus* is shown to contain glycogen in abundance, taurin, hypoxanthin, xanthin, and fermentation lactic acid. Glycocoll was not detected. The muscle extractives of this gasteropod thus closely resemble those obtained from different types of molluscs by Henze and others.

The blood proteids of *Sycotypus* contain both copper and zinc, differing from the familiar hæmocyanins in the occurrence of the latter element and in the proportion of copper present.

Hæmoglobin is present in some of the more active tissues, such as the heart and pharynx muscles. The significance of some of these peculiarities in the distribution of the "respiratory" pigments is briefly discussed.

# ON THE POINT OF ACTION OF DRUGS ON THE HEART WITH SPECIAL REFERENCE TO THE HEART OF LIMULUS.

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## I. INTRODUCTION.

THE study of the action of drugs on the *Limulus* heart was undertaken with a twofold purpose, namely, to compare the pharmacology of this heart with that of the vertebrate heart, and, second, by determining whether a certain drug exerts its specific action on the heart ganglion or on the heart muscle or on both, to throw some light on the mechanism of action of these drugs on hearts in which the nervous and the muscular tissues cannot be separated for experimental purposes. My purpose in comparing the pharmacology of the *Limulus* and the vertebrate hearts was mainly to obtain further data on the fundamental similarity (or, if you please, dissimilarity) between the nature of the heart rhythm in *Limulus* and the vertebrates. In so far this paper is a continuation of the work on the *Limulus* heart already reported in this journal. I have stated before that the demonstrations of the neurogenic nature of the rhythm and conduction in the *Limulus* heart are demonstrations for that heart only; but if it is shown that there are no fundamental differences in the reactions and the behavior of the *Limulus* and the vertebrate hearts, the probability that the mechanism of the heart rhythm is the same in both becomes stronger.

The literature on the pharmacology of the vertebrate heart is very extensive. While there may be said to be a fairly general agreement as to the specific action on the heart of many drugs in therapeutic doses in the intact animal as well as on the isolated heart, the interpretations of the mechanism of this action of the drug on the heart are based almost entirely on analogies and unproven assumptions. The adherents of the myogenic theory of the nature of the heart beat

ascribe the effects of most drugs — except those that are supposed to act mainly on the inhibitory nerve endings — to a direct action on the heart muscle. If the automatism of the heart is due to the peculiar properties of the heart muscle, the mechanism of action of a drug on an isolated heart must be relatively simple. The drug may augment or depress the rhythm by acting directly on the automatic heart muscle or by stimulating the augmentor or inhibitory nerves and nerve endings in the heart. A depressor action of a drug on the augmentor or inhibitory nerves and nerve endings would not affect the rhythm of the isolated heart, as there seems to be no evidence that the extrinsic heart nerves have any "tonic" action on the heart after these nerves have been severed from the central nervous system.

If the neurogenic theory of the nature of the heart rhythm represents the actual facts, however, the possible mechanisms of action of a drug on the isolated heart become more numerous. The heart rhythm may be augmented or inhibited by the drug acting (1) on the heart muscle in a way to increase or decrease excitability and contractility; (2) on the automatic ganglion or ganglia in a way to augment or inhibit both the intensity and the rate of the nervous discharges; (3) on the nerves (and nerve endings) leading from the ganglia to the heart muscle in a way to increase or decrease their excitability and conductivity, and lastly (4) on the vagus and accelerator nerves and nerve endings in connection with the automatic ganglia, the nerve plexus, or the heart muscle. We have practically no direct knowledge enabling us to decide between these or any combination of these possible points of action of the drug on the heart, because no one has so far succeeded in separating the nervous and the muscular tissues in the vertebrate heart for experimental purposes. It is obvious that I refer to the action of minimal or therapeutic doses of the drugs. At sufficient concentration all drugs will apparently act on all forms of protoplasm, but in minimal strengths many drugs appear to have a "selective" action, attacking one tissue while leaving another practically unaffected. This selective action of therapeutic doses must, of course, be due to some physico-chemical differences in the tissues. Accurate determination of this selective action is therefore a valuable step in furthering our knowledge of the mechanisms of actions of the drugs on the living tissues.

For a description of the method of preparing the *Limulus* heart for



these experiments the reader is referred to the preliminary paper and to the paper on the action of the temperature variations on the *Limulus* heart.<sup>1</sup> It will be seen from these descriptions and figures that under conditions allowing ordinary and exceedingly accurate graphic registration of the heart rhythm the action of a drug in solution can be confined either to the heart ganglion or to the heart muscle (and nerve endings) at will. The graphic method was used exclusively, as the action of most of the drugs in dilute solution would escape direct observation. The conclusions as to the action of any one drug are based on experiments on at least ten different hearts. In the cases of most drugs the experiments were repeated on many more preparations, in order to eliminate accidental errors or individual variations.

The drugs were dissolved in *Limulus* plasma or sea water. Isotonicity of the medium is thus maintained, except in so far as the osmotic pressure of the plasma or sea water is directly altered by the drug, or altered in reference to the heart tissues by changing the permeability of the latter.

Errors from temperature variations were excluded by using all solutions at the same temperature, namely, that of running sea water. It has been shown by Newman that the *Limulus* heart tissues exhibit a relatively great resistance to the lack or deficiency of oxygen in the surrounding medium. Slight differences in the oxygen tension of the different solutions employed may therefore be left out of account.

The drugs used were, with the exception of adrenalin and ergot, of Merck's manufacture.

In reporting the results I have adopted the plan of first describing the separate action of the drug on the heart ganglion and the heart muscle as well as on the whole heart of *Limulus*, and then by comparing these results with the known action of the same drug on the vertebrate heart suggest the probable mechanism of action of the drug in the frog and mammal. A comparison between the pharmacology of the *Limulus* heart and the vertebrate heart is not easily made with our present data, because the data on the action of drugs on the intact frog or mammal cannot be used, owing to indirect effect on the heart rhythm from the action of the drug on other tissues. Only the data on the action of the drugs on the isolated heart are available for this comparison. And even these are not

<sup>1</sup> CARLSON: *Science*, 1904, xx, p. 684; this journal, 1906, xi, p. 207.

strictly comparable to my data on the *Limulus* heart, because the rhythm of the whole heart of frog or mammal is much more complicated than that of the isolated *Limulus* heart, particularly in the mechanism of co-ordination and conduction between the different parts of the heart. A series of data on the action of these drugs on the isolated sinus venosus of vertebrates would be comparable with the data on the isolated *Limulus* heart, because in the former the factors of conduction and co-ordination would be to a great extent eliminated. Such a series of observations should be made.

For the comparison of the action of these drugs in *Limulus* and the vertebrates I have received much assistance from the last editions (1906) of the textbooks of pharmacology of Cushney and Sollmann in the way of reference to the literature.

A word in the way of explanation regarding the interpretation of the action of the drugs on the ganglion-free part of the *Limulus* heart is perhaps in place. When the solution of a drug is applied to the ganglion-free anterior end of the heart, the contraction of that part of the heart may continue unchanged, in which case the drug has little or no action either on the muscle or on the motor nerves and nerve endings, or the beats may be weakened or strengthened. This change in the amplitude of the contractions may be due to the action of the drug either directly on the muscle or on the nerves and nerve endings, or on both. It is also conceivable that the influence of the drug on the two tissues may be in opposite directions, that is, stimulation of the one and depression of the other. In fact, I have some evidence pointing to such conditions in the case of some drugs. But in this report I speak, provisionally, of the influence of the drugs on the ganglion-free part of the heart as due to the action on the muscle. Experiments are in progress in this laboratory to determine whether it is purely or even mainly a muscular action.

In the experiments to determine the action of a drug on a ganglion-free part of the heart simultaneous records were taken from the two ends of the heart, so as to be sure that changes in the rhythm of the ganglion-free part were not due to changes in the activity of the ganglion.

## II. THE ACTION OF ALCOHOL.

1. **The heart ganglion of *Limulus*.** *Ethyl alcohol has a primary stimulating action on the *Limulus* heart ganglion.* — The heart ganglion of this animal is, however, not very sensitive to the action of alcohol.

In a solution of 1 c.c. of alcohol to 500 c.c. of plasma or sea water the primary stimulating action of the alcohol is very slight, and the ganglion will continue in activity in this mixture for several hours. One per cent alcohol in plasma or sea water produces immediate stimulation of the ganglion, and in greater concentrations the augmented rhythm becomes irregular and the stimulating phase is soon followed by depression and ultimate paralysis. In the weaker

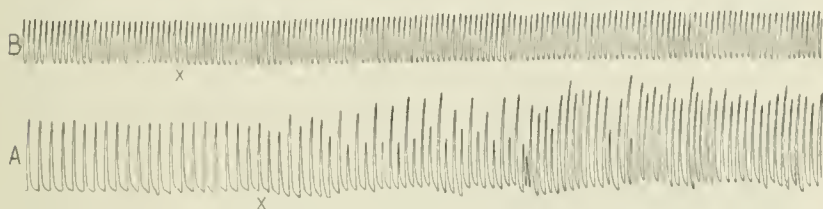


FIGURE 1.—Four-sevenths the original size. Tracings from the anterior end of the *Limulus* heart. *A*,  $x$ ,  $\frac{1}{2}$  per cent alcohol in plasma applied to the isolated ganglion.—Showing primary stimulation. *B* (ganglion extirpated from anterior end of heart),  $x$ , 2 per cent alcohol in plasma applied to anterior end. Showing primary stimulation of ganglion-free part of the heart.

concentrations the stimulating action appears only in increased intensity of the nervous discharges, the rate remaining unaltered, but in the stronger concentrations the rate is also augmented.

**2. The heart muscle of *Limulus*.** *Ethyl alcohol has a primary stimulating action on the Limulus heart muscle.*—This stimulating influence appears in gradually increasing amplitude of the contractions when the ganglion-free anterior end of the heart is bathed in a mixture of plasma and alcohol. The rate of the rhythm can, of course, not be affected when the drug does not reach the heart ganglion. Solutions of alcohol and plasma that are just able to exhibit a stimulating influence on the heart ganglion (1 part alcohol to 500 parts plasma) do not appear to influence the heart muscle at all, but in greater concentrations ( $\frac{1}{2}$  per cent, 1 per cent, 2 per cent) the primary stimulating action becomes evident in the stronger contractions.

Ethyl alcohol has therefore the same action on the two tissues of the *Limulus* heart. The depression and ultimate paralysis caused by the alcohol is preceded by a primary stimulating phase both of the heart muscle and the heart ganglion; the only difference between the two tissues in their reactions to the drug is the relatively greater sensitiveness of the ganglion.

3. **The vertebrate heart.** — The actual primary influence of alcohol on the vertebrate heart has been, and is still, apparently, a matter of controversy. The Schmiedeberg school of pharmacologists contend that alcohol has no primary stimulating action on the heart. According to the Schmiedeberg school, the accelerated rhythm following administration of alcohol in the mammal is an indirect effect of increased muscular activity and depression of cerebral inhibitory centres, and not due to any direct stimulating action on the heart itself. Wood and Hoyt<sup>1</sup> (1905) have, however, recently shown that small amounts of alcohol augment the force of the heart beat in the frog, and Loeb<sup>2</sup> has shown that the same is true for the isolated mammalian heart. It would therefore seem that alcohol in weak concentrations has a slight primary stimulating action on the vertebrate heart, just as it has on the *Limulus* heart. In stronger concentrations this stimulating phase is probably of such a brief duration that it may readily escape observation. There appears to me no good reason for holding that the stimulating action of alcohol on the vertebrate heart is solely on the heart muscle. Lee and Salant<sup>3</sup> have shown that small amounts of alcohol stimulate the skeletal muscle of the frog, and we have seen that alcohol similarly stimulates the *Limulus* heart muscle. Waller has shown that alcohol causes an initial increase in excitability of nerve. Alcohol causes an initial augmentation of voluntary contractions in man. The "stimulant phase" in the action of alcohol is just as readily explained by a stimulation of the cerebral centres as by depression of possible inhibitory centres or nervous impulses. There can be no doubt that alcohol has a primary stimulating action on skeletal muscle. In the *Limulus* heart alcohol similarly stimulates both the muscle and the ganglion. And as alcohol has a similar stimulating action on the vertebrate heart, it seems probable that it acts both on the heart muscle and on the heart ganglia.

### III. THE ACTION OF ETHER, CHLOROFORM, CHLORETON, CHLORAL HYDRATE.

1. **The heart ganglion of *Limulus*.** *The anæsthetics chloroform, ether, chloral hydrate, and chloreton have a primary stimulating action on the*

<sup>1</sup> WOOD and HOYT: University of Pennsylvania medical bulletin, 1905, xviii, p. 70.

<sup>2</sup> LOEB, O: Archiv für experimentelle Pathologie und Pharmakologie, 1905, lii, p. 459.

<sup>3</sup> LEE and SALANT: This journal, 1902, viii, p. 61.

*Limulus* heart ganglion.—The ultimate depression and paralysis of the ganglion produced by these anæsthetics is always preceded by stimulation. This primary stimulating action is the more intense and of briefer duration, the stronger the concentration of the anæsthetic. The stimulating action on the ganglion appears both in the rate and in the intensity of the nervous discharges, mainly in the latter in case

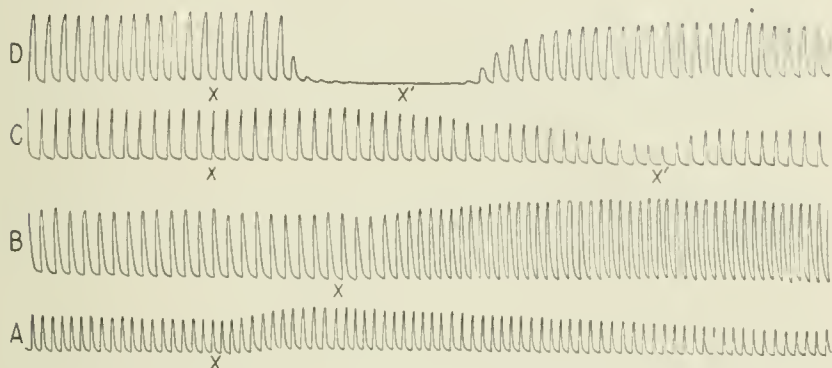


FIGURE 2.—About two-thirds the original size. Tracings from the anterior end of the *Limulus* heart. In *A*, *C*, *D*, the ganglion was removed from the anterior end. *x*, application of the drugs. *x'*, the drugs replaced by plasma or sea water. *A*, chloroform 1-5000 on ganglion-free end. Showing primary stimulation. *B*, chloreton 1-5000 on the isolated ganglion. Primary stimulation. *C*, chloreton 1-1000 on the heart muscle. *D*, chloreton 1-500 on the heart muscle. Primary depression.

of weak concentrations of the drugs. In relatively strong concentrations the augmented rhythm of the heart ganglion becomes irregular prior to the depression and final paralysis. In equal concentrations chloroform stimulates the ganglion much more intensely than ether. Chloroform also acts more strongly than chloreton, the latter occupying a position midway between chloroform and ether. Thus 1 c.c. chloroform dissolved in 10,000 c.c. plasma usually stimulates the ganglion as intensely as the same amount of ether dissolved in 1000 c.c. of the plasma.

2. **The heart muscle of *Limulus*.** *Ether, chloral hydrate, and chloreton in concentrations that appreciably affect the heart muscle produce depression with no indication of a primary stimulation. Chloroform tends to produce tonus contraction prior to and simultaneous with the depression of the beats.*—The depression can, of course, be manifested only in the amplitude of the beats. These drugs, with the possible exception of chloroform, have therefore an opposite action on the

heart muscle from that on the heart ganglion. But the heart muscle is less sensitive to the action of these drugs than is the heart ganglion, as is shown by the fact that concentrations of these drugs too weak to affect appreciably the muscle, except very gradually, cause a distinct stimulation of the ganglion. In consequence of this, the depressor action on the heart muscle does not obscure or counteract the stimulating action on the ganglion when the whole heart is subjected to the action of weak solutions of the drug. These weak solutions always stimulate the whole heart.

3. **The vertebrate heart.** — Chloroform and ether appear to depress the vertebrate heart (frog, dog) without any primary stimulation

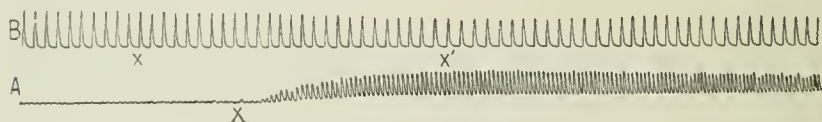


FIGURE 3.— About one-half the original size. Tracings from the anterior end of the *Limulus* heart. *A* (preparation in poor condition), *x*, ether in plasma 1-1000 applied to isolated ganglion. Showing primary stimulation. *B*, ether in plasma 1-500 applied to the ganglion-free anterior end. *x'*, the anæsthetic replaced by sea water. Showing primary depression.

similar to that on the *Limulus* heart ganglion. Hedbom<sup>1</sup> found that chloral hydrate has a primary stimulating action on the isolated mammalian heart, and according to Harnack and Böhme<sup>2</sup> chloral hydrate acts as a primary stimulant to the frog's heart. According to some investigators, however, chloroform and ether have a primary stimulating action on other tissues. Waller found that these anæsthetics increased the excitability of motor nerves. There is some evidence that chloroform stimulates the vaso-constrictor centres. It is also said to constrict the coronary vessels. The "excitement" stage in chloroform and ether anæsthesia may be due to a primary stimulating action of the drugs on the central nervous system. Pickering<sup>3</sup> found that chloroform and ether stimulate the embryonic heart (chick). The stimulating action is particularly marked with ether. In the case of chloroform Pickering concludes that it has a depressant action, but in the experiment he cites the heart rate is increased from 120 to 140 per minute, while the rhythm is at the same time rendered irreg-

<sup>1</sup> HEDBOM: *Skandinavisches Archiv für Physiologie*, 1899, ix, p. 22.

<sup>2</sup> See ROHDE: *Archiv für experimentelle Pathologie und Pharmakologie*, 1905, liv, p. 104.

<sup>3</sup> PICKERING: *Journal of physiology*, 1893, xiv, p. 444.

ular. We have seen that these anæsthetics have an opposite action, on the *Limulus* heart muscle from that on the *Limulus* heart ganglion. It is possible that in the case of the vertebrate heart the depressor action on the muscle obscures or overbalances any slight stimulating action on the heart ganglion.

#### IV. THE ACTION OF STRYCHNIA.

1. **The heart ganglion of *Limulus*.** *Solutions of strychnia sulphate in plasma or sea water act as primary stimulants to the heart ganglion.* — In relatively strong concentrations the stimulating phase is followed by irregularity in the rhythm, depression, and ultimately paralysis of the ganglion. Distinct stimulation of the ganglion is produced by strychnia in dilutions of 1 part of the drug to 5000 parts of plasma. At this dilution the stimulation appears only in the increased intensity of the nervous discharges, the rate of the nervous discharges remaining unaltered. In concentrations of 1 to 4000 or 1 to 2000 the rate of the ganglionic activity is also augmented. The stimulating action of strychnia on the heart ganglion is counteracted by calcium chloride.

2. **The heart muscle of *Limulus*.** *Strychnia has no distinct effect on the heart muscle of *Limulus*, except a gradual depression when applied in strong concentrations.* — In strength of 1 part of the drug to 4000 or 5000 parts of plasma, no specific effect on the muscle could be detected; in concentrations of 1 part to 300 or 400 there appeared to be a gradual depression. In no case did I observe a primary stimulating action of strychnia on the heart muscle, and we are therefore forced to the conclusion that this drug has primarily an opposite action on the two tissues composing the *Limulus* heart, namely, a strong stimulating action on the ganglion, and only a slight action on the muscle (or nerve endings) in the direction of depression of excitability and contractility.

3. **The vertebrate heart.** — The literature on the action of strychnia on the vertebrate heart is very conflicting. It seems probable that the changes in the heart rhythm following the administration of small doses of strychnia in the intact mammal are not due to a direct action of the drug on the heart. Pickering<sup>1</sup> found that strychnia has a primary stimulating action on the embryonic heart. Hedbom<sup>2</sup> has

<sup>1</sup> PICKERING: *Journal of physiology*, 1893, xiv, p. 457.

<sup>2</sup> HEDBOM: *Skandinavisches Archiv für Physiologie*, 1899, ix, p. 45.

shown that in relatively strong doses strychnia augments the rhythm of the isolated mammalian heart. Igerscheimer<sup>1</sup> concludes, however, that strychnia depresses the isolated heart of both frog and mammal without primary stimulation. We are justified in concluding that at least in certain conditions of the heart strychnia has primarily the same action on the vertebrate heart as on the *Limulus* heart, namely, stimulation followed by depression and paralysis. In the case of *Limulus* the stimulating action is confined to the heart ganglion. In the subsequent depression and paralysis the direct action of the drug on the heart muscle is probably a factor. The fact that strychnia stimulates nerve centres, but has scarcely any action on the skeletal muscles in vertebrates is an additional evidence that the stimulating action of the drug on the vertebrate heart is similarly due to its action on the motor heart ganglia.

## V. THE ACTION OF CAFFEIN.

**1. The heart ganglion of *Limulus*.** *Solutions of caffein in plasma or sea water stimulate the Limulus heart ganglion.*—The stimulating action of caffein on the ganglion appears in dilutions represented by 1 part of the drug to 5000 or 10,000 parts of plasma. At this dilution the rate of the ganglionic rhythm remains unchanged, but the intensity of the nervous discharges from the ganglion is augmented. In greater concentrations the rate of the ganglionic rhythm is also increased. In concentrations represented by 1 part of the drug to 200 or 500 parts of plasma the augmented rhythm of the ganglion soon becomes irregular, and this is followed by depression and paralysis. The stimulating action of caffein on the *Limulus* heart ganglion is counteracted by calcium chloride.

**2. The heart muscle of *Limulus*.** *The Limulus heart muscle is less sensitive to the action of caffein than the heart ganglion, but the primary action of the drug is the same on both tissues, i. e., stimulation.*—Solutions of the drug represented by 1 part to 5000 parts of plasma have no appreciable effect on the heart muscle, although even at this dilution the drug is able to stimulate the ganglion. Stronger solutions (1 to 500, 1 to 1000) of caffein strengthen the contractions when applied to the ganglion-free anterior end of the heart.

**3. The vertebrate heart.**—The literature is practically unanimous

<sup>1</sup> IGERSCHEIMER: Archiv für experimentelle Pathologie und Pharmakologie, 1906, liv. p. 73.



in ascribing to caffeine a primary stimulating action on the vertebrate heart. This is true for the isolated mammalian heart (Hedbom), for the embryonic heart of the chick (Pickering), as well as for the heart of cold-blooded vertebrates. But as caffeine has a primary stimulating action both on the central nervous system and on the skeletal muscles in the vertebrates, and the same action, differing only in degree

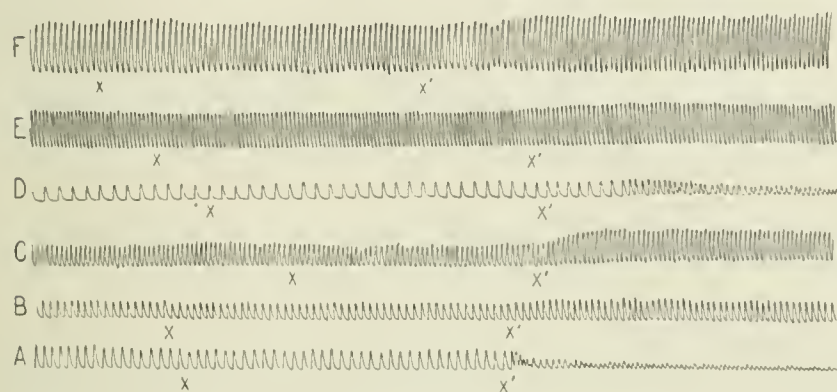


FIGURE 4. — About one-half the original size. Record from the anterior end of the *Limulus* heart. The ganglion extirpated in first two segments, the lateral nerves being left intact. *x*, application of the drug to the ganglion-free anterior end. *x'*, application of the drug to posterior end of the heart containing the ganglion. *A*, nicotin alkaloid in plasma 1-500,000. *B*, atropin sulphate in sea water 1-4000. *C*, strychnia sulphate in plasma 1-2000. *D*, veratrin in plasma 1-800,000. *E*, caffeine in plasma 1-2000. *F*, curare in plasma 1-1000. Showing primary stimulation of the ganglia by these drugs, and greater sensitiveness of the ganglion than the ganglion-free part of the heart.

on the heart muscle and the heart ganglion of *Limulus*, it would seem probable that this drug has primarily the same action on the two tissues of the vertebrate heart. Whether the nervous tissue in the vertebrate heart exhibit greater sensitiveness to caffeine than does the heart muscle must be determined by another line of investigation.

## VI. THE ACTION OF CURARE.

1. **The heart ganglion of *Limulus*.** *Curare* has a primary stimulating action on the *Limulus* heart ganglion. — The weakest concentration of the drug (1 to 10,000) that affects the ganglion at all does not alter the rate of the rhythm, but it augments the intensity of the nervous discharges. In concentrations of 1 to 1000 or 1 to 500 the rate is also augmented, and the rhythm is also liable to become irregular.

Greater concentrations of the drug always produce irregularity of the rhythm simultaneously with the augmentation. No strength of the drug was found that would paralyze the ganglion without the primary stimulating phase. The stimulating action of curare on the heart ganglion is counteracted by calcium chloride.

2. **The heart muscle of *Limulus*.** *Solutions of curare do not seem to have any appreciable effect on the *Limulus* heart muscle.* — Solutions of this drug in plasma or sea water in concentrations up to 1 to 500 have no effect whatever on the muscle. We have seen that these strengths of curare have a strong stimulating action on the heart ganglion. When curare in strengths of  $\frac{1}{2}$  to 1 per cent is applied to the heart muscle, the amplitude of the contractions is gradually diminished. It would therefore seem that so far as curare has any action at all on the heart muscle and the motor nerves and nerve endings in the muscle, this action is the opposite to that on the heart ganglion, or in the direction of depression. I have made numerous observations on the action of curare on the molluscan and the crustacean heart. Curare, when of sufficient strength to affect the heart at all, has a primary stimulating action on the heart of these animals, just as when the drug is applied to the ganglion on the *Limulus* heart. Inasmuch as the drug has practically no action, or only a depressor one, on the *Limulus* heart muscle, it would seem probable that the stimulating action of this drug on the molluscan and crustacean heart is also due to the action on the motor heart ganglia.

3. **The vertebrate heart.** — The vertebrate heart appears to be relatively resistant to the action of curare. According to Boehm and Tillie<sup>1</sup> curare has the same action on the vertebrate heart as strychnia and digitalis, that is, primary stimulation. It is well known that curare acts like strychnia on the central nervous system, and that it depresses skeletal muscle without primary stimulation. It is probable that experiments on the isolated mammalian heart would reveal the same primary stimulating action of curare as has been described for the *Limulus* heart ganglion.

## VII. THE ACTION OF NICOTIN.

1. **The *Limulus* heart ganglion.** *Solutions of nicotin in plasma or sea water have a primary stimulating action on the *Limulus* heart ganglion.*

<sup>1</sup> Cited from BABAK and CHODOUNSKY: *Centralblatt für Physiologie*, 1904, xviii, p. 282.

*In the stronger solutions the primary stimulating action is quickly followed by irregularity in the rhythm, depression, and ultimate paralysis.* — The ganglion is very sensitive to this drug. The stimulating action is obtained from the pure alkaloid in dilution of 1 to 1,000,000, or even 1 to 10,000,000. The salts of nicotin act less intensely. At

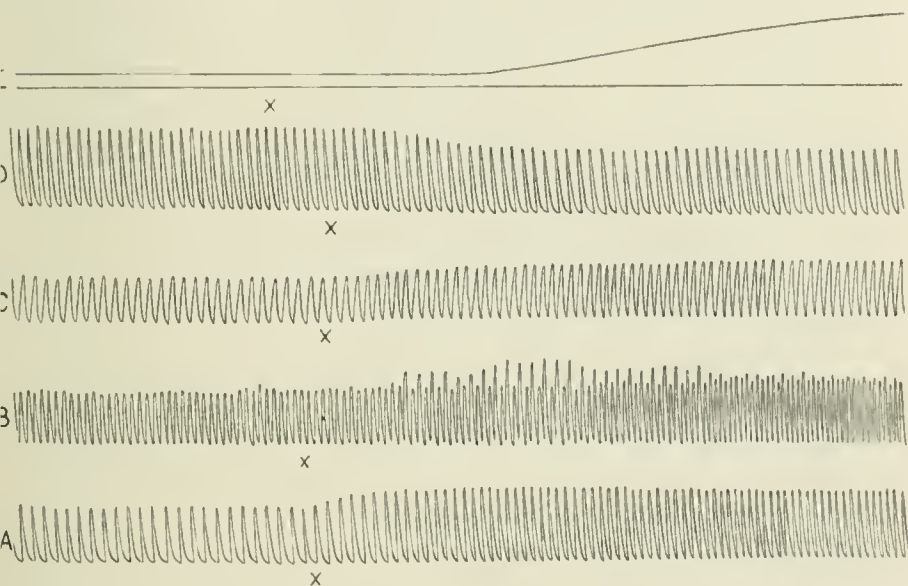


FIGURE 5.—*A* to *D*, tracings from the anterior end of the *Limulus* heart, ganglion isolated posteriorly. *x*, application of the drugs to the isolated ganglion. *A*, nicotin 1-5,000,000. Stimulation. *B*, aconitin 1-500,000. Stimulation. *C*, pilocarpin 1-1000. Stimulation. *D*, ergot 1-10,000. Depression. *E*, ganglion-free part of the heart. *x*, digitalin 1-500 applied to heart muscle. Tonus contraction.

these dilutions the only evidence of stimulation is the augmented intensity of the nervous discharges, the rate of the ganglionic rhythm remaining the same. In stronger solutions the rate of the rhythm is also augmented. In fact, the rate may be augmented, while the intensity is at the same time decreased. When solutions of the strength of 1 part of the drug to 50,000 or 100,000 parts of plasma are applied to the ganglion, the heart muscle is sent into a condition of incomplete tetanus or tonus, which is quickly followed by paralysis of the ganglion. An increase in the tonus of the muscle is apparent, even when nicotin in weaker solutions is applied to the ganglion. In concentrations of 1 to 4000 the heart ganglion is paralyzed within two minutes, after an intense stimulation, as shown by the incom-

plete tetanus and tonus of the muscle. The paralysis of the ganglion is followed by complete tonus relaxation of the heart muscle. The stimulating action of nicotin, except in the stronger concentrations, is counteracted by calcium chloride.

2. **The *Limulus* heart muscle.** *Nicotin in dilute solutions has no appreciable effect on the *Limulus* heart muscle. In strong concentrations it depresses the ganglion-free part of the heart without primary stimulation.* — Distinct depressor effects on the muscle usually begin in concentrations of 1 to 50,000 or 1 to 25,000; this is in the case of using the pure alkaloid. If salts of nicotin are used, much greater concentrations are required to produce the depressor effects on the muscle. Solutions of the alkaloid of the strength of 1 to 5000 or 1 to 1000 may also produce some tonus in the muscle.

The conclusion seems therefore justified that nicotin has opposite actions on the two tissues of the *Limulus* heart. Solutions of the drug too weak to affect the ganglion-free part of the heart have a powerful stimulating action on the ganglion. Every strength of nicotin that affects the ganglion at all produces a primary stimulation, while the concentration of the drug that acts on the muscle depresses its contractility and excitability. No distinct stimulating action on the heart muscle was obtained from nicotin in any strength.

3. **The vertebrate heart.** — All observers seem to agree that nicotin has a primary stimulating action on the vertebrate heart, although this direct action on the heart is in the intact animal ordinarily obscured by the intense stimulating action of the drug on the central nervous system and peripheral ganglia, which in turn affect the heart rhythm indirectly. But as nicotin primarily augments the rhythm of the frog's heart, the embryonic heart (Pickering), and the isolated mammalian heart, it is obvious that this drug has a primary stimulating action on the automatic tissue of the vertebrate heart similar to that on the *Limulus* heart ganglion. In fact, the action of nicotin on the entire *Limulus* heart and on the vertebrate heart appears to be identical. We have the same primary augmentation, followed by irregularity, inco-ordination, depression, and final paralysis in case of the stronger concentrations or on long-continued action of the weak concentrations of the drug. In the *Limulus* heart this stimulation is due solely to the action of the drug on the ganglion, the heart muscle not being affected by the weaker concentrations. The depressor action of stronger concentrations of the

drug may in part be due to the influence of the drug on the heart muscle. That the mechanism of action of nicotin on the vertebrate heart is the same as in the *Limulus* heart is indicated not only by this analogy, but also by the reactions of other vertebrate tissues to nicotin. Nicotin has a primary stimulating action on the central nervous system and apparently on all peripheral ganglia, while it has a depressor action on skeletal and smooth muscle. Nicotin also depresses nerves and nerve endings, usually without any primary stimulation. In the absence of direct evidence positive statements cannot be made, but these facts, together with the results in the *Limulus* heart, make it probable that nicotin does not stimulate the vertebrate heart muscle, but that the primary stimulating action of this drug is due to its influence on the motor ganglia in the heart, just as I have shown to be the case in *Limulus*.

Nicotin stimulates the molluscan and the crustacean heart very much the same as the *Limulus* and the vertebrate heart. This drug is therefore without exception a primary heart stimulant throughout the animal kingdom. There is no good reason for supposing that nicotin affects the heart muscle of molluscs and crustaceans differently from that of *Limulus*. Local ganglia have been described in the heart of most of these invertebrates, and it is therefore highly probable that the stimulating phase of nicotin on these hearts is due to the action of the drug on these ganglia.

#### VIII. THE ACTION OF ATROPIN.

1. **The *Limulus* heart ganglion.** *Atropin sulphate in plasma or sea water has a primary stimulating action on the heart ganglion of Limulus.*—This is uniformly true of every strength of the drug that affects the ganglion at all. Solutions of 1 to 4000 or 5000 augment the intensity of the nervous discharges only, leaving the rate of the ganglionic rhythm unchanged. Stronger solutions also augment the rate of the nervous discharges. In concentrations of 1 to 200 or 300 the primary stimulation approaches incomplete tetanus for a brief period; this is followed by irregularity of the rhythm, depression, and final paralysis. But in weaker strengths the atropin may act on the ganglion for a long period before irregularity and depression set in. The stimulating action of this drug on the heart ganglion is counteracted by calcium chloride.

2. **The heart muscle of *Limulus*.** *Atropin in weak concentrations has*

no distinct effect on the *Limulus* heart muscle. In strong concentrations the drug diminishes the amplitude of the contractions without any primary stimulation. — When atropin in dilute solutions (1 to 4000 or 5000) is applied to the whole heart, the stimulating action on the ganglion is not marked by a simultaneous depressor action on the muscle, for at this dilution atropin does not seem to act on the muscle. In no concentrations of this drug did I obtain a primary stimulating

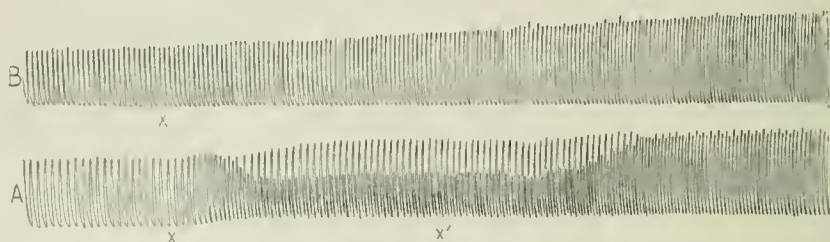


FIGURE 6.—About one-half the original size. Tracings from the fifth segment of the intact *Limulus* heart. A, x, application of nicotin 1-1,000,000 to the heart. x', the nicotin solution replaced by plasma. Showing primary stimulation and irregularity of rhythm by nicotin. B, x, application of digitalin 1-20,000 to the heart. Showing primary stimulation.

action on the heart muscle. The depressor action of the stronger solution (1 to 100 or 1 to 200) on the ganglion-free part of the heart is in all probability due in part to depression of the motor nerves and nerve endings.

3. **The vertebrate heart.**—The literature is not unanimous on the question of the primary action of atropin on the vertebrate heart. The depressor action of this drug on nerve endings to gland cells and smooth muscle (also skeletal muscle, frog) in the vertebrates is so much more striking than the primary stimulating action of the drug on peripheral ganglia that many observers deny the latter. There is no question but that atropin has a primary stimulating action on the central nervous system. A primary stimulating action of atropin on peripheral ganglia is indicated by the primary augmentation of intestinal peristalsis following the administration of small quantities of the drug. Atropin will accelerate the rhythm of the heart in the intact mammal, but this apparent stimulation is usually interpreted as due to the paralysis of the vagi nerve endings in the heart. Hedbom<sup>1</sup> has shown, however, that atropin in certain concentrations has a primary stimulating action on the isolated mammalian heart.

<sup>1</sup> HEDBOM: Skandinavisches Archiv für Physiologie, 1898. viii, p. 185.

In Hedbom's experiments the augmentation of the rhythm could not have been due to vagus paralysis. There is also evidence in the literature that atropin has a slight primary stimulating action on the isolated frog's heart. It would therefore seem that atropin has a direct stimulating action on the vertebrate heart, just as it has on the *Limulus* heart. What is the evidence that this stimulating action in the case of the vertebrate heart is due to the action of the heart ganglia rather than to the muscle? The evidence from my result in *Limulus* need not be restated, and this is strengthened by the fact that atropin has hardly any action on skeletal and smooth muscle in the vertebrates. Strong concentrations depress the excitability and contractility of the muscle, apparently without any primary stimulation, just as we have seen to be the case in the *Limulus* heart muscle. This fact coupled with the undoubted primary stimulating action of the drug on the central nervous system and on at least some peripheral ganglia renders it probable that the mechanism of the direct stimulating action of atropin on the vertebrate heart is through the motor heart ganglia and not through the heart muscle. In the paralyzing phase of the atropin action undoubtedly the motor ganglia, the motor nerve endings, and the heart-muscle play a part.

Atropin, nicotin, and curare paralyze the vagi in the vertebrate heart. I have shown in a previous paper that these drugs have a similar depressor or paralyzing action on the inhibitory nerves to the *Limulus* heart, but in order to produce this paralysis of the inhibitory nerve these drugs must act on the heart ganglion.<sup>1</sup> If allowed to come in contact with the heart muscle and motor nerves only, they have no effect on the inhibitory nerves. This shows that in the *Limulus* heart the point of paralysis of the inhibitory fibres by these drugs is somewhere in the ganglion. The inhibitory nerves are paralyzed by these drugs acting on the heart ganglion long before the ganglion itself is paralyzed, just as the vagi are paralyzed by the action of these alkaloids on the vertebrate heart long before the drugs exert this depressor action on the automatic tissue.

## IX. THE ACTION OF COCAINE.

1. **The *Limulus* heart ganglion.** *Solutions of cocaine hydrochlorate in plasma or sea water have a primary stimulating action on the *Limulus* heart ganglion.*—This salt of cocaine is only very slightly

<sup>1</sup> CARLSON: This journal, 1905, xiii, p. 217.

soluble in *Limulus* plasma or sea water, but the primary stimulating action of this drug on the heart ganglion is obtained with  $\frac{1}{10}$  to  $\frac{1}{20}$  of the saturated solution. In concentrations represented by the saturated solution the augmented rhythm soon becomes irregular, and the stimulating phase is followed by depression and ultimate paralysis.

2. **The heart muscle of *Limulus*.** *Concentrations of cocaine hydrochlorate in plasma or sea water up to saturation have no distinct effect on the *Limulus* heart muscle either in the direction of stimulation or depression.*—No distinct evidence of primary stimulation of the muscle was ever obtained when a saturated solution of the drug in plasma was allowed to act on the ganglion-free part of the heart for a long period. A gradual diminution in the amplitude of the contractions of that part of the heart was usually observed, but this effect was so gradual that it could scarcely be distinguished from the ordinary gradual decline in the cardiac rhythm under the experimental conditions.

When solutions of cocaine in plasma are applied to the whole heart, the rhythm is augmented in just the same manner as when the same solutions are applied to the isolated ganglion. It is evident from the above results that this stimulation is due solely to action of the drug on the ganglion.

3. **The vertebrate heart.**—Physiologists are not agreed as to the direct effect on the heart rhythm of cocaine. Primary augmentation of the rhythm has been described both in the frog and mammal.<sup>1</sup> In the mammal this augmentation is probably in part due to depression of the vagus centres and to stimulation of the augmentor centres in the medulla. In the isolated mammalian heart Hedbom<sup>2</sup> usually obtained depression of the rhythm by cocaine, not preceded by stimulation, while in a few cases indications of a primary stimulation were recorded.

There is, therefore, some indication that cocaine has a primary stimulating action on the vertebrate heart similar to that on the *Limulus* heart ganglion. This is further indicated by the primary stimulating action of this drug on the central nervous system and peripheral ganglia (Auerbach's plexus). Cocaine has a depressor effect on skeletal muscle similar to that on sensory nerves and nerve

<sup>1</sup> MOSSO: *Archiv für experimentelle Pathologie und Pharmakologie*, 1887, xxiii, p. 153; ANREP: *Archiv für die gesammte Physiologie*, 1880, xxi, p. 28.

<sup>2</sup> HEDBOM: *Skandinavisches Archiv für Physiologie*, 1899, ix, p. 61.



endings. According to some investigators, however, the depression is preceded by a stimulating phase. It is obvious that more work is required on the nature of the action of cocaine on the heart and on curarized muscle in the vertebrates before valid comparisons can be made with the mechanism of cocaine action on the heart of *Limulus*.

#### X. THE ACTION OF PILOCARPIN.

1. **The *Limulus* heart ganglion.** *Solutions of pilocarpin hydrochlorate in plasma or sea water have a primary stimulating action on the *Limulus* heart ganglion.* — In weak strengths (1 to 10,000) the stimulation appears only in the augmented intensity of the nervous discharges, the rate of the ganglionic rhythm remaining the same, but in the stronger solutions (1 to 500, 1 to 1000) the rate may be augmented to a point approaching incomplete tetanus. This degree of stimulation of the ganglion is always accompanied by increased tonus of the muscle. The stronger solutions of the drug usually produce some irregularity of the ganglionic rhythm, and this is followed by depression of the rhythm particularly in the intensity of the nervous discharges.

The stimulating action of pilocarpin on the heart ganglion is counteracted by calcium chloride.

2. **The *Limulus* heart muscle.** — *Solutions of pilocarpin in sea water or plasma appear to have no distinct effect on the ganglion-free part of the *Limulus* heart.* — This is true for concentrations up to  $\frac{1}{2}$  per cent. Greater concentrations were not tried. The strength of the drug that causes great primary stimulation when applied to the heart ganglion produces no effect, either in the line of stimulation or depression, when applied to the ganglion-free part of the heart. When these solutions are applied to the whole heart, the same effect is obtained as by bathing the ganglion in the solution. And since pilocarpin has practically no action on the heart muscle, it is evident that the effects produced by the drug action both on the heart muscle and on the heart ganglion at the same time are due solely to the action of the drug on the ganglion.

3. **The vertebrate heart.** — The most striking action of pilocarpin in the vertebrates is the stimulation of the endings of secretory nerves in glands and of the nerve endings in smooth muscle. Pilocarpin also stimulates peripheral ganglia (Auerbach's plexus) as well as the central nervous system. Nothing appears to be definitely established touching the primary action of this drug on skeletal muscle and smooth muscle.

The literature is conflicting touching the primary action of pilocarpin on the heart. According to Pickering,<sup>1</sup> pilocarpin has a primary stimulating action on the embryonic heart (chick). In the case of the isolated mammalian heart Hedbom<sup>2</sup> sometimes observed a primary stimulation of the rhythm following the administration of pilocarpin. This stimulating phase is followed by depression, which may in turn give way to a second phase of stimulation. In some preparations no perceptible augmentation preceded the depressor stage. The usual account of the primary action of pilocarpin on the intact heart of frog and mammal is that the drug causes a primary depression of rhythm. This depression is usually ascribed to the stimulation of inhibitory ganglia and nerve endings in the heart, as it is counteracted by atropin. These conflicting results are probably to be accounted for by the simultaneous stimulation of both inhibitory and motor nervous mechanism in the vertebrate heart, the inhibitory being usually the more powerful in the beginning and obscuring the effect of the simultaneous stimulation of the motor ganglia or plexus. This would be analogous to the simultaneous stimulation of the inhibitory and augmentor fibres in the vagi, in which case the inhibitory effects entirely obscure the action of the augmentor nerves in the beginning. In the hearts where pilocarpin causes augmentation prior to the depression the motor nervous mechanism is probably for some reason the more excitable and powerful. The uniform stimulating action of the alkaloid on the embryonic heart may be due to a later development of the inhibitory nervous mechanism as compared to the motor.<sup>3</sup> The *Limulus* heart ganglion contains inhibitory nerves and nerve endings, but it may not contain any inhibitory ganglion cells. At any rate, the stimulation of the motor cells by the drug completely obscures possible simultaneous stimulation of the inhibitory nervous mechanism.

## XI. THE ACTION OF PHYSOSTIGMIN.

1. **The heart ganglion of *Limulus*.** *Solutions of physostigmin in plasma or sea water have a primary stimulating action on the *Limulus* heart ganglion.*—This alkaloid is not a very strong stimulant to the ganglion, however. In dilutions of 1 to 5000 it has no appreciable effect. In concentrations of 1 to 2000 and stronger the alkaloid in-

<sup>1</sup> PICKERING : Journal of physiology. 1893, xiv, p. 461.

<sup>2</sup> HEDBOM : Skandinavisches Archiv für Physiologie, 1899, ix, p. 56.

<sup>3</sup> CARLSON : This journal, 1905, xiv, p. 52.

variably stimulates the ganglion without any indication of a primary depression. Irregularity and depression of the ganglionic rhythm follow the stimulating phase when strong solutions are employed. The weaker solutions augment only the intensity of the nervous discharges from the ganglion, the rate remaining unchanged.

2. **The heart muscle of *Limulus*.** *Solutions of physostigmin in plasma or sea water in concentrations up to 1 to 500 have no appreciable effect on the ganglion-free part of the heart, either in the direction of depression or stimulation.* — Stronger solutions were not tried, mainly because of lack of time and animals. Whatever be the effect of this alkaloid on the muscle in strong doses, it is obvious that the heart ganglion is more sensitive to the drug, and that the reaction of the whole heart to the moderately strong (up to 1 to 500) solutions of the drug is due to the action of the drug on the ganglion.

3. **The vertebrate heart.** — In the last edition (1906) of his textbook Cushney states that "the action of physostigmin on the vertebrate heart requires further investigation," a statement that any one who has had occasion to consult the literature on the subject will readily assent to. It is well established that physostigmin has a primary stimulating action on the central nervous system, peripheral ganglia (Auerbach's plexus),<sup>1</sup> and nerve endings in skeletal muscle, smooth muscle, and glands. There is some evidence that this drug has a primary stimulating action on muscle, although the evidence is not conclusive.

Application of physostigmin to the frog's heart causes slowing and strengthening of the beats, according to most observers. This is followed by paralysis. The strengthening of the beats is usually interpreted as due to a direct stimulating action of the drug on the automatic heart tissue. Primary augmentation as well as primary depression of the heart rhythm has been observed to follow the administration of physostigmin in the intact mammal, and the same results were obtained by Hedbom on the isolated mammalian heart. In most of Hedbom's experiments the effects of the drug were a primary slowing and an increase in the strength of the beats.

It does not seem probable that a drug having a primary stimulating action on perhaps all the tissues should have a primary depressant action on the heart of vertebrates. The primary slowing of the heart caused by the administration of this drug is therefore in all probability due to the stimulation of an inhibitory nervous mechanism

<sup>1</sup> MAGNUS: *Archiv für die gesammte Physiologie*, 1905, cviii, p. 28.

in the heart. It seems also reasonable to suppose that the local motor nervous mechanism is at the same time stimulated, but that the effects of this stimulation are usually marked by the more powerful influence of inhibitory mechanism. In some conditions of the heart the former may be more powerful than the latter, and we have in consequence a primary augmentation of the rhythm, as recorded in some of Hedbom's experiments. This interpretation brings the action of physostigmin on the vertebrate heart in complete accord with its action on the *Limulus* heart, the only difference being that in the *Limulus* heart the motor nervous mechanism is invariably more powerful than the inhibitory.

Physostigmin acts more powerfully on the *Limulus* heart ganglion than on the heart muscle, and in the vertebrates the drug certainly acts more powerfully on the nervous tissues than on muscle, even granted that in therapeutic doses it acts on the muscle at all. These facts make it probable that the primary action of physostigmin on the vertebrate heart is also due solely to its action on the local nerve centres.

## XII. THE ACTION OF ACONITIN.

1. **The heart ganglion of *Limulus*.** *Aconitin alkaloid in plasma or sea water has an intense stimulating action on the *Limulus* heart ganglion.*—This primary stimulation appears even with dilutions of 1 to 1,000,000. At this dilution the stimulating action appears mainly in the augmented intensity of the nervous discharges, the rate remaining the same or being only slightly accelerated. In strengths of 1 to 500,000 the ganglion is thrown into incomplete tetanus, and the tonus of the muscle is increased. The augmented ganglionic rhythm soon becomes irregular and depression and ultimate paralysis follow within a few minutes. The paralyzed ganglion may be revived by replacing it in sea water or plasma, provided it is removed from the solution containing the drug as soon as complete paralysis sets in.

The stimulating action of the weaker solutions of aconitin on the ganglion is counteracted by calcium chloride.

2. **The heart muscle of *Limulus*.** *Solutions of aconitin in plasma or sea water in concentrations up to 1 in 100,000 have no appreciable effect on the *Limulus* heart muscle.*—Stronger solutions (1 to 50,000) appear to gradually depress the strength of the contractions of the ganglion-free part of the heart. No indications of a primary stimu-

lation of the heart muscle or the motor nerve and nerve endings were ever obtained. But my experiments with the effects of the strong solutions on the ganglion-free part of the heart were not extensive, because after I had found that the concentrations of the drug that were sufficient to stimulate the ganglion to the point of paralysis within a few minutes had no effect on the heart muscle, it was obvious that the heart action of the alkaloid in "therapeutic" doses is solely due to its action on the ganglion.

3. **The vertebrate heart.**— In the vertebrates aconitin acts primarily on the nervous system. Its primary action is stimulation followed by depression and paralysis, and this is true both for the central nervous system, peripheral ganglia (Auerbach's plexus), sensory, secretory, or motor nerve endings. As far as I can learn from the literature, this alkaloid has little or no action on skeletal or smooth muscle, at least in the concentrations that act strongly on the above nervous structure.

The literature is unanimous that aconitin has a primary stimulating action on the vertebrate heart, frog as well as mammal; the stimulating phase is followed by irregularity, depression, and final paralysis of the heart. In other words, the action of aconitin on the vertebrate heart is identical with the action of aconitin on the *Limulus* heart ganglion. And since this alkaloid has practically no action on the *Limulus* heart muscle in dilute solution, nor on skeletal or smooth muscle in vertebrates, but acts primarily as a stimulant on nerve centres and nerve endings, in the vertebrates, it seems reasonable to conclude that the stimulating action of the drug on the vertebrate heart is similarly due to its action on the motor ganglia in the heart and not to any stimulating action directly on the heart muscle.

### XIII. THE ACTION OF VERATRIN.

1. **The *Limulus* heart ganglion.** *Solutions of veratrin in plasma or sea water have a primary stimulating action on the *Limulus* heart ganglion.*— Veratrin proved to have even a more intense action on the heart ganglion than aconitin, as its stimulating action is very intense, even at dilutions of 1 to 10,000,000. At strengths of 1 to 500,000 or 1 to 100,000 the stimulation approaches incomplete tetanus, the rhythm at the same time becoming irregular. In these strong solutions depression and paralysis of the ganglion supervenes within two or three minutes. If, following complete paralysis, the ganglion is

quickly transferred to plasma or sea water, the automaticity may be restored. In two preparations that were in poor condition veratrin solution of the strength of 1 to 500,000 paralyzed the ganglia instantaneously without any indication of a primary stimulation.

The stimulating action of veratrin on the heart ganglion is counteracted by calcium chloride.

2. **The heart muscle of *Limulus*.** *Solutions of veratrin of the strength of 1 to 5 or 10 million appear to have no effect on the ganglion-free part of the heart.*— Concentrations between 1 to 1,000,000 and 1 to 100,000 diminish the contractions without any indication of a primary stimulation, while the still stronger solutions cause tonus contractions of the heart muscle. The depression of the contractions without primary stimulation by the medium-strength solutions is probably due to depression of the motor nerves or nerve endings, while the very strong solutions cause tonus contractions by direct action on the heart muscle. Further work will be done to decide this point. The moderately strong solutions of the alkaloid applied to the ganglion also cause increased tonus of the heart muscle accompanied by augmented ganglionic rhythm, but this tonus subsides as depression and paralysis of the ganglion supervene. Following Kolliker, veratrin is usually designated as a "muscle poison." In the *Limulus* heart it certainly acts more strongly on the ganglion than on the muscle.

3. **The vertebrate heart.**— Veratrin stimulates the central nervous system and peripheral ganglia (Auerbach's plexus), but motor nerves and nerve endings are paralyzed without any primary stimulation. The alkaloid also stimulates sensory nerve endings and primarily increases the excitability and contractility of skeletal muscle. The action of veratrin on the vertebrate heart is more difficult to interpret. In the case of the isolated mammalian heart Hedbom obtained sometimes a primary augmentation of the rhythm followed by slowing and irregularity of the beats; at other times the primary action was a slowing of the rhythm followed by augmentation, irregularity, depression, and final paralysis. This is also the usual action on the frog heart. The explanation of these phenomena is in all probability the same as that suggested in the case of physostigmin. Veratrin stimulates both motor and inhibitory nervous mechanism in the heart, the acceleration of the rate being due to the stimulation of the former, the slowing of the rate to the stimulation of the latter. On simultaneous stimulation the inhibitory effects usually overshadow

the motor in the beginning, but the reverse may also take place. The increase in the strength of the beats may be due either to the action on the heart muscle directly or to action on motor ganglia. The only difference between the action of veratrin on the vertebrate heart and the *Limulus* heart appears to be this, that in the latter the local inhibitory mechanism is less developed, in consequence of which only the augmentive action becomes apparent. Veratrin does not lengthen the contraction time of the *Limulus* heart muscle to any appreciable extent in the manner exhibited by the frog's ventricle. When the concentration of the alkaloid is strong enough to act on the heart muscle, it produces tonus contraction, on which the fundamental beat may be superimposed for a minute or so, but this strength of the drug suppresses the normal contraction so rapidly, evidently by paralyzing the motor nerves, that comparison cannot here be made with the frog or the mammalian heart. It is true, however, that the *Limulus* heart muscle contracts and relaxes much slower than normally, when in condition of veratrin tonus.

#### XIV. THE ACTION OF SAPONIN.

1. **The *Limulus* heart ganglion.** *Solutions of saponin in plasma or sea water have a primary depressant action on the *Limulus* heart ganglion.*—In weak concentrations (1 to 200,000, 1 to 500,000) the primary depression of the ganglion appears only in the diminished intensity of the nervous discharges, the rate remaining the same. *On continued action of saponin solutions the ganglionic rhythm soon becomes irregular, and in some stages of this phase the rate of the nervous discharges may be slightly greater than the normal.* The intensity of the nervous discharges continues weaker than the normal until paralysis sets in. In no case did I obtain a distinct primary augmentation of the ganglionic rhythm with any strength of saponin. We may therefore conclude that saponin depresses the heart ganglion without primary stimulation. The depression of the intensity of the nervous discharges is in most cases uniform, but the rate of the nervous discharges from the ganglion may be momentarily augmented.

2. **The *Limulus* heart muscle.** *Solutions of saponin in plasma or sea water depress the heart muscle without any primary stimulation.*—The action of this alkaloid on the ganglion-free part of the heart is therefore the same as on the heart ganglion, the only difference being that the ganglion is much more sensitive to the action of

saponin than is the heart muscle. Concentrations of 1 to 200,000 or 1 to 500,000 appear to have no effect on the heart muscle. But decided depressor action is obtained with  $\frac{1}{10}$ ,  $\frac{1}{5}$ , or  $\frac{1}{2}$  per cent strength.

3. **The vertebrate heart.**—Saponin appears to depress and paralyze all tissues in the vertebrates without any primary stimula-

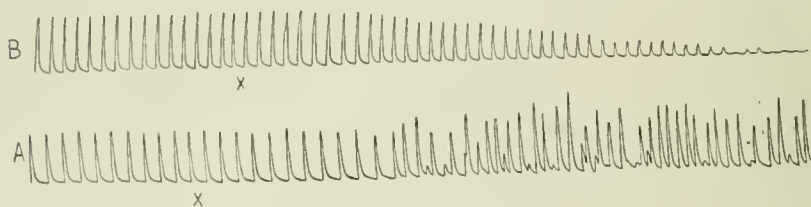


FIGURE 7.—About two-thirds the original size. Tracings from the anterior end of the *Limulus* heart. *A* (ganglion isolated posteriorly), *x*, application of saponin 1-200,000 to the isolated ganglion. Showing primary depression. *B*, *x*, application of saponin 1-50,000 to the ganglion-free anterior end. Showing primary depression.

tion. This is also true of the heart, although the heart is more resistant to the action of saponin than the central nervous system and most peripheral nervous tissues. Saponin thus acts exactly the same on the *Limulus* and vertebrate heart, and since the drug has the same action on nervous tissues and muscular tissues the question of point of action in the heart is of little moment.

Saponin increases the permeability of red blood corpuscles and of pus corpuscles to ions.<sup>1</sup> It is probable that saponin has the same action on the cells of other tissues. The depressor action may therefore be casually related to the disturbance of the normal osmotic relations between the cells and the lymph. This question is being investigated.

## XV. THE ACTION OF QUININE.

1. **The *Limulus* heart ganglion.**—Quinine sulphate is but slightly soluble in the *Limulus* plasma or sea water, the actual strength of the various solutions worked can therefore not be given. In the weaker concentrations I sometimes obtained a slight augmentation of the intensity of the nervous discharges prior to the depression, but with stronger solutions such primary stimulation was never observed. *The action of this alkaloid on the heart ganglion is therefore depressant, with a possible slight initial stimulation, but the latter is so slight and*

<sup>1</sup> STEWART: *The journal of experimental medicine*, 1902, vi, p. 257.



*of so short duration that it is not always detected.* The depressor action appears mainly in the intensity of the nervous discharges, the rate of the ganglion rhythm remaining the same or being but little altered. This is variably true for the weaker solutions. In the later stages of the action of the weaker solution the rate of the ganglionic rhythm is also diminished. The stronger solutions (one-half saturated) may depress the rate almost from the very beginning. The slight indication of primary stimulation never appeared in accelerated rhythm, nor was any acceleration of the rate of the nervous discharges obtained during any stage preceding complete paralysis of the ganglion.

**2. The heart muscle of *Limulus*.** *The action of quinine on the heart muscle is the same as on the heart ganglion, with the difference that the latter is much more sensitive to the drug than the former.*—The weaker solutions that still have a decided depressant action on the ganglion have no influence on the ganglion-free part of the heart, or the action is so gradual that it cannot be distinguished from the very gradual decline of the heart rhythm under the experimental conditions. Stronger solutions (one-half saturated) depress the heart muscle gradually. In some preparations a slight indication of a primary stimulation was obtained, lasting for a few beats.

**3. The vertebrate heart.**—So far as I can make out from the literature, the action of quinine on the vertebrate heart is identical with that on the *Limulus* heart, that is, depressor with a possible slight initial stimulation. The acceleration of the heart following administration of quinine in the intact mammal is in all probability due to depression of the vagus centre in the medulla. Both in the isolated frog's heart<sup>1</sup> and the isolated mammalian heart<sup>2</sup> the paramount action is depression of the rhythm. The slight indication of primary stimulation sometimes observed in the *Limulus* heart is either absent in the vertebrate heart or else of so brief duration as to escape detection. Quinine tends to produce somewhat greater irregularity of the heart rhythm in the vertebrates than in the *Limulus*.

Hedbom interprets the depressor action of quinine on the isolated mammalian heart as due to the stimulation of the inhibitory nervous mechanism in the heart. That interpretation appears to me in all probability incorrect. The depressor action is persistent till paraly-

<sup>1</sup> SANTESSON: *Archiv für experimentelle Pathologie und Pharmakologie*, xxxiii, p. 321.

<sup>2</sup> HEDBOM: *Skandinavisches Archiv für Physiologie*, 1899, ix, p. 33.

sis supervenes, except for occasional periods of more rapid rhythm during the irregular phase, while we know that the primary stimulating action is of very brief duration. The depression of the rhythm is probably due to depression both of the motor nervous mechanism and the heart muscle, in the case of the dilute solutions perhaps mainly due to the former, as we have seen is the fact in the *Limulus* heart. The point of action of quinine on the heart has little bearing on the question of the nature of the heart rhythm, since this drug acts in the same way both on the nervous tissue and the muscular tissue in the heart.

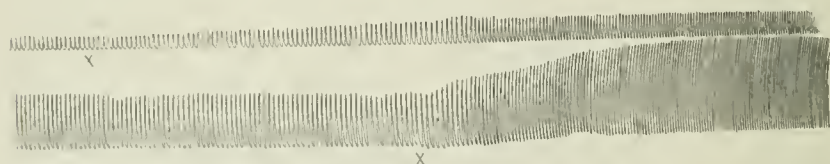


FIGURE 8.—About one-third the original size. Simultaneous tracings from the anterior (upper record) and posterior (lower record) end of the *Limulus* heart. The ganglion extirpated in the first two segments and the heart muscle transected, leaving the two ends of the heart connected only by the lateral nerves. Upper record, *x*, application of adrenalin chloride 1-200,000 to the ganglion-free anterior end. Lower record, *x*, application of same strength of adrenalin to posterior end of heart. Showing primary stimulation both of the ganglion and the ganglion-free part of the heart.

## XVI. THE ACTION OF DIGITALIN.

1. **The heart ganglion of *Limulus*.** *Solutions of digitalin in plasma or sea water have a primary stimulating action on the Limulus heart ganglion.*—Primary depression of the ganglionic rhythm was never obtained. In dilutions of 1 to 10,000 or 15,000 the stimulation of the ganglion appears only in the increased intensity of the nervous discharges, the rate remaining the same. In greater concentrations both the rate and the intensity of the nervous discharges from the ganglion are augmented. In strengths of 1 to 500 or 1 to 1000 the stimulation of the ganglion may approach incomplete tetanus, the tonus of the heart muscle is increased, and the rhythm soon becomes irregular followed by depression and final paralysis. The irregularity in the rhythm appears both in the rate and the intensity of the nervous discharges, mainly in the latter. Thus, by the action of the stronger solutions of digitalin on the heart ganglion of *Limulus*, tracings may be obtained which closely resemble the records

from the isolated mammalian heart in the irregular phase of the digitalin action.

The stimulating action of digitalin on the *Limulus* heart ganglion is counteracted by calcium chloride. Concentrations of digitalin of 1 to 1,000 paralyze the ganglion within seven to ten minutes.

2. **The heart muscle of *Limulus*.** *Solutions of digitalin stronger than 1 to 1000 cause tonus contractions in the *Limulus* heart muscle, and the increased tonus is usually accompanied by stronger beats for a short period, but this stimulating phase is quickly followed by a diminution and complete cessation of the beats while the increased tonus of the muscle persists. In some cases the tonus of the muscle produced by strong solutions of digitalin was not accompanied by stronger contractions of the ganglion-free part of the heart, the strength of the contractions being diminished from the beginning.*

The tonus contraction of the muscle produced by strong digitalin is counteracted by calcium chloride.

The weaker strengths of digitalin (1 in 2000 down to 1 in 15,000) depress the contractions of the ganglion-free part of the heart without any, or, at the most, with only the slightest indication of primary stimulation. The slight indication of primary stimulation was obtained in only two preparations with solutions of the strength of 1 in 10,000. These weaker solutions cause no tonus contraction of the muscle. The weakest strength of the drug that will affect the ganglion has practically no effect on the ganglion-free part of the heart. Stronger concentrations than 1 in 15,000 are required to produce the typical depression just described, although there are great individual variations in the degree of susceptibility to the action of the drug, the degree of depression obtained by solutions of 1 in 10,000 is obtained only by a strength of 1 in 5,000 in another heart.

I have some data which indicate that the primary depressant action of digitalin in medium strength on the ganglion-free part of the heart is due to depression (possibly preceded by a transient stimulation) of the motor nerves and nerve endings, and the stronger solutions that cause tonus contraction accompanied by a temporary increase in excitability and contractility do so by acting directly on the heart muscle. It is therefore probable that digitalin has a primary stimulating action both on the heart muscle and the heart ganglion, the latter being, however, much more susceptible to the drug than the former.

3. **The vertebrate heart.** — The main point of interest in the action

of digitalin in the higher animals has been its action on the heart, and the literature on that subject is considerable. The drug has a primary stimulating action on the central nervous system and possibly on peripheral ganglia. In strong concentrations it paralyzes nerves and skeletal muscle, the latter after a primary stimulation. The action of the drug on the isolated heart appears to be slightly different in the frog and in the mammal. The primary action in the frog's heart is an increase in the strength of the beat accompanied by a slowing of the rhythm. This phase is followed by irregularity, lack of co-ordination, periodic acceleration of the rate and ultimate paralysis, the ventricle stopping in extreme systole. Instead of the primary slowing of the rate, the drug may produce a primary acceleration. In the isolated mammalian heart digitalin causes an initial augmentation of the strength of the beat, usually without marked change in the rate.<sup>1</sup> The initial change in the rate, when present, may be either slowing, as in the frog, or actual acceleration followed by slowing.<sup>2</sup> The fundamental and typical action of digitalin on the vertebrate heart is therefore the primary increase in the strength of the beats, and this may be accompanied by a slowing or an acceleration of the rhythm or attended by no change in the rate.

It is, therefore, evident that *this primary action of digitalin on the vertebrate heart is almost duplicated by its action on the Limulus heart ganglion.* The only difference is this, that the initial increased intensity of the nervous discharges from the Limulus heart ganglion is never accompanied by a slowing of the rate. The strengthened nervous discharges may be accompanied by an accelerated rhythm, in case the concentration of the drug is sufficiently strong, but in no case did I observe a preliminary slowing. This preliminary slowing is not a necessary result of the action of digitalin on the isolated vertebrate heart, for while it is usual in the frog's heart, it is not usual in the isolated mammalian heart. The further phenomena of irregularity in the rhythm and grouping of the beats are the same as the irregularities following the strong doses on the Limulus ganglion. In view of the results on the Limulus heart I would interpret the mechanism of the action of digitalin on the isolated vertebrate heart as follows: the primary strengthening of the beats and quickened rhythm are due to stimulation of the motor nervous

<sup>1</sup> GOTTLIEB and MAGNUS: Archiv für experimentelle Pathologie und Pharmakologie, 1905, ii, p. 30.

<sup>2</sup> HEDBOM: Skandinavisches Archiv für Physiologie, 1898, viii, p. 185.

elements. The local inhibitory nervous apparatus is stimulated at the same time, and in some cases this results in a slowing of the rate. The irregularity of the rhythm results from the stimulation of parts of the motor nervous mechanism not normally automatic, that is, the parts that normally respond to impulses from the sinus or from the auricles.

The increased tonus of the muscle in the frog's heart appears to be identical with the tonus contraction produced in the *Limulus* heart muscle by strong doses of digitalin. These phenomena are in all probability due to a direct action of the drug on the heart muscle rather than on the motor nervous tissue. The paralysis of the heart by strong doses of digitalin is probably due to the paralysis of the motor ganglia in the heart. This is certainly the case in the heart of *Limulus*.

## XVII. THE ACTION OF ADRENALIN.

1. **The heart ganglion of *Limulus*.** *Adrenalin is a very powerful stimulant to the Limulus heart ganglion.* — This stimulating action is evident with dilutions of 1 to 500,000. The greater the concentration of the drug, the greater the intensity of its stimulating action on the ganglion; but adrenalin differs from drugs like aconitin, digitalin, nicotin, or veratrin in that the rhythm does not become irregular. In very strong solutions, however, the depression following the stimulating phase may be accompanied by some irregularity. The stimulating action of adrenalin on the ganglion appears both in the rate and in the intensity of the nervous discharges. It is counteracted by calcium chloride.

2. **The heart muscle of *Limulus*.** *Solutions of adrenalin chloride in plasma or sea water have a primary stimulating action on the ganglion-free part of the Limulus heart just as they have on the ganglion.* — Where such solutions are applied to the ganglion-free anterior end of the heart the amplitude of the beats is gradually strengthened and this stimulating action may last for hours. The only difference between the heart ganglion and the ganglion-free part of the heart in this reaction to adrenalin is the greater sensitiveness of the ganglion. Solutions too weak to affect appreciably the ganglion-free part still act on the ganglion, and any given strength of the drug acts more quickly on the heart ganglion than on the ganglion-free part of the heart.

It is, of course, impossible to say whether the stimulating action

of adrenalin on the ganglion-free part of the heart is due to its action on the nerves or on the muscle or on both.

3. **The vertebrate heart.**— On the vertebrate heart adrenalin acts in a manner similar to digitalin, that is, as a primary stimulant. This stimulating action appears in increased strength of the beats rather than in any augmentation of the rate, in the intact mammal; in fact, the rate is diminished by stimulation of the vagus centre in the medulla.

Adrenalin has a primary stimulating action on the central nervous system, and, according to the best supported view, on nerve endings

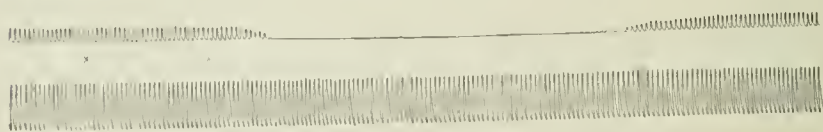


FIGURE 9.— About one-third the original size. Simultaneous record from the two ends of the *Limulus* heart prepared as in Figure 8. Upper record, ganglion-free anterior end. Lower record. Posterior end of heart containing the ganglion. *x*, nicotin alkaloid 1-40,000 applied to anterior end. *x'*, nicotin 1-10,000 applied to anterior end. *x''*, nicotin solution replaced by plasma. Showing primary depression of the ganglion-free part of the heart by concentrations of nicotin that strongly stimulate the ganglion.

of the sympathetic system.<sup>1</sup> It is not supposed to act directly on the muscle cells, though that is a disputed point. The supporters of the myogenic theory ascribe the stimulating action of adrenalin on the heart to a direct action on the muscle, though the evidence seems to show that this drug acts on the nervous rather than on the muscular tissues. The action of adrenalin on the *Limulus* heart is identical with that on the mammalian heart. The results on *Limulus*, however, furnish no clue to the interpretation of the point of action of the drug in the vertebrate heart, since the heart ganglion and the ganglion-free part of the heart react to adrenalin in the same manner.

#### XVIII. THE ACTION OF ERGOT.

1. **The heart ganglion of *Limulus*.** *Extract of ergot in plasma or sea water has a primary depressant action on the heart ganglion of *Limulus*.*— The depression is confined almost entirely to the intensity of the nervous discharges, the rate of ganglionic rhythm remaining the same. The degree of depression is proportional to the strength of the drug, the weakest solution having any appreciable action on

<sup>1</sup> See ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 401.

the ganglion being 1 in 10,000. There is usually a gradual recovery of the ganglion in case of the weaker solutions, even when the drug is allowed to act on the ganglion continuously. The degree of recovery may reach the normal rhythm, that is, in the strength of the nervous discharges, but in no case did the ganglionic rhythm become stronger than the normal after the primary depression.

**2. The Limulus heart muscle.** *Extract of ergot in plasma or sea water up to the strength of 1 in 1000 has no action on the ganglion-free part of the Limulus heart.*—Stronger solutions were not tried. It is therefore evident that the heart ganglion is more sensitive to the

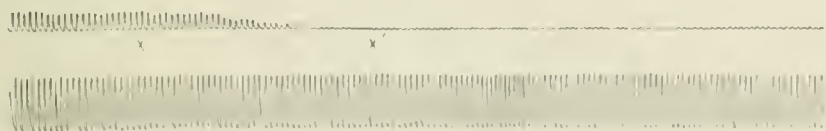


FIGURE 10.—Simultaneous tracings from the anterior (upper record) and the posterior (lower record) ends of the *Limulus* heart prepared as in Figure 8. *x*, digitalin 1-2000 applied to the ganglion-free anterior end of the heart. *x'*, the digitalin solution replaced by plasma. Showing primary depression of the ganglion-free part of the heart by this concentration of digitalin.

drug than is the heart muscle or the motor nerves. The ergot solutions have the same action on the intact *Limulus* heart as on the isolated heart ganglion. This action is consequently due to the influence of the drug on the ganglion.

**3. The vertebrate heart.**—The commercial extracts of ergot contain several toxic substances, some of which have a primary stimulating action both on parts of the central nervous system and on peripheral ganglia, and possibly on nerve endings, while others are said to act on muscular tissue in a manner similar to veratrin. Sollmann and Brown<sup>1</sup> have recently made exhaustive studies of the action of ergot on the intact as well as on the isolated mammalian heart, and I will confine myself to a comparison of their results with those on the *Limulus* heart. Direct application, that is, intravenously, of solutions of ergot produce quickly a primary weakening of the heart beats without any changes in the rate. The heart recovers from this depressor action almost as quickly, and the degree of recovery usually exceeds the normal strength of the beats; that is, there is some indication of a secondary stimulation following the depression. As these effects are obtained both in the intact and

<sup>1</sup> SOLLMANN and BROWN: *Journal of American medical association*, 1905, xlv, p. 229.

in the isolated mammalian heart, Sollmann and Brown interpret them as due to a direct action of the ergot on the heart muscle.

The direct action of ergot is therefore practically identical in the mammalian heart and the *Limulus* heart. The mechanism by which these changes in the heart rhythm are produced may or may not be the same in the hearts from these two widely separated groups of animals, but in the case of *Limulus* we have seen that they are due solely to the action of the drug on the heart ganglion.

The mechanisms of action of the foregoing drugs on the *Limulus* heart need not be restated. Moreover, these results on the *Limulus* heart do not warrant any general deductions as to the mechanisms of action of the same drugs on the vertebrate heart, apart from the interpretations already suggested in the case of each individual drug. As was pointed out in the beginning of the paper, this is partly due to the fact that the literature is not a unit as regards the primary action of many drugs on the vertebrate heart, as well as to the fact that we have scanty data on the pharmacology of the heart under conditions eliminating, as far as possible, the factor of conduction and co-ordination in the heart. Such data only can be directly compared with the above results in *Limulus*.

This fact is, nevertheless, apparent that *the primary heart action of most of these drugs is the same in Limulus and in the vertebrates, and that in case of the drugs showing discrepancies, the literature on the vertebrate heart is conflicting.* We have seen that the primary action of these drugs on the *Limulus* heart is due to their action on the local motor ganglion. But this is suggestive for further work rather than a warrant for further generalizations; as the neurogenic theory of the heart beat may be true for the vertebrates, and yet the mechanisms of primary action of drugs in the *Limulus* and the vertebrate hearts be very dissimilar, because in the vertebrates the motor heart ganglia, the heart nerves, and the heart muscle may not exhibit the relative excitabilities that we found in the *Limulus* heart. And further investigation will probably demonstrate a local inhibitory nervous mechanism in the vertebrate heart, a mechanism that appears to be absent in the heart of *Limulus*. It seems to me, however, that the interpretations of the mechanisms of action of drugs in the vertebrate heart suggested by the pharmacology of the *Limulus* heart bring, on the whole, the heart action of these drugs in line with their known action on nervous and muscular tissues respectively.



# THE EFFECT OF CASTRATION ON THE METABOLISM IN OSTEOMALACIA.<sup>1</sup>

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## INTRODUCTION.

CASTRATION was first recommended as a curative measure in osteomalacia by Febling,<sup>2</sup> who obtained apparently permanent cures in several cases of puerperal osteomalacia. The operation has since been performed a number of times with good results upon patients suffering from this disease. In the present paper an account is given of the results of a metabolism experiment performed on a young woman who had suffered from osteomalacia and had been castrated a year and a half previously in an attempt to cure the disease. A similar metabolism experiment had been performed on the patient just before the operation and another a few months after.<sup>3</sup> The operation caused a marked change in the metabolism, and there was apparently a marked improvement in the condition of the patient, leading to union of a fracture of the femur. The patient was then lost sight of for over a year. At the end of that time the disease seemed to be in an acute condition again, and two fractures occurred, one just before and one during the third metabolism experiment. It is believed that the results throw some light on the mechanism of the pathological processes which take place.

<sup>1</sup> This is a continuation of the study of osteomalacia started under the direction of Drs. GOLDTHWAIT, PAINTER, and OSGOOD of Boston over two years ago. The expenses were contributed in part by the Procter Fund for the Study of Chronic Diseases.

<sup>2</sup> H. FEBLING: *Archiv für Gynäkologie*, 1891, xxxix, p. 171; H. FEHLING: *Zeitschrift für Geburtshilfe und Gynäkologie*, 1894, xxx, p. 471; H. FEHLING: *Archiv für Gynäkologie*, 1895, l, p. 472.

<sup>3</sup> GOLDTHWAIT, PAINTER, OSGOOD, and MCCRUDDEN: *This journal*, 1905, xiv, p. 389.

## EXPERIMENTAL RESULTS.

**Third metabolism experiment diet.** *First day.* — Breakfast: bread, 52.0 gm., butter, 12.6 gm.; oatmeal, 148.5 gm.; coffee, 150.0 c.c.; cream, 50.0 c.c. Dinner: liver, 38.0 gm.; potato, 94.3 gm.; bread, butter. Supper: jam, 59.0 gm.; gingerbread, 79.0 gm.; cocoa, 146.0 c.c.

*Second day.* — Breakfast: oatmeal, 109.0 gm.; omelette, 40.1 gm.; coffee, 153.0 c.c.; milk, 58.0 c.c.; bread, butter. Dinner: fish, 46.8 gm.;

TABLE I.

Urine.	CaO.	MgO.	P <sub>2</sub> O <sub>5</sub> .	S.	N.
1st day . . . . .	0.175	0.0942	0.9018	0.3168	5.03
2d day . . . . .	0.363	0.1482	0.9682	0.3976	6.84
3d day . . . . .	0.325	0.1243	0.9146	0.3358	5.86
4th day . . . . .	0.228	0.0917	0.8642	0.3252	5.37
5th day . . . . .	0.252	0.0964	0.9184	0.3902	4.97
6th day . . . . .	0.241	0.0924	0.9108	0.298	5.9
Total . . . . .	1.584	0.648	5.478	2.0636	33.97
Fæces . . . . .	6.69	1.116	4.873	0.729	0.705
Total outgo . . . . .	8.27	1.764	10.35	2.793	34.68
In food. . . . .	3.44	1.504	12.28	2.897	38.85

potato, 95.0 gm.; jam, 44.5 gm.; bread, butter. Supper: oyster broth, 160.0 c.c.; cocoa, 139.0 c.c.; jam, 48.5 gm.; bread, butter.

*Third day.* — Breakfast: oatmeal, 17.5 gm.; omelette, 44.8 gm.; milk, 50.0 c.c.; coffee, 153.0 c.c.; bread, butter. Dinner: potato, 95.1 gm.; cabbage, 64.5 gm.; broth, 121.0 c.c.; jelly, 68.3 gm.; milk, 60.0 c.c.; bread, butter. Supper: cocoa, 137.0 c.c.; jam, 69.2 gm.; bread, butter.

*Fourth day.* — Breakfast: baked beans, 74.5 gm.; coffee, 152.0 c.c.; bread, butter. Dinner: beef, 38.0 gm.; potato, 124.1 gm.; peas, 56.7 gm.; ice cream, 55.1 gm.; bread, butter. Supper: pears, 58.5 gm.; cocoa, 128.0 c.c.; bread, butter.

*Fifth day.* — Breakfast: oatmeal, 93.3 gm.; omelette, 132.0 gm.; coffee, 152.0 c.c.; bread, butter. Dinner: lamb, 27.0 gm.; potato, 93.7 gm.; ice cream 56.2 gm.; bread, butter. Supper: apple butter, 61.5 gm.; cocoa, 125.0 c.c.; bread, butter.

*Sixth day.*— Breakfast: sausage, 30.1 gm.; oatmeal, 110.0 gm.; coffee, 127.0 c.c.; milk, 60.0 c.c.; bread, butter. Dinner: beef, 48.0 gm.; potato, 92.5 gm.; milk, 68.0 c.c.; bread, butter.

Bread for the week, 551 gm.; butter, 86.5 gm.; sugar, 144.8 gm.; salt, 3.82 gm.; water, 2820.0 c.c. The analyses<sup>1</sup> are given in Table I.

For the sake of comparison a brief résumé of the analyses obtained in Experiments 1 and 2 is given in Tables II and III.

TABLE II.

EXPERIMENT 1. PERFORMED BEFORE THE OPERATION. DURATION 8 DAYS.

	CaO.	MgO.	P <sub>2</sub> O <sub>5</sub> .	S.	N.
Daily average in urine . . . . .	0.483	0.083	1.1	0.19	6.38
Total in urine . . . . .	3.859	0.667	8.81	1.517	51.04
Fæces . . . . .	1.8	1.348	3.56	1.166	11.98
Total excreted . . . . .	5.66	2.015	12.37	2.68	63.02
Food . . . . .	4.56	2.207	12.05	7.15	69.12

TABLE III.

EXPERIMENT 2. PERFORMED A FEW MONTHS AFTER THE OPERATION. DURATION 14 DAYS.

	N.	S.	CaO.
Daily average in urine . . . . .	6.32	0.245	0.386
Total in urine . . . . .	88.5	3.425	5.397
Fæces . . . . .	15.78	1.414	1.8
Total excreted . . . . .	104.28	4.84	7.2
Food . . . . .	127.	10.54	10.03

Several points may be noted in the third metabolism experiment.

There is an excess of 4.83 gm. of calcium oxide in the excreta over

<sup>1</sup> For details of the methods, see GOLDTHWAIT, PAINTER, OSGOOD, and McCRUDDEN: This journal, 1905, xiv, p. 389.

that in the food. The loss of calcium by the body is more marked even than in the first experiment, in which case 1.10 gm. were returned. The daily excretion of calcium in the urine is high. The average in this case is 0.264. According to Neubauer and Vogel,<sup>1</sup> the normal average is 0.16 gm.

The magnesium in the urine is lower than normal. According to Neubauer and Vogel, the normal average is from 0.18 to 0.28 gm. per day. We obtained in this case 0.108 gm. per day as an average. Further, it may be seen that there is about twice as much magnesium in the fæces as in the urine, whereas we know that normally magnesium is excreted chiefly in the urine.

The metabolism of the calcium and magnesium in this experiment is essentially the same as in the first experiment, when the patient was suffering from the disease and before the operation had been performed. One noticeable difference in the metabolism in the two cases may be noted, however: in the first experiment there is an enormous retention of sulphur; in the third experiment the retention is almost negligible.

There is a retention of nitrogen and phosphorus in the third experiment. Satisfactory studies of the effect of castration on the inorganic metabolism have not yet been carried out, so that we do not know the reason for this retention. A possible explanation lies in the lowering of the metabolism, which, according to Loewy,<sup>2</sup> castration brings about. On account of our lack of knowledge concerning the effect of castration on the metabolism the author is about to begin a careful study of the effect of castration on the inorganic metabolism of normal adult animals.

#### DISCUSSION.

According to Fehling, osteomalacia is a tropho-neurosis of bone due primarily to a diseased condition of the ovaries. Removal of the ovaries does not, however, always effect a permanent cure, as Neumann<sup>3</sup> as well as we ourselves have observed. Further, beyond a slight hyperemia in some cases, the ovaries are not found to be pathological; and cases of osteomalacia occur in young women

<sup>1</sup> NEUBAUER und VOGEL: *Analyse des Harns*, 1898, p. 45.

<sup>2</sup> A. LOEWY: *Ergebnisse der Physiologie*, 1903, ii, p. 130.

<sup>3</sup> NEUMANN: *Archiv für Gynäkologie*, 1896, li, p. 130.

whose ovaries have not yet become functional,<sup>1</sup> and also in men. This disease therefore cannot be due in all cases to a pathological condition of the ovaries.

Among others we can imagine three possible processes in osteomalacia, namely, (1) an incapacity of the inorganic ground substance to enter into combination with and fix the inorganic salts, (2) a deficiency of lime in the nutritive material supplied to the bones, and (3) an especially active katabolism of bony tissue.

The first possibility might be thought of in connection with the fact mentioned in our last paper that the composition of the organic matrix is abnormal in this disease. It is improbable, however, that a slight change in the chemical composition of the organic matrix would affect its power of fixing lime. This process seems to be a physical one, and dead bone<sup>2</sup> and even gelatin can take calcium away from its salts in a similar manner. Further, this theory would not explain the excess of calcium in the excreta over that in the food. Again, as we shall see further on, the apposition of new osteoid tissue is not the primary feature, and seems to come only after the loss of calcium.

Deficiency of calcium in the food supply alone cannot be the cause of the disease, for the absolute quantity of calcium excreted daily is decidedly higher than normal, and not lower, as we should expect on a diet too poor in calcium to maintain normal equilibrium. Further, such an hypothesis does not explain why one person should suffer severely and others living on the same diet be perfectly well; on the other hand, artificial softening of bone has been produced in animals, and especially in birds, where the osseous metabolism is especially active by means of a diet poor in calcium,<sup>3</sup> so that we can conclude that a diet poor in lime or a condition of poor absorption of calcium would probably act as a contributing cause if there were primarily a condition of increased bone absorption.

In regard to the third cause we know that bones as well as other tissues have an active metabolism until well into adult life, and that absorption of old and building up of new bone is continuously taking place. Further, the exceedingly large quantity of calcium excreted

<sup>1</sup> This is the case in our patient.

<sup>2</sup> M. PFAUNDLER: *Münchener medizinische Wochenschrift*, 1903, l. p. 1577.

<sup>3</sup> VOIT: *Zeitschrift für Biologie*, 1880, xvi, p. 55; RÖLL: *Pathologie und Therapie der Haustiere*, 2d ed., p. 411; CHOSSAT: *Comptes rendus*, 1842, xiv, p. 51.

in both urine and fæces establishes the fact that in osteomalacia absorption of bone is particularly active. The cause of the active katabolism of bone in puerperal osteomalacia is undoubtedly the condition of pregnancy, during which the developing foetus requires large quantities of lime. Hanau<sup>1</sup> found at autopsy, especially in the bones of the pelvis, a condition similar to that found in osteomalacia even in pregnant women who seemed to be free from all signs of unsound bones.

In the non-puerperal cases we do not know the cause of the active bone katabolism. It is certainly not to be accounted for, as we have already shown,<sup>2</sup> by the old theory of solution of the calcium by an acid.

The abnormal sulphur metabolism may be explained as a condition secondary to that of the loss of calcium, — an attempt to make up for the great loss of osseous tissue by an excessive production of osteoid material. In the first experiment, while the disease was in progress, there was a loss of calcium with a retention of sulphur. The removal of the ovaries immediately affected the calcium metabolism and caused a retention. The change in the sulphur metabolism did not respond so rapidly, — just as we should expect in accordance with the view that the abnormal sulphur metabolism is secondary to the loss of calcium, — so that in the second experiment there is still a retention of sulphur, but not so great a retention as in the first experiment. By the time of the third experiment the sulphur metabolism had finally reached a condition of equilibrium. Just previous to this time the patient began to show symptoms of the disease again, and in this experiment, although there is a considerable loss of calcium, a marked new formation of osteoid tissue (which would be indicated by a retention of sulphur) has not yet begun. This combination of the two unfavorable circumstances probably accounts for the spontaneous fractures at just this time.

It seems probable that the loss of lime and consequent softening of bone in osteomalacia is merely one of the symptoms of the disease. We may relieve this symptom for a time by removing the ovaries. In the case of puerperal osteomalacia this operation removes also the possibility of the condition — namely, pregnancy — which is one of the causes of the disease. In non-puerperal osteomalacia,

<sup>1</sup> HANAU: *Fortschritte der Medicin*, 1892, x, p. 236.

<sup>2</sup> GOLDTHWAIT, PAINTER, OSGOOD, and MCCRUDDEN: *This journal*, 1905, xiv, p. 389.

castration likewise masks for a time the symptom of loss of calcium, but does not have any influence on the ultimate cause of the disease.

I wish to thank Drs. Goldthwait, Painter, and Osgood for obtaining the patient and maintaining her in the hospital during the course of the experiment.

## ON CONDUCTION AND CONTRACTION IN SKELETAL MUSCLE IN WATER RIGOR.

BY CLYDE BROOKS.

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

THE object of this paper is to ascertain whether contraction or conduction ceases first in skeletal muscle on going into water rigor.

Biedermann<sup>1</sup> bathed one end of the frog's sartorius in a hypotonic solution till local water rigor was induced. He does not state whether he used curarized frogs or not. On stimulating the rigid part of the muscle, visible contractions occurred only in the other end. He concluded from this that skeletal muscle in water rigor may conduct without contracting.

Engelmann,<sup>2</sup> using the curarized sartorius, repeated and confirmed Biedermann's observations and extended them to the frog's heart. He bathed the auricles in a hypotonic solution till no visible contractions could be obtained from them on stimulation; but on exciting the auricles, the ventricles contracted. He concluded from this that cardiac muscle in water rigor may conduct without contracting.

Three objections have been made to the above conclusions: —

1. It may be that nerve fibres resist the action of water for a longer time than muscle fibres do. Therefore in the heart the impulses may have been carried by the intrinsic nerves. Unless the nerve endings in the sartorius were completely curarized, this *may* have occurred in the case of the skeletal muscle too.

2. It is possible by the technique of the above experiments that there was complete rigor in the outer fibres, and yet that the inner fibres at this stage were relatively unaffected and able to contract on artificial stimulation, and that such contractions were masked by the rigidity of the outer fibres.

<sup>1</sup> BIEDERMANN: Sitzungsberichte der Wiener Akademie der Wissenschaften, 1888-89, xc, Abtheilung 3, p. 101; Electrophysiologie, 1895, p. 140.

<sup>2</sup> ENGELMANN: Archiv für die gesammte Physiologie, 1894, lvi, p. 199.



3. It is possible that the contractions observed were caused by the spread of the electrical current (actual escape, or electrotonus) instead of by physiological conduction.

Weight is given the first objection by the fact that in *Limulus* heart in a certain stage of water rigor, the intrinsic nerves still conduct even after the muscle fibres have lost their contractility.<sup>1</sup>

In order to test this point on skeletal muscle, a retractor muscle of the neck of the tortoise was used to make a preparation such as was made of the *Limulus* heart by Carlson in the article referred to above. The retractor muscle is supplied by a large nerve running very near the surface of the muscle from before backward, and giving off branches to the muscle fibres



FIGURE 1.—Method of preparing a retractor muscle of the neck of the tortoise for testing conduction through the intramuscular nerves.

at frequent intervals. In two places the main nerve was dissected out and the muscle transected into three parts connected only by the main nerve which was left intact (Fig. 1). The middle segment was then placed in distilled water. When the nerve was stimulated after this segment had become rigid, no visible contractions occurred in the middle segment itself, but both the other segments contracted strongly.

This shows that the intramuscular nerves of skeletal muscles may conduct even when the adjacent muscle fibres are in water rigor. This is analogous to the results of Carlson on the *Limulus* heart.

Therefore, if the uncurarized or the incompletely curarized sartorius was used, Biedermann's results may have been due to the stimulation of the intramuscular nerves. Although on examination of May's drawings<sup>2</sup> of the nerve supply of the frog's sartorius, it seemed possible that Biedermann's results were due to double conduction; yet several trials to show splitting of the axis cylinders by dissecting out the nerve and transecting the muscle at the point where the nerve enters it, and then stimulating one segment, failed, for there were no visible contractions in the other segment.

Since Engelmann used the curarized sartorius in confirming Biedermann's experiment, this objection does not apply to this part of his work. But, as pointed out by Carlson, his results on the frog's heart may have been due to the stimulation of the intrinsic nerves of the auricles.

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 112.

<sup>2</sup> MAY: *Zeitschrift für Biologie*, 1884, xx, p. 470.

On *a priori* grounds the second objection is reasonable, for, since the water would touch the outer fibres first, they should become rigid first. It is known that water rigor takes an appreciable time to become well established.<sup>1</sup>

In order to obtain exact data on this point the retractor of the tortoise was placed in distilled water and attached to a light lever which magnified the contractions about ten times. After the muscle had been acted upon by the water so that on stimulation no contractions could be detected even by the aid of a lens, tracings about 3 mm. in height were still recorded on the drum. After immersion of three or four hours the record showed no contractions whatever, even on stimulation with a much stronger current. The muscle was then pared on all sides, leaving only a small core of clear-looking tissue from the centre. On stimulation of this core with a much weaker current than that first used, tracings 3 cm. in height were recorded, the magnification being the same as before.

This shows that when skeletal muscle is allowed to stay for a time in distilled water, the outer part loses its contractility a measurable time before the inner part.

Therefore Biedermann's results *may* have been due to contractions of a core of fibres in the centre of the muscle, such contractions being masked by the rigidity of the outer fibres.

The third objection, as pointed out by Kaiser,<sup>2</sup> applies to the work of Biedermann and of Engelmann on the frog's sartorius.

In order to test this point experimentally, a curarized frog's sartorius was isolated, placed in a vertical position with each end attached to a light lever, and the middle held by a screw clamp. In order that this clamp should be insulated from the muscle, two strips of cork were partly imbedded in a layer of paraffin covering the surface of the clamp next to the muscle. Two fine platinum wire electrodes were thrust through the muscles near its lower end. The electrodes were connected with an induction machine supplied by a cell with an E. M. F. of about one volt. The strength of current was adjusted so that it should be more than sufficient to set up maximal contractions in the directly stimulated muscle and yet should not be strong enough for the current to escape to the other end of the muscle. With the muscle so lightly clamped that there was no interference with the passage of the contraction wave, the adjustment was made by gradually increasing from a very weak current to the point where there were maximal contractions. This fixed the minimal limit

<sup>1</sup> BIEDERMANN: *Electrophysiologie*, 1895, p. 305.

<sup>2</sup> KAISER: *Zeitschrift für Biologie*, 1895, xxxi, p. 244.

of the current to be used. Then the muscle was so strongly crushed by tightening the clamp that no physiological conduction could occur. The lower end of the muscle was then stimulated with increasingly strong currents till contractions began in the other half. This fixed the maximal limit of the strength of stimulus to be used. The crushed muscle was next replaced by an intact one. A dish of distilled water was brought up around the lower half of the muscle submerging all that part of it below the clamp. The upper part of the muscle was kept moist with 0.7 per cent sodium chloride solution. The preparation was allowed to stand in this condition except at certain intervals when the water was drawn off and the muscle stimulated.

The tracings (Fig. 2) show that the part of the muscle in water gradually shortens and loses contractility and also conductivity, as measured by contractions in the upper half of the muscle, so that in about twenty minutes all visible contractions in both parts of the muscle have ceased. On stimulating the upper part of the muscle directly, there were strong contractions. After this the rigid half was again stimulated with gradually increasing currents. When the maximal limit for stimulation, as determined above, was exceeded, the upper half of the muscle gave full contractions. But these were due to the spread of the current and not to physiological conduction, for after crushing the muscle with the clamp the contractions persisted. This agrees with the results of Kaiser, and is not necessarily contrary to those of H. E. Hering.<sup>1</sup>

It would seem, therefore, that the conclusions of Biedermann and of Engelmann do not follow from their observations.

In order to avoid the above three sources of error, an effort was made to study microscopically the effect of water on contractility and conductivity in an isolated muscle fibre of several cm. in length.

Two shallow cells were constructed of paraffin side by side on a glass slide which was placed on the stage of a microscope. One cell was filled with distilled water; the other with 0.07 per cent NaCl or with Ringer's solution. Next

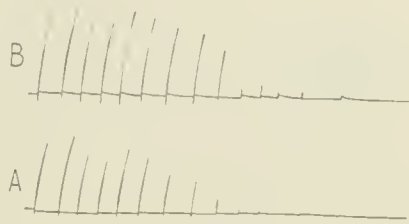


FIGURE 2.—Showing the effect of water on contraction and conduction in the curarized frog's sartorius. A. Contractions of the part in water on direct stimulation. B. Contractions of the part not in water on stimulation of the part in water. The stimulations are at one minute intervals.

<sup>1</sup> HERING: *Archiv für die gesammte Physiologie*, 1901, lxxxvi, p. 540.

the fibres of the retractor were separated by the aid of a microscope. This muscle was used on account of the great length of its fibres. Various methods for isolating a fibre were tried. The stripping method, which was the most successful, consisted in separating the fibres at one end of the muscle and then splitting them apart throughout their entire length. For this purpose fine pointed forceps were most useful. A single fibre thus isolated was placed on the slide with its anterior third submerged in the cell containing the solution;



FIGURE 3. — Cross section of a strip of eleven muscle fibres, about 5 cm. in length, used to test the effect of water on contractility and conductivity in skeletal muscle.

its middle third in the distilled water cell, while the posterior third was laid across two small electrodes connected with the secondary cell of an induction machine. On stimulation no change was observed in any of the preparations consisting of a single fibre. This negative result may be due to injuries during the process of isolation. For when a number of small strips of muscle tissue containing only a few fibres and several cm. in length (Fig. 3), was prepared in the same way, strong contractions were observed in all the fibres of some of the strips, although only a somewhat small proportion of

strips were successful. This was also due to unavoidable injuries. In order to minimize the danger of conduction through the intramuscular nerves, the strip was taken from the upper edge of the muscle where there are terminal nerve fibres only. For the same reason the electrodes were placed on the posterior end of the strip so that when it was stimulated the impulse would travel in the muscle opposite to the direction of the nerve fibres.

The experiment showed that the part of the fibres acted upon by the water gradually lost their contractility and conductivity in the same measure in which they became swollen and opaque. All visible contraction had disappeared in about three minutes. Conduction through that part of the fibres in the water, as judged by the contractions of the part in the saline solution, ceased at the same time.

*Therefore it is probable that when skeletal muscle goes into water rigor, it loses both contractility and conductivity at practically the same time.*

My thanks are due Dr. A. J. Carlson for suggesting the problem and for assistance in the work, and to Professor Stewart for criticism of the manuscript.

# THE CHEMISTRY OF THE PROTEIN BODIES OF THE WHEAT KERNEL. — PART II.<sup>1</sup>

## PREPARATION OF THE PROTEINS IN QUANTITY FOR HYDROLYSIS.

BY THOMAS B. OSBORNE AND ISAAC F. HARRIS.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

THE recent advance in our knowledge of the several decomposition products of the proteins and the methods for determining their amount has given us a new means for differentiating our various proteins and establishing their individuality. As the first step in this direction, we have undertaken the present work, and in this paper describe the methods which we have used in preparing our material for hydrolysis.

Before undertaking to determine the nature and amount of the products yielded by proteins when decomposed by boiling with strong acids, it is essential that the large quantity of material necessary for this work shall be as pure as possible, both as respects admixture with non-protein as well as with other protein substances. We have therefore endeavored to make preparations of the wheat proteins to be used for a study of their decomposition products of the highest degree of purity which our experience with them has made possible. In regard to the chemical individuality of the several wheat proteins which have been studied in the past in this laboratory we refer to papers already published which deal with this subject.<sup>2</sup> Although positive evidence of chemical individuality of protein substances cannot, with the means at present available, be obtained, there can be

<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 VOORHEES: *American chemical journal*, 1893, xv, p. 392; *Ibid.*, 1897, xix, 236; *Journal American Chemical Society*, 1900, xxii, 379; *This journal*, 1905, xiii, p. 36.

no question that protein substances can be isolated from seeds and animal tissues which are beyond doubt distinctly different substances.

Thus five unquestionably different forms of protein, differing in composition, solubility, and physical characters, can be isolated from the wheat kernel. Whether each of these is itself a chemical individual or a mixture of two or more very similar substances cannot at present be asserted. All that can be said is that it has not yet been possible to separate them into fractions the properties of which indicate a mixture.

Owing to the extreme sensitiveness of proteins to the action of acids, alkalis, and salts, minor differences in solubility are not to be depended upon as a basis for characterizing individual proteins. Thus the protein edestin, which in pure water is entirely insoluble, in the presence of a slight amount of acid is freely soluble therein. The addition of a small quantity of a neutral salt throws the protein out of this acid solution, while a larger quantity of salt at once redissolves it.

Such differences in solubility have nothing to do with the protein molecule proper, but depend on the formation of protein salts, the solubility of which is different from that of the free protein itself. As the formation of such protein salts depends on conditions that in most cases cannot be taken into account, such differences in solubility cannot be made a basis for characterizing the different individual proteins. We are therefore limited in dealing with such problems to the more marked differences in solubility, such as that in alcohol, strong saline solutions, or alkalis, and to constant ultimate composition of successive fractional precipitations.

Thus, when proteins have been separated into fractions which have the same composition, general solubility, and physical properties, we are not justified in concluding that we have in hand a single individual protein. All we can conclude is that we have reached the limit of separation attainable with the means now available, and that for the present we must accept such products as the simplest units with which we can now deal and which for the present must serve as our basis for further study. If, on the other hand, protein preparations, characterized in the manner above described, show distinct and constant differences from one another, we are justified in considering them to be different substances.

That the wheat kernel contains at least five such distinct protein substances has been shown by extensive researches in this laboratory,

the first of which<sup>1</sup> described the isolation of each of these and gave the results of attempts to establish their individuality, so far as the means then available permitted.

The proteins described were *gliadin*, insoluble in neutral aqueous solutions but distinguished from all the others by its ready solubility in neutral 70 per cent alcohol; *glutenin*,<sup>2</sup> a protein having a similar elementary percentage composition to gliadin, soluble in very dilute acid and alkaline solutions but insoluble in dilute alcohol or neutral aqueous solutions; *leucosin*, an albumin-like protein, freely soluble in pure water and coagulated by heating its solution to 50°-60°; a globulin similar in composition and properties to many globulins found in other seeds; and one or more proteoses which were present in very small quantity. In a later paper<sup>3</sup> the proteins obtained from the embryo of the wheat were described, and it was there shown that the globulin, albumin, and proteose above mentioned formed nearly all of the protein substance of this part of the seed. It thus appeared that these three proteins were contained chiefly in the embryo, and that gliadin and glutenin formed nearly the whole of the proteins of the endosperm, or over 90 per cent of the total protein matter of the seed.

#### PREPARATION OF THE ALBUMIN, LEUCOSIN.

The embryo of the wheat contains about 10 per cent of leucosin, while the whole kernel yields about 0.3 to 0.4 per cent. The commercial wheat-germ meal, which consists almost wholly of the embryo, together with a small amount of endosperm and bran, was therefore used in preparing the leucosin in quantity. The freshly ground meal was extracted with water, and, as the gummy solution could not be filtered within a reasonable time, an equal volume of saturated ammonium sulphate solution was added. The precipitate thus produced was filtered out, dissolved in water, the solution filtered perfectly clear, and the leucosin coagulated by heating the dilute solution to 65° in a water bath at 70°. As the only other protein substance present in this solution was a relatively insignificant quantity of proteose, the product obtained was practically free from any other pro-

<sup>1</sup> OSBORNE and VOORHEES: American chemical journal, 1893, xv, p. 392.

<sup>2</sup> This is the protein which RITTHAUSEN called gluten-casein.

<sup>3</sup> OSBORNE and CAMPBELL: Journal American Chemical Society, 1900, xxii, p. 379.

tein. This coagulum was thoroughly washed with hot water, in order to remove any admixed proteose, and dehydrated with absolute alcohol. The preparation formed a light white powder. In physical properties and elementary composition leucosin resembles the proteins of animal tissues more nearly than the reserve proteins of the endosperm. It is much less stable than the latter and more readily converted into an insoluble product.

ELEMENTARY COMPOSITION OF LEUCOSIN.

Carbon . . . . .	53.02 per cent.
Hydrogen . . . . .	6.84 " "
Nitrogen . . . . .	16.80 " "
Sulphur . . . . .	1.28 " "
Oxygen . . . . .	<u>22.06</u> " "
	100.00

Proteins of similar composition and properties to leucosin occur in small quantity in many cereals and legumes, and it is possible that these, like most of the animal proteins, form a part of the physiologically active tissue of the embryo and serve a different purpose in the metabolism of the seed from that of the reserve protein of the endosperm. It is therefore a matter of interest to compare the results of quantitative determinations of its decomposition products, both with those of the endosperm proteins and with those of proteins from physiologically active animal tissues.

PREPARATION OF THE GLOBULIN.

Whole wheat flour, when treated with 10 per cent sodium chloride solution, yields to the latter about 0.6 per cent of protein, which, on dialysis, is thrown down in the form of minute spheroids that are for the most part readily soluble again in sodium chloride brine. This protein has the properties characteristic of a large number of globulins obtained from other seeds and also resembles these in composition. Whether this resemblance extends further we do not at present know. We hoped therefore to be able to obtain a sufficient amount of this protein to enable us to make quantitative determinations of its several decomposition products, but in this we were disappointed. Owing to the small proportion in which this globulin is obtained from the flour of the entire seed, it is not practicable to prepare large quantities of it therefrom. We have therefore used freshly prepared



embryo meal from which the globulin can be extracted in considerable quantity. Unfortunately, when thus extracted, it is combined with triticonucleic acid, although the preparations from the entire seed contained none of this acid. Our attempts to separate the globulin from the nucleic acid resulted in such great loss that we were compelled to abandon our efforts to prepare sufficient quantities for the present investigation.

#### THE PROTEOSES.

Aqueous extracts of the wheat flour always contain a small proportion of protein matter which closely resembles in its properties the substances which are obtained by the action of pepsin-hydrochloric acid on native proteins. It is probable that these are to some extent, if not wholly, due to the action of the enzymes of the seed on the reserve proteins of the endosperm, as well as on the tissue proteins of the embryo. As we have no means of determining the origin of these proteoses or of separating the mixture into products of probable chemical individuality, we have made no attempt to prepare them for further investigation.

#### GLIADIN.

This important protein has been the subject of the first paper of this series<sup>1</sup> in which we reviewed the literature relating to gliadin published since 1893 and gave our reasons for rejecting such evidence as had been presented concerning the existence of more than one alcohol soluble protein in this seed. We also showed that fractional precipitates of gliadin from alcoholic solutions of various concentrations yielded similar proportions of glutaminic acid.

There being no sufficient evidence that more than one alcohol soluble protein occurs in the wheat kernel, we have made no attempt, in preparing large quantities of gliadin for our present investigation, to subject the protein matter extracted by alcohol to any fractional precipitation, but have undertaken to separate it as completely as possible from all other substances soluble in water, alcohol, and ether.

Wheat flour, unlike that of any other known seed, when mixed with sufficient water, forms a doughy mass which, on kneading in a current of water, yields to the latter nearly all the starch and water

<sup>1</sup> OSBORNE and HARRIS: This journal, 1905, xiii, p. 35.

soluble substances and finally assumes a coherent and elastic character. This latter product is "wheat gluten," and consists of a mixture of two proteins, gliadin and glutenin, together with more or less of the other constituents of the seed.

We have prepared the gliadin for this investigation entirely from gluten, as thereby the water-soluble constituents of the seed are more completely removed than by any other method of preparation which can be readily used on a large scale. The wheat flour was kneaded into dough in a domestic "bread mixer," and then under water in a specially constructed kneading-machine. After frequently decanting and renewing the water, a thoroughly coherent gluten was obtained. This was washed practically starch free in a current of water and, while moist, was ground by passing through a "drug press" which we found to be the most ready means of reducing it to comparatively small pieces. The ground gluten was then extracted with alcohol of such strength that, with the combined water of the gluten, a solvent of 60-70 volume per cent resulted. The extracts were filtered *perfectly* clear, through thick felts of filter paper pulp, and the water-clear solution, free from any trace of opalescence or turbidity, was evaporated to a small volume on a water bath. The thick syrup that resulted was cooled and then poured, with constant and rapid stirring, into a large volume of distilled ice water containing a very little sodium chloride. The gliadin was thus precipitated as a filament, which, on stirring, united to a coherent plastic mass. This gliadin was next dissolved by stirring with strong alcohol until all had gone into solution, the water combined with the precipitated gliadin being sufficient to dilute the alcohol to the proper degree. The resulting solution was evaporated to a thick syrup, absolute alcohol being added from time to time in order to hold the gliadin in the solution, since this, during the evaporation, became constantly more aqueous. The thick syrup was then poured in a very fine stream into a large volume of absolute alcohol, under rapid and constant stirring. In this way a porous mass of protein was obtained which was at once reduced to small pieces and digested under fresh absolute alcohol. When well dehydrated, the gliadin was digested with ether, partially dried over sulphuric acid, ground to a coarse powder, and then dried thoroughly over sulphuric acid. When thus prepared, gliadin forms a snow-white, friable mass which is easily reduced to a powder. If dried by slow evaporation of its solution in dilute alcohol, it forms perfectly clear, transparent sheets that closely resemble pure animal gelatin.

In consequence of this property gliadin was long known as plant gelatin.

In cold water gliadin is slightly soluble, especially if a very little acid is present. It is most probable that this solubility is due to the formation of soluble gliadin salts, and that the apparent slight solubility of preparations obtained in the above manner is due to the existence of such salts in the seed or to their formation during extraction.

Gliadin is insoluble in absolute alcohol and, so far as known, in all water-free organic liquids with the exception of glacial acetic acid. In aqueous alcohol it is soluble, the degree of solubility increasing with the amount of water until this reaches 30-40 per cent and then diminishing. The elementary composition of gliadin is shown by the following figures, which are averages of many accordant analyses of numerous fractional precipitations which have been made in this laboratory.

PERCENTAGE COMPOSITION OF GLIADIN.

	Gliadin-wheat; av. 25 analyses.	Gliadin-rye; av. 13 analyses.
Carbon . . . . .	52.72	52.72
Hydrogen . . . . .	6.86	6.84
Nitrogen . . . . .	17.66	17.72
Sulphur . . . . .	1.14	1.21
Oxygen . . . . .	<u>21.62</u>	<u>21.48</u>
	100.00	100.00

GLUTENIN.

Glutenin was prepared from the residue of the wheat gluten after extracting the gliadin by alcohol. This residue was dried at room temperature and then ground to a powder which was extracted first with absolute alcohol and then with ether as long as either solvent removed anything from it. The alcohol was then removed at room temperature and the residual powder treated with just enough 0.2 per cent solution of potassium hydroxide to dissolve it. The resulting turbid solution was then filtered perfectly clear and neutralized with very dilute hydrochloric acid. The precipitate produced was extracted with 70 per cent alcohol as long as any gliadin was removed, then thoroughly dehydrated with absolute alcohol and dried over sulphuric acid.

When thus prepared, glutenin has the following composition: —

## PERCENTAGE COMPOSITION OF GLUTENIN.

Carbon . . . . .	52.34
Hydrogen . . . . .	6.83
Nitrogen . . . . .	17.49
Sulphur . . . . .	1.08
Oxygen . . . . .	22.26
	<hr/>
	100.00

Unless the solution from which glutenin is precipitated is filtered perfectly clear, the product obtained contains much less nitrogen and more carbon. This fact is discussed in a former paper from this laboratory.<sup>1</sup>

The agreement in composition between glutenin and gliadin is close, and has in the past led several investigators to consider them to have a common origin or to be derived one from the other. That this is not so was first proved by Kossel and Kutscher,<sup>2</sup> who found lysine among the decomposition products of glutenin, but none among those of gliadin. This difference has been still further emphasized by the results of our present investigation which shows marked differences in the proportion of several of the decomposition products.

<sup>1</sup> OSBORNE and VOORHEES: *American chemical journal*, 1893, xv, p. 455.

<sup>2</sup> KOSSEL and KUTSCHER: *Zeitschrift für physiologische Chemie*, 1901, xxxi, p. 165.

# THE CHEMISTRY OF THE PROTEIN BODIES OF THE WHEAT KERNEL. — PART III.<sup>1</sup>

## HYDROLYSIS OF THE WHEAT PROTEINS.

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THE extensive use of wheat flour makes it of great importance to know as much as possible regarding its chemistry and especially regarding the chemistry of its protein constituents. This is of importance not only in relation to problems of vegetable chemistry and physiology as well as to problems of protein chemistry, but especially so in connection with the nutrition of man. In connection with the latter question it has become of fundamental importance to know the nature and proportion of the products which the food proteins yield when decomposed by boiling acids, for the recent progress in our knowledge of digestion has shown that the digestive enzymes convert the food protein very largely into the same substances as those produced by boiling acids. These final products of hydrolysis are consequently the units with which the process of assimilation chiefly deals.

Furthermore, as the wheat kernel is the only seed from which practically all of the protein constituents have been isolated and of which we have the most definite knowledge both in respect to their kind and proportion, it is especially desirable to supplement our present knowledge by as full information as possible concerning the primary decomposition products of these proteins in order that something in regard to their structural relations may be known and the kind and amount of the possible products of the metabolism of this seed may be ascertained.

The investigation of this problem has accordingly been undertaken, the results of which are given in the following pages.

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

As stated in Part II of this series of papers,<sup>1</sup> gliadin, glutenin, and leucosin are the only proteins of the wheat kernel which could be obtained in sufficient amount for such quantitative estimations of their decomposition products.

The determinations of the mono-amino acids have been made, for the most part, according to the methods recently proposed by E. Fischer,<sup>2</sup> and those of the bases essentially according to the methods given by Kossel and his associates.<sup>3</sup> The general plan of these analyses has been that followed by Abderhalden<sup>4</sup> in analyzing oxyhæmoglobin.

In carrying out this work care has been taken to make the identification of the substances determined as complete as possible, and to weigh only such products as examination showed to be essentially pure, so that the figures given in the following table are to be taken as minimal, except possibly those given for prolin, which may be slightly too high owing to the well-known difficulty of separating *all* of the other amino-acids from its alcoholic solution.

The results of these analyses are given in Table I.

The analyses show that these three proteins are distinguished from one another by differences so great that there can be no question that decided differences in structure exist between them. Thus gliadin differs from glutenin and leucosin by yielding no glycooll and lysine and much more prolin and glutaminic acid. Gliadin and glutenin each yield very much more glutaminic acid and ammonia than leucosin and less leucine. In respect to the amount of these amino-acids, leucosin more nearly resembles the animal proteins than the seed proteins thus far examined, and in this connection it is interesting to note that leucosin occurs chiefly if not wholly in the embryo of this seed and is probably one of its "tissue" proteins, in contrast to the "reserve" proteins of the endosperm of which gliadin and glutenin form the chief part.

Glycooll is not given among the products of hydrolysis of gliadin, for careful efforts to detect its presence in the preparation used for this analysis failed. Another hydrolysis of another lot of gliadin prepared in essentially the same way did yield 0.02 per cent of glycooll,

<sup>1</sup> OSBORNE and HARRIS: This journal, 1906, xvii, p. 245.

<sup>2</sup> FISCHER, E.: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 151.

<sup>3</sup> KOSSEL and KUTSCHER: *Ibid.*, 1900, xxxi, p. 161; and KOSSEL and PATTEN: *Ibid.*, 1903, xxxviii, p. 39.

<sup>4</sup> ABDERHALDEN: *Ibid.*, 1903, xxxvii, p. 484.

TABLE 1.

	Gladiin. <sup>1</sup>	Glutenin.	Lencosin.
	per cent ..... <sup>2</sup>	per cent	per cent
Glycocoll . . . . .		0.89	0.94
Alanine . . . . .	2.00	4.65	4.45
Amino-valerianic acid . . .	0.21	0.24	0.18
Leucine . . . . .	5.61	5.95	11.34
α prolin . . . . .	7.06	4.23	3.18
Phenyl alanine . . . . .	2.35	1.97	3.83
Aspartic acid . . . . .	0.58	0.91	3.35
Glutamic acid . . . . .	37.33	23.42	6.73
Serine . . . . .	0.13	0.74	....
Tyrosine . . . . .	1.20	4.25	3.34
Cystine . . . . .	0.45	0.02	....
Oxyprolin . . . . .	....	....	....
Lysine . . . . .	0.00	1.92	2.75
Histidine . . . . .	0.61 <sup>3</sup>	1.76 <sup>3</sup>	2.83 <sup>3</sup>
Arginine . . . . .	3.16	4.72	5.94
Ammonia <sup>4</sup> . . . . .	5.11	4.01	1.41
Tryptophane <sup>5</sup> . . . . .	present	present	present
Total . . . . .	65.81	59.66	50.32

<sup>1</sup> After this analysis of gliadin was nearly finished, ARDERHALDEN and SAMUELY (*Zeitschrift für physiologische Chemie*, 1905, xliv, p. 276) published an analysis of this protein. As their preparation yielded 12 per cent of humus while ours yielded only traces, and as their results differed from ours in several respects, we thought it desirable to complete our analysis and publish the results.

<sup>2</sup> See page 244.

<sup>3</sup> See page 257.

<sup>4</sup> Cf. OSBORNE and HARRIS: *Journal American Chemical Society*, 1903, xxv, p. 323.

<sup>5</sup> OSBORNE and HARRIS: *Ibid.*, 1903, xxv, p. 853.

the identity of which was certainly established. This small quantity was most probably due to a small admixture of glutenin in the gliadin used for this determination, which may have been dissolved in very small amount by the large quantity of dilute alcohol used for extract-

ing the gliadin. A contamination of this preparation with 2 per cent of glutenin would have sufficed to give the amount of glycooll found. The amount of prolin yielded by gliadin is larger than that as yet obtained from any other protein, those coming nearest being the albuminoids spongin and gelatin. As the amount of prolin from glutenin is relatively high, the wheat proteins as a whole, so far as we yet know, are particularly rich in this peculiar amino-acid.

Glutaminic acid is the chief product from the endosperm proteins of this seed, gliadin yielding more of this acid than any protein yet examined and, so far as known, very much more than the average of our food proteins. The average amount of glutaminic acid from the total protein matter of this seed is more than 30 per cent. The yield of ammonia is likewise relatively very high, more than 4.5 per cent for the total protein, while the aggregate amount of hexone bases is correspondingly small. In respect therefore to the proportion of several of their decomposition products, the wheat proteins present marked and important differences from other food proteins.

#### HYDROLYSIS OF GLIADIN.

Eleven hundred grams of gliadin equal to 998.6 gm., dried at  $110^{\circ}$ , were heated with a mixture of 1000 c.c. of concentrated hydrochloric acid and 1000 c.c. of water on the water bath for several hours, until the gliadin had dissolved and frothing had ceased. The solution was boiled in an oil bath having a temperature of  $115^{\circ}$  for ten hours, cooled with ice, and saturated with gaseous hydrochloric acid. After remaining on ice for two days, the glutaminic acid hydrochloride that had separated was filtered out, washed with ice-cold alcoholic hydrochloric acid, dissolved in water, the solution treated with bone black and freed from ammonia by boiling with an excess of barium hydroxide. After removing the barium with an equivalent amount of sulphuric acid, the glutaminic acid was separated as hydrochloride, and, when recrystallized and thoroughly dried, weighed 374.3 gm., equivalent to 300 gm. of free glutaminic acid. This was converted into the free acid, which melted at  $202^{\circ}$ - $203^{\circ}$ .

*Carbon and hydrogen*, 0.6218 gm. subst., gave 0.9276 gm.  $\text{CO}_2$  and 0.3494 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.4574 gm. subst. gave  $\text{NH}_3 = 4.3$  c.c.  $\text{HCl}$  (1 c.c.  $\text{HCl} = 0.01$  gm. N).

Calculated for  $\text{C}_5\text{H}_9\text{O}_1\text{N} = \text{C } 40.78$ ;  $\text{H } 6.18$ ;  $\text{N } 9.54$ .

Found . . . . =  $\text{C } 40.69$ ;  $\text{H } 6.24$ ;  $\text{N } 9.40$ .



The mother liquor from the recrystallized glutaminic acid was added to the filtrate from the first separation of the glutaminic acid, and the entire solution concentrated to a syrup under strongly reduced pressure. Three litres of alcohol, previously saturated with hydrochloric acid at a low temperature, were added to the syrup, and dry hydrochloric acid gas was passed into the solution until it was saturated. The mixture was again concentrated as before, under reduced pressure, the syrup again taken up in three litres of alcoholic hydrochloric acid, and, after standing several hours, again concentrated to a syrup, taken up in alcoholic hydrochloric acid and, after some hours, concentrated to a syrup on a bath of 40° and under a pressure of from 5 to 10 mm. The neutralization, extraction, and drying of the esters were conducted according to the method described by Emil Fischer.<sup>1</sup>

DISTILLATION A.

Fraction.	Temp. of bath up to	Vapor.	Pressure.	Weight.
I	93°	....	12.0 mm.	28.18 gm.
II	100°	75-76°	12.0 "	47.93 "
III	120°	....	0.8 "	64.68 "
IV	160°	....	0.8 "	40.00 "
Total . . . . .				179.80 "

The undistilled residue weighed 180.0 gm.

The residue, which remained after extracting the esters with ether, was made strongly acid with hydrochloric acid, freed from sodium and potassium salts by repeated evaporations with alcoholic hydrochloric acid, and thorough extraction of the precipitated chlorides with the latter. The alcoholic solution of the chlorides of the amino-acids was evaporated to a syrup, and esterification repeated as in the first instance. After extracting the esters with ether and drying them, the entire process was repeated, and the ethereal solution of the esters resulting from this third treatment was united with that from the second, thus following the method applied by Abderhalden to oxyhæmoglobin.<sup>2</sup>

<sup>1</sup> FISCHER, EMIL: *Zeitschrift für physiologische Chemie*, 1901, xxxiii, p. 151.

<sup>2</sup> ABDERHALDEN: *Ibid.*, 1903, xxxvii, p. 484

## DISTILLATION B.

Fraction.	Temp of bath up to	Pressure.	Weight.
I	83°	12.0 mm.	20.13 gm.
II	100°	12.0 "	36.81 "
III	120°	0.8 "	62.70 "
IV	200°	0.8 "	33.00 "
Total			152.64 "

**Fraction I.** *Distillation A.*—This was saponified at once by evaporation with concentrated hydrochloric acid on a water bath, the residue taken up in alcohol, the solution saturated with dry hydrochloric acid gas, and a crystal of glycocoll ester hydrochloride added. After prolonged standing on ice no separation occurred.

The solution was then evaporated on the water bath with concentrated hydrochloric acid, the latter removed with lead oxide and the lead with hydrogen sulphide. The amino-acids were subjected to fractional crystallization.

**Fraction I.** *Distillation B* was treated in substantially the same way, but although several attempts were made to isolate the hydrochloride of glycocoll ester, none was found. By systematic fractionation there were obtained from fraction I of the two distillations, A and B, 6.68 gm. alanine and 0.86 gm. leucine.

The leucine, when recrystallized from dilute alcohol, decomposed at about 298°.

*Carbon and hydrogen*, 0.1778 gm. subst., dried at 110°, gave 0.3577 gm. CO<sub>2</sub> and 0.1608 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>11</sub>O<sub>2</sub>N = C 54.89; H 10.01 per cent.

Found . . . . = C 54.87; H 10.04 " "

The alanine, when recrystallized by dissolving in a little hot water and gradually adding alcohol, decomposed at about 290°.

*Carbon and hydrogen*, 0.2404 gm. subst., dried at 110°. gave 0.3571 gm. CO<sub>2</sub> and 0.1712 gm. H<sub>2</sub>O.

*Nitrogen*, 0.3980 gm. subst. gave NH<sub>3</sub> equal to 6.2 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.40; H 7.93; N 15.75 per cent.

Found . . . . = C 40.51; H 7.91; N 15.58 " "

**Fraction II.** — Temp. of bath to 100°. Pressure, 12 mm. Weight, 83.84 gm. — *Distillations A and B.* Each was saponified by boiling for five and one-half hours with five parts of water.

The solution was evaporated to dryness under reduced pressure, the dried residue boiled up with absolute alcohol, and 13.37 gm. were dissolved. This solution was united with a similar one obtained from fraction III. The substance, insoluble in alcohol, after systematic fractional crystallization, gave

I. 22.1 gm. of leucine, which decomposed at about 298° and had the following composition:

*Carbon and hydrogen.* 0.3128 gm. subst., dried at 110°, gave 0.6310 gm. CO<sub>2</sub> and 0.2794 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.89; H 10.01 per cent.

Found . . . = C 55.01; H 9.92 " "

II. A fraction of 3.45 gm. which by fractional crystallization could not be further separated and gave results on analysis which agreed best for a mixture of leucine and amino-valerianic acid.

*Carbon and Hydrogen.* 0.2857 gm. subst., dried at 110°, gave 0.5519 gm. CO<sub>2</sub> and 0.2577 gm. H<sub>2</sub>O.

Calculated for equal molecules of leucine and amino-valerianic acid  
= C 53.05; H 9.74 per cent.

Found . . . = C 52.68; H 10.02 " "

III. 2.1 gm. substance which had the properties and composition of amino-valerianic acid.

*Carbon and hydrogen.* I. 0.2115 gm. subst., dried at 110°, gave 0.3957 gm. CO<sub>2</sub> and 0.1870 gm. H<sub>2</sub>O.

II. 0.4597 gm. subst., dried at 110°, gave 0.8643 gm. CO<sub>2</sub> and 0.3956 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.22; H 9.48 per cent.

Found . . . { I = C 51.03; H 9.82 " "  
                  { II = C 51.27; H 9.56 " "

**Specific rotation.** — Dissolved in 20 per cent hydrochloric acid ( $\alpha$ )<sub>D</sub><sup>20</sup> = +25.79°. E. Fischer and Dörpinghaus<sup>1</sup> found +25.9° for their preparation from horn, and Schulze and Winterstein<sup>2</sup> found +28.2° and +27.9° for preparations from lupine seedlings.

<sup>1</sup> FISCHER and DÖRPINGHAUS: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 462.

<sup>2</sup> SCHULZE and WINTERSTEIN: *Ibid.*, 1902, xxxv, p. 300.

The chlorine was removed from the solution used for determining the specific rotation, and the substance racemized by heating with 20 c.c. of water and 7 gm. of crystallized barium hydroxide for 19 hours in an autoclave at 175°. The barium was quantitatively removed with sulphuric acid, and the *a*-naphthyl-hydantoic acid prepared according to the directions of Neuberg and Manasse.<sup>1</sup> This crystallized in long needles and melted constantly, on repeated recrystallization from 40 per cent alcohol at 180°–181°.

*Carbon and hydrogen*, 0.3235 gm. subst., dried at 90°, gave 0.7936 gm. CO<sub>2</sub> and 0.1909 gm. H<sub>2</sub>O.

Calculated for C<sub>16</sub>H<sub>15</sub>O<sub>3</sub>N<sub>2</sub> = C 67.07; H 6.35 per cent.

Found . . . . = C 66.90; H 6.56 " "

By racemizing the remaining mixture of undetermined amino-acids we were unable to isolate any more amino-valerianic acid.

IV. 8.6 gm. alanine. — This was racemized by heating with an excess of barium hydroxide and coupled with *a*-naphthylisocyanate according to the directions of Neuberg and Manasse.<sup>2</sup>

The hydantoic acid, which crystallized in prisms, melted at 197°.<sup>3</sup>

*Carbon and hydrogen*, 0.3146 gm. subst., dried at 110°, gave 0.7480 gm. CO<sub>2</sub> and 0.1560 gm. H<sub>2</sub>O.

Calculated for C<sub>14</sub>H<sub>11</sub>O<sub>3</sub>N<sub>2</sub> = C 65.06; H 5.48 per cent.

Found . . . . = C 64.84; H 5.51 " "

**Fraction III.** Temp. of bath up to 120°. Pressure, 0.8 mm. Weight, 127.38 gm. This fraction was boiled for five and one-half hours with eight parts of water. The solution, evaporated to dryness under reduced pressure, gave 98 gm. of amino-acids or 80 per cent of the esters. Of this 59.94 gm. were soluble in alcohol. From the part insoluble in alcohol, by systematic fractional crystallization there were isolated 33.06 gm. of leucine and 4.57 gm. of alanine. The leucine decomposed at about 298°.

*Carbon and hydrogen*, 0.2416 gm. subst., dried at 110°, gave 0.4872 gm. CO<sub>2</sub> and 0.2166 gm. H<sub>2</sub>O.

*Nitrogen*, 0.2690 gm. subst., gave NH<sub>3</sub> = 2.85 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.89; H 10.01; N 10.70 per cent.

Found . . . . = C 54.99; H 9.96; N 10.59 " "

<sup>1</sup> NEUBERG and MANASSE: Berichte der deutschen chemischen Gesellschaft, 1905, xxxviii, p. 2359.

<sup>2</sup> *Ibid.*

<sup>3</sup> *Ibid.*

The alcohol soluble substance from fraction II was united with that from fraction III. The solution was evaporated to dryness under reduced pressure, the residue taken up in water and boiled gently for about an hour with an excess of copper hydroxide. The filtered solution was evaporated to dryness under reduced pressure and the residue boiled with absolute alcohol. The undissolved part was dissolved in water, freed from copper by hydrogen sulphide, and the solution again evaporated to dryness under reduced pressure. The residue was boiled with absolute alcohol in which all of it dissolved. The alcohol was evaporated off under reduced pressure, the residue dissolved in 500 c.c. of water and again converted into the copper salt. By concentration 19.91 gm. of crystalline racemic prolin copper salt were obtained, which is equal to 13.98 gm. of *a*-prolin. This was recrystallized from water and dried in the air.

*Water*, 0.7789 gm. subst., lost 0.0856 gm. H<sub>2</sub>O at 110°.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub> Cu · 2 H<sub>2</sub>O = H<sub>2</sub>O 11.00 per cent.

Found . . . . . = H<sub>2</sub>O 10.98 " "

*Carbon and hydrogen*, 0.6851 gm. subst., dried at 110°, gave 1.0310 gm. CO<sub>2</sub> and 0.3450 gm. H<sub>2</sub>O.

*Copper*, 0.2926 gm. subst. gave 0.0799 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = C 41.11; H 5.54; Cu 21.79 per cent.

Found . . . . . = C 41.04; H 5.59; Cu 21.81 " "

The *r*-prolin copper salt was freed from copper with hydrogen sulphide, its solution evaporated to dryness and the residue recrystallized from alcohol. After drying in vacuo over sulphuric acid the *r*-prolin melted at 203°–205°.<sup>1</sup>

*Carbon and hydrogen*, 0.3373 gm. subst., gave 0.6424 gm. CO<sub>2</sub> and 0.2453 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>2</sub>N = C 52.12; H 7.90 per cent.

Found . . . . . = C 51.94; H 8.08 " "

The solution of the alcohol soluble copper salt was evaporated to dryness and left a residue of *l*-prolin copper salt which, dried at 120°, weighed 71.62 gm. which is equal to 56.51 gm. of free *l*-*a*-prolin.

*Copper*, 0.2850 gm. subst., dried at 110°, gave 0.0760 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 21.79 per cent.

Found . . . . . = Cu 21.30 " "

<sup>1</sup> WILLSTAETER, R.: Berichte der deutschen chemischen Gesellschaft, 1900, xxxiii, p. 1100; FISCHER, EMIL: *Ibid.*, 1901, xxxiv, p. 458.

One-half of this prolin copper salt was freed from copper, and the prolin racemized by heating with 150 c.c. of water containing 80 gm. of crystallized barium hydroxide for five hours at 150°. The barium was removed quantitatively with sulphuric acid, the solution concentrated, and the prolin again converted into the copper salt. There was thus obtained 20.1 gm. of very nearly pure racemic  $\alpha$ -prolin copper.

*Water*, 0.3356 gm. subst., air dry, lost 0.0369 gm. H<sub>2</sub>O at 110°.

*Copper*, 0.2640 gm. subst., gave 0.0640 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu · 2 H<sub>2</sub>O = H<sub>2</sub>O 11.00; Cu 19.40 per cent.

Found . . . . . = H<sub>2</sub>O 11.00; Cu 19.35 " "

The total crystalline racemic copper salt was equal to 41.96 gm. of  $\alpha$ -prolin.

From the other half of l-prolin copper salt the free prolin was regenerated and recrystallized from alcohol. A small part only was obtained in a crystalline condition which melted at 205°-206°.

*Carbon and hydrogen*, 0.2729 gm. subst., dried over H<sub>2</sub>SO<sub>4</sub>, gave 0.5198 gm. CO<sub>2</sub> and 0.1977 gm. H<sub>2</sub>O.

*Nitrogen*, 0.2453 gm. subst., gave NH<sub>3</sub> = 2.95 c.c. HCl (1 c.c. HCl = 0.01 gm. N),

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>2</sub>N = C 52.12; H 7.90; N 12.20 per cent.

Found . . . . . = C 51.95; H 8.04; N 12.03 " "

From fraction III there were isolated 4.57 gm. alanine, 33.06 gm. leucine, and 70.49 gm.  $\alpha$ -prolin, including in this last that from fraction II which was not weighed separately.

Fraction.	Temp. of bath to	Pressure.	Weight.
IV, A	160°	0.8 mm.	39.96 gm.
B	200°	0.8 "	33.00 "
Total . . . . .			72.96 "

This was treated with water and shaken out with ether according to the procedure described by Emil Fischer.<sup>1</sup>

The ether was carefully removed by evaporation and the residual phenylalanine ester saponified by dissolving in concentrated hydrochloric acid, and evaporating on a water bath. The phenylalanine hydrochloride weighed 29.14 gm., equivalent to 23.87 gm. of free

<sup>1</sup> FISCHER, E.: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 274.

phenylalanine. The phenylalanine hydrochloride was recrystallized from strong hydrochloric acid. It was decomposed by evaporating with an excess of ammonia and the phenylalanine recrystallized from water. It melted, on slow heating, at  $263^{\circ}$ – $265^{\circ}$ .<sup>1</sup>

*Carbon and hydrogen*, 0.3051 gm. subst., dried at  $110^{\circ}$ , gave 0.7322 gm.  $\text{CO}_2$  and 0.1792 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.3020 gm. subst. gave  $\text{NH}_3 = 2.53$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_2\text{N} = \text{C } 65.40$ ;  $\text{H } 6.73$ ;  $\text{N } 8.50$  per cent.

Found . . . . = C 65.44; H 6.53; N 8.38 " "

The aqueous layer was heated with an excess of barium hydroxide on a water bath for five hours. After standing sometime the barium salt that had separated was filtered out and decomposed by an equivalent amount of sulphuric acid. The solution on concentration yielded 5.76 gm. of aspartic acid.

*Carbon and hydrogen*, 0.3866 gm. subst., dried at  $110^{\circ}$ , gave 0.5109 gm.  $\text{CO}_2$  and 0.1852 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.3637 gm. subst. gave  $\text{NH}_3 = 3.78$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_4\text{H}_7\text{O}_4\text{N} = \text{C } 36.09$ ;  $\text{H } 5.26$ ;  $\text{N } 10.53$  per cent.

Found . . . . = C 36.04; H 5.32; N 10.39 " " "

The filtrate from the barium aspartate was freed from barium, concentrated to small volume and saturated with hydrochloric acid. On prolonged standing a trace of phenylalanine hydrochloride separated, but no glutamic acid hydrochloride was obtained. After removing the hydrochloric acid with silver sulphate and the sulphuric acid with barium hydroxide, the solution was boiled with an excess of copper hydroxide, but no copper salt could be separated from it, even after concentrating to a very small volume. The copper was then removed and the solution treated with bone black and, when concentrated in vacuo over sulphuric acid, gave crystals, which, on fractional crystallization from water, gave 0.42 gm. of serine, which, in an open capillary, browned at about  $218^{\circ}$  and decomposed to a brownish mass at about  $240^{\circ}$ .

<sup>1</sup> FISCHER, EMIL, and ABDERHALDEN: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 268; ERLÉNMEYER and LIPP: *Annalen der Chemie*, 1883, ccxix, p. 197.

Carbon and hydrogen, 0.2577 gm. subst. dried at 110°, gave 0.3224 gm. CO<sub>2</sub> and 0.1602 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>3</sub>N = C 34.24; H 6.73 per cent.

Found . . . . = C 34.12; H 6.91 " "

In the filtrate from the serine there was obtained about 5.32 gm. of crystalline substance from which nothing definite could be isolated.

#### DISTILLATION RESIDUE.

The residues from distillations A and B were dissolved in boiling alcohol and the solutions united. On cooling, 5.76 gm. of long hair-like crystals separated.

The filtrate from this substance was freed from alcohol and saponified by heating with 200 gm. of crystallized barium hydroxide; the barium removed, the solution concentrated under reduced pressure to small volume, saturated with hydrochloric acid, and, after standing on ice for some time, yielded 74.21 gm. glutaminic acid hydrochloride, equal to 59.46 gm.<sup>1</sup>

The glutaminic acid hydrochloride melted at about 198° with effervescence.

Carbon and hydrogen, 0.4028 gm. subst., dried over H<sub>2</sub>SO<sub>4</sub>, gave 0.4802 gm.

CO<sub>2</sub> and 0.2056 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>10</sub>O<sub>4</sub>NCl = C 32.67; H 5.50 per cent.

Found . . . . = C 32.51; H 5.67 " "

Specific rotation. — Dissolved in 20 per cent hydrochloric acid.

$$(a) \frac{20^\circ}{D} = + 31.47^\circ.$$

Fischer and Dörpinghaus found +31.91° for a preparation from horn, +30.45° from gelatin, and +28.20° for one from casein.<sup>2</sup>

The residue which remained after removing the esters with ether from the original solution of the products of hydrolysis was treated in the way described by Emil Fischer,<sup>3</sup> for the isolation of oxy-prolin. The only substance, however, that could be isolated was serine, of

<sup>1</sup> ABDERHALDEN and WELLS: Zeitschrift für physiologische Chemie, 1905, xlv, p. 31.

<sup>2</sup> Cf. FISCHER, EMIL, and DÖRPINGHAUS: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 475.

<sup>3</sup> FISCHER, EMIL: Bericht der deutschen chemischen Gesellschaft, 1902, xxxv, p. 2660.



which 0.87 gm. was obtained which browned at about 219° and decomposed at about 240°.

*Carbon and hydrogen.* 0.2987 gm. subst., dried at 110°, gave 0.3743 gm. CO<sub>2</sub> and 0.1844 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>3</sub>N = C 34.24; H 6.73 per cent.

Found . . . . = C 34.18; H 6.85 " "

In the filtrate from this serine β-naphthalene-sulphone chloride failed to give any definite product.

We thus isolated, in the two distillations, from fraction I, 6.68 gm., from fraction II, 8.59 gm., and from fraction III, 4.57 gm. of alanine, or 19.84 gm. in all; from fraction II, 2.1 gm. of amino-valerianic acid; from fraction I, 0.86 gm., from fraction II, 22.10 gm., from fraction III, 33.06 gm. of leucine, — in all 56.02 gm.; from fractions II and III, 70.49 gm. of prolin; from fraction IV, 23.47 gm. of phenylalanine, 5.76 gm. of aspartic acid, and 0.42 gm. of serine, and from the residue which remained after extracting the esters, 0.87 gm. of serine, or 1.29 gm. in all. From the main solution of the total products of hydrolysis we obtained 300 gm. of glutaminic acid, and from the residues after distilling the esters 59.5 gm., or in all 359.5 gm.

As the amount of prolin which we found in this hydrolysis was so great, we have undertaken to confirm our result by a second hydrolysis, and have also made another effort to obtain glycocoll, which, if present in very small amount, might have escaped detection.

For this purpose we hydrolyzed 500 gm. of gliadin, air dry, equal to 439.6 gm. dried at 110°, in the same way as in the preceding hydrolysis. After esterifying and shaking out the esters three times, as before, the ether was removed by distillation at 760 mm. and the esters distilled.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	95°	17.00 mm.	20.20 gm.
II	80°	5.00 "	46.08 "
III	A	2.00 "	{ 44.14 gm. 15.70 "
	B	0.88 "	
IV	180°	0.88 "	54.70 "
V	200°	0.78 "	29.69 "
Total . . . . .			210.51 "

**Fraction I** was immediately evaporated on the water bath with hydrochloric acid, the residue dissolved in alcohol, and the solution

saturated with dry hydrochloric acid gas. The solution was concentrated to a small volume at a low temperature under a pressure of 10 mm., the residue taken up in alcohol, its solution cooled to 0°, and saturated with hydrochloric acid gas. On prolonged standing 0.22 gm. of glycocoll ester hydrochloride separated, which melted at 144°-145°. When mixed with pure glycocoll ester hydrochloride, the melting-point was unchanged.

*Chlorine*, 0.1058 gm. subst. gave 0.1063 gm. AgCl = Cl 24.86 per cent.

Calculated for  $C_4H_{10}O_2NCl$  = Cl 25.40 per cent.

Neither fraction II nor the ether distilled from the esters gave evidence of glycocoll.

This preparation of gliadin did, in fact, contain a very small amount of glycocoll, which is possibly due to a slight contamination with glutenin, in which we have since found a notable quantity of this amino-acid.

**Fraction II** was saponified, and the solution evaporated, under highly reduced pressure from a bath at 40°, and the residue extracted with alcohol, in which about 18 gm. dissolved.

**Fraction III**, by similar treatment, yielded 24 gm. of alcohol soluble substance. The alcoholic solutions were united and evaporated to dryness from a bath at 40°. The crystalline residue, when dried to constant weight in vacuo, weighed 39.59 gm. From this, by extraction with alcohol, 8.7 gm. of substance insoluble therein were separated.

We expect to return to the further examination of this prolin, but for the present we have accepted the weight of the substance soluble in alcohol as a measure of its amount.<sup>1</sup>

#### CYSTINE.

Three hundred grams of gliadin were digested for two to three hours at 85° with 900 c.c. of hydrochloric acid, sp. gr. 1.19, and the solution boiled for three hours. This was then concentrated to a syrup under diminished pressure, diluted to 900 c.c. with cold water and neutralized with 50 per cent sodium hydroxide solution. After

<sup>1</sup> EMIL FISCHER employs this method for estimating the proportion of prolin in proteins, but states that the result obtained is too high (Berichte der deutschen chemischen Gesellschaft, 1906, xxxix, p. 530).

boiling with a large amount of bone black and concentrating to 800 c.c., much substance separated which was recrystallized from about 300 c.c. of water. The recrystallized product was dissolved in five per cent sulphuric acid and precipitated by mercuric sulphate solution.<sup>1</sup>

The mercury precipitate was decomposed by hydrogen sulphide, the solution freed from hydrogen sulphide, neutralized with sodium hydroxide and acidified with acetic acid. On standing, cystine separated from the solution in hexagonal plates, and by adding alcohol to the filtrate more was obtained. When no more cystine could be thus obtained, the precipitation with mercuric sulphate was repeated. By several repetitions of this process 1.18 gm. of cystine were finally isolated, which, when recrystallized by dissolving in dilute ammonia and acidifying with acetic acid, gave the following analysis :

*Carbon and hydrogen*, 0.3063 gm. subst., dried at 110°, gave 0.3379 gm.

CO<sub>2</sub> and 0.1444 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>12</sub>O<sub>4</sub>N<sub>2</sub>S<sub>2</sub> = C 29.96 ; H 5.04 per cent.

Found . . . . = C 30.08 ; H 5.23 “ “

#### TYROSINE.

Two hundred and nineteen grams of gliadin, equal to 200 gm. dried at 110°, were treated with 600 c.c. of concentrated hydrochloric acid, digested for some time on a water bath and the solution boiled for twelve hours on an oil bath. The solution was freed from most of the glutaminic acid by saturating with hydrochloric acid, and the filtrate from the glutaminic acid was diluted, boiled with bone black, and then concentrated strongly to remove as much hydrochloric acid as possible. The rest of the acid was neutralized with 50 per cent sodium hydroxide solution. On standing, a considerable precipitate separated, which was filtered out and dissolved in ammonia. The resulting solution was boiled until most of the ammonia had been removed and the tyrosine that separated was filtered out. When dried, this weighed 2.4 gm., equal to 1.2 per cent of the gliadin. Recrystallized from boiling water, this gave the following results on analysis :

*Carbon and hydrogen*, 0.3661 gm. subst., dried at 110°, gave 0.7981 gm.

CO<sub>2</sub> and 0.2160 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>3</sub>N = C 59.62 ; H 6.13 per cent.

Found . . . . = C 59.45 ; H 6.56 “ “

<sup>1</sup> Cf. HOPKINS and COLE: *Journal of physiology*, 1901, xxvii, p. 418.

Tyrosine separated from our hydrolysis solutions of gliadin with very great difficulty. Two other attempts to determine its proportion, which were made by hydrolyzing with sulphuric acid, gave lower results, and the solutions from which the tyrosine separated still continued to give a strong Millon's reaction. Kutscher<sup>1</sup> found 2.09 per cent of tyrosine in gliadin, and Abderhalden and Samuely<sup>2</sup> found 2.37 per cent. We consider our result to be too low, and expect to have an opportunity to return to this determination at an early date.

#### ARGININE AND HISTIDINE.

Fifty grams of gliadin, equal to 43.97 gm., dried at 110°, were hydrolyzed according to the directions of Kossel and Kutscher,<sup>3</sup> and the determination of the bases carried out according to the method of Kossel and Patten.<sup>4</sup>

The solution of the histidine was made up to 500 c.c., and nitrogen determined in 100 c.c. of it.

100 c.c. of solution gave ammonia = 1.37 c.c. HCl (1 c.c. HCl = 0.01 gm. N.) = 0.0137 gm. N. = 0.0685 gm. N. in 500 c.c. = 0.2524 gm. histidine = 0.58 per cent of the gliadin.

The identity of this histidine was not established, as the quantity was too small to permit the preparation of a satisfactory product.

The arginine solution was made up to 500 c.c. and nitrogen determined in 50 c.c. of it.

50 c.c. of solution gave ammonia = 4.15 c.c. HCl (1 c.c. HCl = 0.01 gm. N.) = 0.0415 gm. N. or 0.415 gm. in 500 c.c. = 1.39 gm. arginine, or 3.16 per cent of the gliadin.

The remaining solution was treated as Kossel and Kutscher direct, and the arginine obtained as carbonate. A portion of this carbonate was converted into the picrolonate according to the directions of Steudel.<sup>5</sup> This melted at 226°–227°, while Steudel gives 225°.

<sup>1</sup> KUTSCHER: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 111.

<sup>2</sup> ABDERHALDEN and SAMUELY: *Ibid.*, 1905, xlv, p. 276.

<sup>3</sup> KOSSEL and KUTSCHER: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 165.

<sup>4</sup> KOSSEL and PATTEN: *Ibid.*, 1903, xxxviii, p. 39.

<sup>5</sup> STEUDEL: *Zeitschrift für physiologische Chemie*, 1902, xxxvii, p. 219.

Nitrogen, 0.0832 gm. subst., dried at 110°, gave 18.8 c.c. moist N at 765 mm. and 25°.

Calculated for  $C_6H_{14}O_2N_4 \cdot C_{10}H_9O_5N_4 = N$  25.62 per cent.

Found . . . . . = N 25.40 " "

The filtrate from the silver precipitate of arginine and histidine was freed from silver, precipitated with phosphotungstic acid and lysine tested for with picric acid in the usual way, but none was found.

Kossel and Kutscher<sup>1</sup> found, in the three fractions of the alcohol-soluble protein of the wheat kernel which they examined, 1.2, 0.43, and 1.53 per cent of histidine. Our determination falls between these. Kutscher,<sup>2</sup> in discussing the individuality of these three fractions, considers the differences in the amount of histidine found in them to be within the limits of accuracy of these determinations.

Kossel and Kutscher<sup>3</sup> found 3.05 per cent of arginine in the fraction which they called gluten fibrin, 2.75 per cent in their gliadin, and 3.13 per cent in their mucedin. In determining the amount of protein hydrolyzed they calculated the weight from the nitrogen in solution. If, as we believe, only one alcohol-soluble protein exists in this seed,<sup>4</sup> namely, gliadin, with 17.5 per cent of nitrogen, and that the nitrogen of their solutions belonged to this protein, the proportion of arginine found by them would be, respectively, 3.13, 2.79, and 3.25 per cent, with which our determination of 3.16 per cent agrees very closely.

#### HYDROLYSIS OF GLUTENIN.

Nine hundred and forty grams, equal to 839.32 gm. of glutenin, ash, and water, free, were hydrolyzed by heating for several hours on a water bath with a mixture of 950 c.c. concentrated HCl and 950 c.c. water. After standing over night, the solution was boiled on an oil bath for nine hours, and then saturated with hydrochloric acid gas. By the same treatment as that applied to gliadin (page 234) 202.73 gm. glutamic acid hydrochloride, equal to 162.40 gm. of the free acid, were obtained. Recrystallized once from concentrated hydrochloric acid, this melted at 198°.

<sup>1</sup> KOSSEL and KUTSCHER: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 165.

<sup>2</sup> KUTSCHER: *Ibid.*, 1903, xxxviii, p. 111.

<sup>3</sup> KOSSEL and KUTSCHER: *Loc. cit.*

<sup>4</sup> Cf. OSBORNE and HARRIS: *This journal*, 1905, xiii, p. 35.

Chlorine, 0.5386 gm. subst., gave 0.4211 gm. AgCl.

Nitrogen, 0.5911 gm. subst., gave  $\text{NH}_3 = 4.53$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_5\text{H}_9\text{O}_4\text{NCl} = \text{Cl } 19.35$ ; N 7.65 per cent.

Found . . . . = Cl 19.33; N 7.66 " "

The united filtrates and washings were concentrated to a syrup on a water bath under reduced pressure, and the hydrochlorides of the amino-acids esterified three times, as in the case of gliadin. The hydrochlorides of the esters were neutralized and the free esters shaken out with ether, as in gliadin. After drying the ether solution of the esters with potassium carbonate, it was kept two days over anhydrous sodium sulphate. The ether was then removed by distillation from a water bath at atmospheric pressure, and the esters distilled with the following results:

#### DISTILLATION A.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	65°	12.0 mm.	61.00 gm.
II	100°	12.0 "	43.74 "
III { a	100°	4.0 "	45.94 "
	b	110°	1.5 "
IV	155°	1.5 "	43.32 "
V	200°	0.8 "	32.56 "
Total . . . . .			259.57 "

The undistilled residue weighed 211.5 gm.

The residue from which the esters had been removed by ether was subjected to two more esterifications, as in the case of gliadin, and the esters distilled.

#### DISTILLATION B.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	65°	12.0 mm.	28.74 gm.
II	88°	12.0 "	24.27 "
III { a	100°	10.0 "	19.18 "
	b	120°	0.8 "
IV	180°	0.8 "	23.92 "
Total . . . . .			119.34 "

The undistilled residue weighed 93 gm.

The different fractions from the two distillations were worked up as follows:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I { A	65°	12 mm.	61.00 gm.
{ B	65°	12 "	28.74 "

This was saponified at once by evaporating to a syrup with concentrated hydrochloric acid and the residue dissolved in alcohol and esterified with dry hydrochloric acid gas. The glycocoll ester hydrochloride which separated weighed 6.68 gm. Recrystallized from alcohol, this melted at 144°.

*Carbon and Hydrogen.* 0.3179 gm. subst., gave 0.3984 gm. CO<sub>2</sub> and 0.2151 gm. H<sub>2</sub>O.

Calculated for C<sub>4</sub>H<sub>10</sub>O<sub>2</sub>NCl = C 34.39; H 7.23 per cent.

Found . . . . = C 34.18; H 7.52 " "

The filtrate from the glycocoll ester hydrochloride was saponified by evaporating on the water bath with hydrochloric acid, the latter removed with silver sulphate, and the solution freed from sulphuric acid with an equivalent quantity of barium hydroxide. By fractional crystallization this solution yielded 11.83 gm. of alanine which melted at about 290°.

*Carbon and hydrogen,* 0.2701 gm. subst., dried at 110°, gave 0.4001 gm. CO<sub>2</sub> and 0.1946 gm. H<sub>2</sub>O.

*Nitrogen,* 0.3460 gm. subst., dried at 110°, gave NH<sub>3</sub> = 5.44 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.40; H 7.93; N 15.75 per cent.

Found . . . . = C 40.40; H 8.01; N 15.73 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
II { A	100°	12 mm.	43.74 gm.
{ B	88°	10 "	24.27 "

The united esters were saponified by boiling with ten parts of water for five hours, when their solution reacted neutral to litmus. This solution was evaporated to dryness under reduced pressure and boiled up with absolute alcohol, whereby 0.5 gm. substance was dissolved. By repeated fractional crystallization of the substance

insoluble in alcohol, 3.5 gm. leucine were obtained. The isolated leucine decomposed at about 298°.

*Carbon and hydrogen*, 0.2408 gm. subst., dried at 110°, gave 0.4852 gm.

CO<sub>2</sub> and 0.2169 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.89; H 10.01 per cent.

Found . . . . = C 54.95; H 10.01 " "

After adding to the filtrate from this leucine, the most soluble portion of fraction III, and further fractioning, 25.76 gm. of alanine and 3.84 gm. of glycocoll were obtained. The latter was isolated as the hydrochloride of the ester, which melted at 144°. The alanine was racemized and the *α*-naphthyl-hydantoic acid prepared according to the directions of Neuberger and Manasse.<sup>1</sup> When recrystallized from dilute alcohol, this melted at 197°.

*Carbon and Hydrogen*, 0.2527 gm. subst., dried at 90°, gave 0.5998 gm.

CO<sub>2</sub> and 0.1280 gm. H<sub>2</sub>O.

*Nitrogen*, 0.3515 gm. subst., dried at 90°, gave NH<sub>3</sub> = 3.74 c.c. of HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>14</sub>H<sub>14</sub>O<sub>3</sub>N<sub>2</sub> = C 65.06; H 5.48; N 10.87 per cent.

Found . . . . = C 64.74; H 5.61; N 10.61 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
III { A	110°	1.5 mm.	78.95 gm.
{ B	120°	0.8 "	42.41 "

These esters were saponified by boiling with six parts of water for five hours, when their solution reacted neutral to litmus. After evaporating to dryness under reduced pressure, the dried residue was extracted with boiling absolute alcohol. The part insoluble in alcohol, by a systematic fractional crystallization, gave 32.42 gm. of leucine which decomposed at about 298°.

*Carbon and hydrogen*, 0.4255 gm. subst., dried at 110°, gave 0.8535 gm.

CO<sub>2</sub> and 0.3926 gm. H<sub>2</sub>O.

*Nitrogen*, 0.1488 gm. subst., gave NH<sub>3</sub> = 1.60 c.c. HCl. (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.89; H 10.01; N 10.70 per cent.

Found . . . . = C 54.71; H 10.25; N 10.77 " "

<sup>1</sup> NEUBERGER and MANASSE: Berichte der deutschen chemischen Gesellschaft, 1905, xxxviii, p. 2359.



The filtrate from this leucine yielded 2 gm. of substance which had the composition and properties of amino-valerianic acid.

*Carbon and hydrogen*, 0.2216 gm. subst., gave 0.4144 gm. CO<sub>2</sub> and 0.1967 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.22; H 9.48 per cent.

Found . . . . = C 51.00; H 9.86 " "

**Specific rotation.** — Dissolved in 20 per cent hydrochloric acid,

$$(a) \frac{20^\circ}{D} = +25.63^\circ.$$

A similar preparation from gliadin gave +25.79°. E. Fischer and Dörpinghaus<sup>1</sup> found +25.9° for a preparation from horn, and E. Schulze and Winterstein<sup>2</sup> found +28.2° and +27.9° for a preparation from lupine seedlings.

The solution used for determining the specific rotation was freed from hydrochloric acid with silver sulphate, and the amino-acids racemized by heating with an excess of barium hydroxide in an autoclave at 175° for nineteen hours. After removing the barium quantitatively with sulphuric acid, the substance was coupled with  $\alpha$ -naphthylisocyanate. The hydantoic acid melted constantly on repeated recrystallization from dilute alcohol at 183°–184°. Heated side by side with the corresponding substance obtained from gliadin, the hydantoic acid from glutenin melted 2° higher.

*Carbon and hydrogen*, 0.3277 gm. subst., dried at 80°, gave 0.8067 gm. CO<sub>2</sub> and 0.1891 gm. H<sub>2</sub>O.

Calculated for C<sub>16</sub>H<sub>18</sub>O<sub>3</sub>N<sub>2</sub> = C 67.07; H 6.35 per cent.

Found . . . . = C 67.14; H 6.42 " "

From the filtrate from the amino-valerianic acid there was further obtained 1.41 gm. of alanine. The alcoholic solution which contained the prolin was evaporated to dryness under reduced pressure and the dried residue again treated with boiling absolute alcohol. Even after several repetitions of this process no substance insoluble in alcohol could be obtained. The alcohol soluble substance, when dried, weighed 35.54 gm. A copper salt was prepared from this in the usual

<sup>1</sup> FISCHER, E., and DÖRPINGHAUS: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 462.

<sup>2</sup> SCHULZE, E., and WINTERSTEIN: *Ibid.*, 1902, xxxv, p. 300.

manner, and its solution evaporated to dryness under reduced pressure. The dried residue was extracted with boiling absolute alcohol in order to remove the l-prolin copper. The residue insoluble in alcohol, when recrystallized from water, gave 15.53 gm. of racemic prolin copper, equivalent to 10.9 gm. of prolin.

*Water.* 0.3618 gm. subst., lost at 110° 0.0399 gm. of H<sub>2</sub>O.

*Copper.* 0.3176 gm. subst., dried at 110°, gave 0.0856 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu · 2 H<sub>2</sub>O = H<sub>2</sub>O 11.00 per cent.

Found . . . . . = H<sub>2</sub>O 11.03 " "

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 21.79 per cent.

Found . . . . . = Cu 21.54 " "

The alcoholic solution of the l-prolin copper salt was evaporated to dryness, the copper removed, and the prolin identified as the phenylhydantoin which was prepared according to the directions of Fischer.<sup>1</sup> The substance thus prepared was at once pure and melted at 143°.

*Carbon and hydrogen.* 0.2822 gm. subst., dried in vacuum over H<sub>2</sub>SO<sub>4</sub>. gave 0.6866 gm. CO<sub>2</sub>, and 0.1494 gm. H<sub>2</sub>O.

*Nitrogen.* 0.1025 gm. subst., gave NH<sub>3</sub> = 1.33 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.60; H 5.61; N 12.99 per cent.

Found . . . . . = C 66.36; H 5.88; N 12.98 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
IV, A	155°	1.5 mm.	43.32 gm.

This fraction was shaken out with ether in the usual way and the ether allowed to evaporate spontaneously. No evidence of the presence of phenylalanine was obtained. The residue of ester was saponified with concentrated hydrochloric acid and the hydrochloride decomposed with ammonia. The free acid crystallized in the characteristic form of leucine and decomposed at 298°. There were obtained 13.99 gm. of leucine.

*Carbon and hydrogen.* 0.2906 gm. subst., dried at 110°, gave 0.5825 gm. CO<sub>2</sub> and 0.2690 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.89; H 10.01 per cent.

Found . . . . . = C 54.67; H 10.28 " "

The aqueous layer was saponified by heating on a water bath with an excess of barium hydroxide for five hours. The barium aspartate,

<sup>1</sup> FISCHER, E.: Zeitschrift für physiologische Chemie, 1901. xxxiii, p. 151.

which separated in considerable quantity on standing, was united with that obtained from fraction V and treated as will be described later.

From the filtrate from the barium aspartate no definite substance could be obtained. It appeared to contain serine, but none could be isolated, even by the use of  $\beta$  naphthalene-sulphone-chloride.

Fraction.	Temp. of bath up to	Pressure.	Weight.
V, A	200°	0.8 mm.	32.56 gm.
IV, B	180°	0.8 "	23.92 "

These esters were shaken out with ether, and the substance extracted was saponified with hydrochloric acid. The hydrochloride thus obtained, which weighed 20.21 gm., equal to 16.55 gm. free phenylalanine, was converted into the free acid with ammonia and then into the copper salt by boiling its solution with copper hydroxide.<sup>1</sup>

*Copper.* 0.2099 gm. subst., dried at 110°, gave 0.0425 gm. CuO.

*Nitrogen.* 0.2283 gm. subst., gave NH<sub>3</sub> = 1.63 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>18</sub>H<sub>20</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 16.23 ; N 7.17 per cent.

Found . . . . = Cu 16.18 ; N 7.14 " "

The free phenylalanine, isolated from this copper salt, melted at 263°-265°.

*Nitrogen.* 0.1085 gm. subst., gave NH<sub>3</sub> = 0.92 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N = N 8.50 per cent.

Found . . . . = N 8.48 " "

The aqueous layer was saponified by heating for five hours with an excess of barium hydroxide on the water bath. The barium aspartate, which separated on standing, was united with that previously obtained from fraction IV A, decomposed with an equivalent amount of sulphuric acid, and 7.12 gm. of free aspartic acid was obtained from the solution.

*Carbon and hydrogen.* 0.3293 gm. subst., dried at 110°, gave 0.4335 gm. CO<sub>2</sub> and 0.1625 gm. H<sub>2</sub>O.

*Nitrogen.* 0.2997 gm. subst., gave NH<sub>3</sub> = 3.17 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>11</sub>H<sub>7</sub>O<sub>4</sub>N = C 36.05 ; H 5.31 ; N 10.55 per cent.

Found . . . . = C 35.90 ; H 5.48 ; N 10.58 " "

<sup>1</sup> Cf. SCHULZE and WINTERSTEIN: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 210.

The filtrate from barium aspartate was freed from barium, concentrated under reduced pressure, and saturated with hydrochloric acid. On long standing on ice a trace of phenylalanine hydrochloride separated, but no glutaminic acid hydrochloride could be obtained. After removing the hydrochloride acid with silver sulphate, the solution was boiled with a solution of copper hydroxide and 1.1 gm. of copper aspartate was isolated.

*Copper*, 0.3991 gm. subst., air dried, gave 0.1163 gm. CuO.

*Nitrogen*, 0.2066 gm. subst. gave  $\text{NH}_3 = 1.06$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_4\text{H}_6\text{O}_4\text{NCu} \cdot 4\frac{1}{2} \text{HO}^1 = \text{Cu } 23.06$ ; N 5.09 per cent.

Found . . . . . = Cu 23.27; N 5.13 " "

The filtrate from this copper aspartate, when freed from copper by hydrogen sulphide and concentrated to small volume, yielded, by fractional crystallization, 4.35 gm. of nearly pure serine.

*Carbon and hydrogen*, 0.2847 gm. subst., gave 0.3559 gm.  $\text{CO}_2$  and 0.1751 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_3\text{H}_7\text{O}_3\text{N} = \text{C } 34.29$ ; H 6.67 per cent.

Found . . . . . = C 34.09; H 6.83 " "

This substance browned at about  $213^\circ$  and decomposed to a brownish mass at about  $243^\circ$ .

#### RESIDUE FROM DISTILLATION.

The residues which remained after distilling off the esters weighed 30.45 gm. These were dissolved in hot alcohol, and from their united solutions 3.16 gm. of substance separated on cooling. The filtrate from this substance was evaporated under reduced pressure, the residue dissolved in water and saponified by heating with an excess of barium hydroxide for nine hours. After removing the barium quantitatively, the solution was concentrated and saturated with hydrochloric acid. On standing for some time on ice, 38.25 gm. of glutaminic acid hydrochloride, which melted at  $198^\circ$ , were obtained. This was equivalent to 30.64 gm. of free glutaminic acid, and, with that previously isolated, makes a total of 193.04 gm., or 23.0 per cent of the glutenin. The glutaminic acid hydrochloride was decomposed with an equivalent quantity of potassium hydroxide, and the free

<sup>1</sup> RITTHAUSEN: Die Eiweisskoerper, etc., Bonn, 1872, p. 219.

glutaminic acid was recrystallized from water. It melted at 202°–203° with effervescence.

*Carbon and hydrogen.* 0.3504 gm. subst., dried at 110°, gave 0.5275 gm. CO<sub>2</sub> and 0.2003 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.82 ; H 6.12 per cent.

Found . . . . = C 41.06 ; H 6.35 “ “

#### RESIDUE AFTER ESTERIFICATION.

The residue which remained after the third esterification and extraction of the esters was treated in the same way as the corresponding residue from gliadin (page 242), and the solution, freed from all mineral salts and bases precipitable by phosphotungstic acid, was concentrated under reduced pressure to a small volume and then left for some time over sulphuric acid. After removing a little tyrosine that first separated, 1.87 gm. of serine crystallized out, which, when recrystallized from water, browned at about 210° and decomposed at 240°.

*Carbon and hydrogen.* 0.2308 gm. subst., dried at 110°, gave 0.2894 gm. CO<sub>2</sub> and 0.1407 gm. H<sub>2</sub>O.

Calculated for C<sub>2</sub>H<sub>7</sub>O<sub>3</sub>N = C 34.29 ; H 6.67 per cent.

Found . . . . = C 34.19 ; H 6.77 “ “

This, with the serine obtained from fraction V, gives a total of 6.22 gm. of serine isolated. The mother liquor from the serine contained considerable substance, but no oxyprolin or other definite substance could be obtained from it.

#### CYSTINE.

Three hundred grams of glutenin were hydrolyzed in the way described for gliadin (page 244). After evaporating at low pressure to a syrup, neutralizing the remaining excess of acid with sodium hydroxide and decolorizing the solution with bone black, a considerable quantity of tyrosine separated out, which, on examination, was found to contain nearly all the cystine that could be detected in the solution. It was therefore dissolved in five per cent sulphuric acid and the cystine precipitated with mercuric sulphate. The mercury precipitate was decomposed with hydrogen sulphide, the solution concentrated somewhat, made alkaline with ammonia and then acid with acetic acid, and an equal volume of alcohol added. The cystine, which separated on

standing in characteristic hexagonal plates, weighed only 0.17 gm. No more could be obtained. This was dissolved in ammonia and reprecipitated by acetic acid.

*Sulphur*, 0.0897 gm. subst., dried at  $110^{\circ}$ , gave 0.1730 gm.  $\text{BaSO}_4$

Calculated for  $\text{C}_6\text{H}_{12}\text{O}_4\text{N}_2\text{S}_2 = \text{S } 26.68$  per cent.

Found . . . . = S 26.53 " "

Although glutenin contains about the same amount of sulphur as gliadin, the amount of cystine obtained from the latter under similar conditions was very much greater. It would seem as if glutenin in fact yields less cystine, though the uncertainties attending the isolation of this substance will not permit of a positive conclusion.

#### TYROSINE.

Two hundred and fifty grams of glutenin were boiled with a mixture of 750 gm. sulphuric acid and 1500 gm. of water for twelve hours. The solution was freed from barium by an equivalent amount of sulphuric acid, and after concentrating to 800 c.c., allowed to stand for some time. A considerable quantity of tyrosine separated, which was filtered out, the filtrate boiled with barium carbonate in order to expel ammonia, and then concentrated to one-half its original volume. After cooling, the residue of barium carbonate and other substances which had separated were extracted with hot dilute ammonia and the filtered extract concentrated and cooled. On standing, a little more tyrosine separated, which was added to that first obtained. No more tyrosine could be isolated from the solution of the hydrolytic decomposition products. All of the tyrosine which had separated was dissolved in five per cent sulphuric acid, and phosphotungstic acid was added to the solution. Only a small precipitate resulted. After removing the phosphotungstic acid with barium hydroxide, the solution was concentrated strongly and allowed to cool. After standing for some time 9.62 gm. of tyrosine were obtained, which is equal to 4.25 per cent of the glutenin.

*Carbon and hydrogen*, 0.2922 gm. subst., dried at  $110^{\circ}$ , gave 0.6370 gm.  $\text{CO}_2$  and 0.1634 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_3\text{N} = \text{C } 59.62$ ; H 6.13 per cent.

Found . . . . = C 59.45; H 6.21 " "

Kutscher<sup>1</sup> found 2.75 per cent of tyrosine in "gluten-casein."

<sup>1</sup> KUTSCHER: *Zeitschrift für physiologische Chemie*, 1903, xxxvi, p. 114.

ARGININE, HISTIDINE, AND LYSINE.

Fifty grams of glutenin, equal to 43.39 gm., dried at 110°, were hydrolyzed, and the arginine and histidine separated in the same way as that described for gliadin (page 246).

The solution containing the histidine was made up to 500 c.c.

*Nitrogen*, 100 c.c. solution gave  $\text{NH}_3 = 4.15$  c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 0.0415 gm. N = 0.2075 gm. in 500 c.c. = 0.7645 gm. histidine = 1.76 per cent.

The amount of histidine in the remaining solution was too small for identification.

The filtrate from the histidine precipitate yielded 500 c.c. of solution containing the arginine, in which was found the following amount of nitrogen:

*Nitrogen*, 50 c.c. solution gave  $\text{NH}_3 = 6.79$  c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 0.0679 gm. N, or 0.679 gm. in 500 c.c., or 2.107 gm. arginine = 4.72 per cent.

The remaining solution, treated as Kossel directs, yielded the arginine as carbonate. This was converted into the copper salt which gave the following results on analysis:

*Carbon and hydrogen*, 0.2118 gm. subst., air dried, lost 0.0210 gm.  $\text{H}_2\text{O}$  at 100°.

Calculated for  $\text{C}_{12}\text{H}_{23}\text{N}_{10}\text{O}_{10} \text{Cu} \cdot 3 \text{H}_2\text{O} = \text{H}_2\text{O}$  9.15 per cent.

Found . . . . . =  $\text{H}_2\text{O}$  9.92 " "

*Copper*, I, 0.1858 gm. subst., dried at 100°, gave 0.0275 gm.  $\text{CuO}$ .

II, 0.1808 gm., subst., dried at 100°, gave 0.0267 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_{12}\text{H}_{23}\text{N}_{10}\text{O}_{10}\text{Cu} = \text{Cu}$  11.85 per cent.

Found . . . . .  $\text{Cu} = \text{I}, 11.84; \text{II}, 11.78$  per cent.

Kossel and Kutscher<sup>1</sup> found in three separate determinations of arginine in glutenin, 4.50, 4.02, and 4.54 per cent. They base their determinations on the supposition that glutenin contains 16.2 per cent nitrogen. If their results are recalculated to a basis of 17.5 per cent of nitrogen in this protein, which one of us has found,<sup>2</sup> they become 4.82, 4.52, and 4.84 per cent, with which our result, 4.72 per cent, agrees very closely.

<sup>1</sup> KOSSEL and KUTSCHER: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 165.

<sup>2</sup> OSBORNE and VOORHEES: *American chemical journal*, 1893, xv, 392.

## LYSINE.

The filtrate from the arginine silver was treated as Kossel directs, and after precipitating the lysine with phosphotungstic acid it was converted into the picrate, and 2.33 gm., equivalent to 0.907 gm. of free lysine, were obtained. This is equal to 1.92 per cent of the glutenin. Kossel and Kutscher<sup>1</sup> found in the three determinations of this substance which they made in this protein 1.9, 2.29, and 2 per cent, or, recalculating to a basis of 17.5 per cent of nitrogen in this protein, 2.05, 2.15, and 2.40 per cent.

Nitrogen, 0.1225 gm. subst., dried at 120°, gave 23.1 c.c. moist N at 760 mm. and 29°.

Calculated for  $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3 = N$  18.70 per cent.

Found . . . . . = N 18.76 " "

## HYDROLYSIS OF LEUCOSIN.

Owing to the difficulty of preparing large quantities of this protein, which occurs in very small quantity in the wheat kernel, we were limited in this hydrolysis to 257.8 gm. of water and ash-free leucosin. This was hydrolyzed and the glutaminic acid separated as hydrochloride in the way described for gliadin. When dried in vacuo over sulphuric acid, 12.39 gm. of the hydrochloride were obtained, which melted at 198° with effervescence.

Nitrogen, 0.5482 gm. subst., gave  $NH_3 = 4.17$  c.c. HCl (1 c.c. HCl = 0.01 gm. N.)

Chlorine, 0.2528 gm. subst., gave 0.1989 gm. AgCl.

Calculated for  $C_5H_{10}O_4NCl = N$  7.64 ; Cl 19.35 per cent.

Found . . . . . = N 7.61 ; Cl 19.45 " "

The filtrate from the glutaminic acid hydrochloride was concentrated to a syrup under reduced pressure, the residue taken up in alcohol and saturated with dry hydrochloric acid gas. The solution was then evaporated to a thick syrup under reduced pressure, the residue again esterified with alcohol and hydrochloric acid, and the solution concentrated as before. The esterification was again repeated, the final concentration being made at a pressure of 10 mm.

<sup>1</sup> KOSSEL and KUTSCHER: *Loc. cit.*



from a bath the temperature of which did not rise above 40°. The free esters of the amino-acids were then liberated from the residue, extracted with ether, and dried with potassium carbonate and anhydrous sodium sulphate in the usual way. The aqueous layer was then made strongly acid with hydrochloric acid and the salts removed by concentration and treatment with alcoholic hydrochloric acid. The alcoholic extracts containing the hydrochlorides of the amino-acids were evaporated to a thick syrup under reduced pressure and the residue esterified as above described. The free esters were then liberated and their ether solution dried as before. After distilling off the ether on the water bath, at atmospheric pressure, the residue was distilled with the following results:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	75°	12.0 mm.	21.76 gm.
II	80°	10.0 "	25.86 "
III	125°	0.8 "	49.60 "
IV	200°	0.8 "	49.39 "
Total . . . . .			146.61 "

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	75°	12 mm.	21.76 gm.

This fraction was saponified directly after collection by evaporating on the water bath with concentrated hydrochloric acid. The residue was esterified with alcohol and hydrochloric acid and allowed to stand for several days on ice. The glycooll ester hydrochloride which separated weighed 1.73 gm. and melted at 145°.

*Nitrogen*, 0.2851 gm. subst., dried in vacuo, gave  $\text{NH}_3 = 2.88$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

*Chlorine*, 0.2843 gm. subst., dried in vacuo, gave 0.2889 gm. AgCl.

Calculated for  $\text{C}_4\text{H}_{10}\text{O}_2\text{NCl} = \text{N } 10.05$ ; Cl 25.40 per cent.

Found . . . . . = N 10.12; Cl 25.12 " "

In the filtrate from the glycooll ester hydrochloride the free amino-acids were regenerated and subjected to fractional crystallization in water and alcohol. There were obtained 3.06 gm. of nearly pure alanine.

Carbon and hydrogen, 0.3682 gm. subst., gave 0.5460 gm. CO<sub>2</sub> and 0.2649 gm. H<sub>2</sub>O.

Nitrogen, 0.2508 gm. subst., gave NH<sub>3</sub> = 3.92 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.40; H 7.93; N 15.75 per cent.

Found . . . . = C 40.44; H 7.99; N 15.63 " "

The alanine decomposed at 290°.

Fraction.	Temp. of bath up to.	Pressure.	Weight.
II	80°	10 mm.	25.86 gm.

After saponifying with boiling water the solution of the amino-acids was evaporated to dryness under reduced pressure. The dried residue, which weighed 18.78 gm., was extracted with boiling alcohol, whereby 1.68 gm. went into solution. From the part insoluble in alcohol there were isolated, by fractional crystallization, 5.53 gm. of leucine, 5.79 gm., alanine, and 0.47 gm. of substance which had the percentage composition of amino-valerianic acid.

Carbon and hydrogen, 0.2094 gm. subst., gave 0.3913 gm. CO<sub>2</sub> and 0.1793 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.22; H 9.48 per cent.

Found . . . . = C 50.96; H 9.51 " "

From the more soluble part of this fraction glycocoll was isolated as the hydrochloride of the ester. This weighed 2.78 gm., equivalent to 1.50 gm. of glycocoll, and melted at 144°-145°.

Fraction.	Temp. of bath up to	Pressure.	Weight.
III	125°	0.8 mm.	49.6 gm.

This was saponified by boiling with ten parts of water for ten hours, and the solution evaporated to dryness under reduced pressure. After extracting the prolin with boiling alcohol the insoluble part was fractionally crystallized. There were obtained 23.72 gm. of leucine and 2.62 gm. of alanine.

The isolated leucine decomposed at 298°.

Carbon and hydrogen, 0.1835 gm. subst., dried at 110°, gave 0.3670 gm. CO<sub>2</sub> and 0.1670 gm. H<sub>2</sub>O.

Nitrogen, 0.3086 gm. subst., gave NH<sub>3</sub> = 3.34 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.89; H 10.01; N 10.70 per cent.

Found . . . . = C 54.63; H 10.11; N 10.82 " "

The combined alcoholic solutions from fractions II and III, which contained the prolin, were evaporated to dryness under reduced pressure, and the residue extracted with boiling alcohol, in which 2.3 gm. did not dissolve. The solution filtered from this was again evaporated to dryness under reduced pressure, the residue dissolved in water, and the copper salts prepared by boiling for an hour with an excess of copper hydroxide. The deep blue solution was evaporated to dryness under reduced pressure, and the residue boiled with absolute alcohol, which dissolved the l-prolin copper salt. The residue of racemic copper salt, insoluble in alcohol, was dissolved in water and the solution concentrated. Of racemic prolin copper 2.41 gm. were obtained, equivalent to 1.69 gm. of *a*-prolin.

*Water*, 0.3190 gm. subst., air dried, lost 0.0353 gm. H<sub>2</sub>O at 110°.

*Copper*, 0.2827 gm. subst., dried at 110°, gave 0.0766 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu · 2 H<sub>2</sub>O = H<sub>2</sub>O 11.00 per cent.

Found . . . . . = H<sub>2</sub>O 11.07 " "

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 21.79 per cent.

Found . . . . . = Cu 21.65 " "

The alcoholic solution of the copper salt of the l-prolin was evaporated to dryness under reduced pressure. The dried residue weighed 8.24 gm., equivalent to 6.5 gm. of l-prolin.

For identification a small portion was freed from copper with hydrogen sulphide, and the free prolin converted into the phenylhydantoin, according to the directions of Emil Fischer.<sup>1</sup>

The hydantoin melted sharply at 143° and gave the following analysis:

*Carbon and hydrogen*, 0.2334 gm. subst., gave 0.5676 gm. CO<sub>2</sub> and 0.1204 gm. H<sub>2</sub>O.

*Nitrogen*, 0.1373 gm. subst., gave NH<sub>3</sub> = 1.78 c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.60; H 5.61; N 12.99 per cent.

Found . . . . . = C 66.32; H 5.73; N 12.96 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
IV.	200°	0.8 mm.	49.39 gm.

From this the ester of phenylalanine was removed in the usual manner by shaking out with ether, and after freeing from ether the resid-

<sup>1</sup> FISCHER, E.: Zeitschrift für physiologische Chemie. 1901, xxxiii, p. 251.

ual ester was saponified by dissolving in concentrated hydrochloric acid and evaporating on the water bath. Of phenylalanine hydrochloride 12.12 gm. were obtained, which is equivalent to 9.93 gm. of phenylalanine. For identification the hydrochloride was recrystallized from strong hydrochloric acid and converted into the free acid by evaporation with excess of ammonia. When once recrystallized from water, it melted at  $263^{\circ}$ - $265^{\circ}$ .

*Carbon and hydrogen*, 0.2962 gm. subst., dried at  $110^{\circ}$ , gave 0.7089 gm.  $\text{CO}_2$  and 0.1801 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.1924 gm. subst., gave  $\text{NH}_3 = 1.65$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_2\text{N} = \text{C } 65.39$ ;  $\text{H } 6.73$ ;  $\text{N } 8.50$  per cent.

Found . . . . = C 65.27; H 6.76; N 8.57 " "

The aqueous layer was saponified by warming with an excess of barium hydroxide on the water bath for seven hours. After standing for some time the crystals of racemic barium aspartate were filtered out and decomposed with an equivalent quantity of sulphuric acid. The filtrate from the barium sulphate gave, on concentration, 5.06 gm. of aspartic acid, which, when recrystallized from water, was analyzed.

*Carbon and hydrogen*, 0.2483 gm. subst., dried at  $110^{\circ}$ , gave 0.3278 gm.  $\text{CO}_2$  and 0.1215 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.2769 gm. subst., gave  $\text{NH}_3 = 2.93$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_4\text{H}_7\text{O}_4\text{N} = \text{C } 36.06$ ;  $\text{H } 5.31$ ;  $\text{N } 10.55$  per cent.

Found . . . . = C 36.00; H 5.44; N 10.59 " "

The filtrate from barium aspartate was freed from barium quantitatively with sulphuric acid and the filtrate from the barium sulphate concentrated to small volume and saturated with hydrochloric acid gas. After long standing on ice, 2.15 gm. of glutamic acid hydrochloride separated.

The filtrate from this was evaporated under reduced pressure, the residue taken up in water, and the chlorine removed with silver sulphate.

After removing the sulphuric acid with an equivalent quantity of barium hydroxide, the solution was boiled with an excess of copper hydroxide. The filtered solution, on standing, separated tyrosine-like needles of copper laevo-aspartate which weighed 7.41 gm., equivalent to 3.57 gm. of aspartic acid.

*Nitrogen*, 0.1853 gm. subst., air dried, gave  $\text{NH}_3 = 0.93$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

*Copper*, 0.1677 gm. subst., air dried, gave 0.0491 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_4\text{H}_5\text{O}_4\text{N Cu} \cdot 4\frac{1}{2} \text{H}_2\text{O} = \text{N } 5.09$ ; Cu 23.06 per cent.

Found . . . . . = N 5.02; Cu 23.37 " "

From the copper salt the free acid was regenerated and analyzed.

*Carbon and hydrogen*, 0.2041 gm. subst., gave 0.2715 gm.  $\text{CO}_2$  and 0.1054 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_4\text{H}_7\text{O}_4\text{N} = \text{C } 36.06$ ; H 5.31 per cent.

Found . . . = C 36.29; H 5.74 " "

**Specific rotation.** — Dissolved in 20 per cent hydrochloric acid,

$$[\alpha] \frac{20^\circ}{D} = +23.8^\circ.$$

Fischer and Dörpinghaus<sup>1</sup> found

$$[\alpha] \frac{20^\circ}{D} = +22.0^\circ.$$

An effort to isolate serine in the filtrate from the copper aspartate failed.

#### THE RESIDUE AFTER DISTILLATION.

The residue remaining after distillation of the esters weighed 57 gm. This was dissolved in boiling alcohol, and after cooling 1.98 gm. of needle crystals were filtered out. The filtrate was evaporated to a syrup under reduced pressure, saponified by heating with an excess of barium hydroxide, and, after removing the barium, evaporated to small volume under reduced pressure. The solution was then saturated with hydrochloric acid, and, after standing on ice for a long time, yielded 7.39 gm. of glutaminic acid, hydrochloride. The free acid prepared by evaporating with an exactly equivalent quantity of potassium hydroxide, when recrystallized from water, melted at  $202^\circ\text{--}203^\circ$  with effervescence.

*Carbon and hydrogen*, 0.3646 gm. subst., dried at  $110^\circ$ , gave 0.5432 gm.  $\text{CO}_2$  and 0.2019 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.3696 gm. subst., gave  $\text{NH}_3 = 3.55$  c.c. HCl (1 c.c. HCl = 0.01 gm. N).

Calculated for  $\text{C}_5\text{H}_9\text{O}_4\text{N} = \text{C } 40.82$ ; H 6.12; N 9.52 per cent.

Found . . . . . = C 40.63; H 6.15; N 9.60 " "

<sup>1</sup> FISCHER and DÖRPINGHAUS: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 462.

The total glutaminic acid obtained from leucosin was 17.5 gm., or 6.73 per cent. This result is higher than that recently recorded from this laboratory, namely, 5.72.<sup>1</sup>

This protein is one from which the glutaminic acid hydrochloride can be directly obtained only with great difficulty. In the former paper attention was directed to this fact, and the statement made that it is possible that the result given was too low.

#### TYROSINE.

Forty grams of leucosin, equal to 34.96 gm. dried at 110°, were boiled for twelve hours with a mixture of 120 gm. of sulphuric acid and 240 gm. of water. After removing the sulphuric acid with an equivalent quantity of barium hydroxide, the solution was evaporated with an excess of barium carbonate in order to remove ammonia. After removing the barium the solution was concentrated to a small volume on the water bath and allowed to stand for some time. The substance which separated was washed with cold water, dissolved in ammonia, the solution treated with bone black, and evaporated. On cooling 1.0360 gm. tyrosine separated in colorless needles. The filtrate from this, on further concentration, yielded 0.13 gm. more tyrosine, making a total of 1.1660 gm., or 3.33 per cent. This was recrystallized and analyzed.

*Carbon and hydrogen*, 0.4573 gm. subst., dried at 110°, gave 0.9994 gm. CO<sub>2</sub> and 0.2813 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>3</sub>N = C 59.62; H 6.13 per cent.

Found . . . . = C 59.60; H 6.11 " "

#### ARGININE, HISTIDINE, AND LYSINE.

The filtrate from the tyrosine, by the method applied to gliadin (page 246), yielded a solution in which the nitrogen found corresponded to 0.99 gm. of histidine, or 2.83 per cent.

The identity of the histidine could not be established owing to its very small amount.

The solution of the arginine contained nitrogen equal to 0.6720 gm., which is equal to 2.08 gm. of arginine, or 5.94 per cent. A part was converted into the copper salt by boiling with an excess of copper hydroxide and the copper salt recrystallized from water.

<sup>1</sup> OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

*Water*, 0.1984 gm. subst., air dried, lost 0.0191 gm. H<sub>2</sub>O at 110°.

*Copper*, 0.1766 gm. subst., dried at 110°, gave 0.0262 gm. CuO.

Calculated for C<sub>12</sub>H<sub>28</sub>O<sub>4</sub>N<sub>8</sub>Cu (NO<sub>3</sub>)<sub>2</sub> · 3 H<sub>2</sub>O = H<sub>2</sub>O 9.15 per cent.

Found . . . . . = H<sub>2</sub>O 9.62 “ “

Calculated for C<sub>12</sub>H<sub>28</sub>O<sub>4</sub>N<sub>8</sub>Cu (NO<sub>3</sub>)<sub>2</sub> = Cu 11.85 per cent.

Found . . . . . = Cu 11.83 “ “

The filtrate from the copper salt was freed from copper and the solution evaporated with an excess of sulphuric acid under reduced pressure. The sulphuric acid was removed with an excess of barium hydroxide, and the barium with carbonic acid. The filtrate from the barium carbonate was evaporated to dryness and the arginine converted into the picrolonate, according to the directions of Studel.

This melted at 226°-227°. Studel gives 225°.

*Nitrogen*, 0.0516 gm. subst., dried at 110°, gave 11.8 c.c. moist N<sub>2</sub> at 25° and 765 mm.

Calculated for C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>N<sub>4</sub> · C<sub>10</sub>H<sub>5</sub>O<sub>5</sub>N<sub>4</sub> = N 25.62 per cent.

Found . . . . . = N 25.71 “ “

The filtrate from the first silver precipitate which contained the arginine and histidine was freed from silver and barium, and the lysine precipitated with phosphotungstic acid and then converted into the picrate, of which 2.47 gm. equal to 0.9616 gm. of lysine, or 2.75 per cent, was obtained. This was recrystallized from water and analyzed.

*Nitrogen*, 0.2288 gm. subst., gave 38.6 c.c. moist N at 25.5° and 759 mm.

Calculated for C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>N<sub>2</sub> · C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>N<sub>3</sub> = N 18.70 per cent.

Found . . . . . = N 18.77 “ “

## THE NATURE OF CHEMICAL AND ELECTRICAL STIMULATION.

BY WILLIAM SUTHERLAND.

UNDER this heading A. P. Mathews has given a suggestive interpretation of his numerous experiments on the nerve-stimulating action of electrolytic solutions.<sup>1</sup> He shows that a fair measure of the stimulating power of an ion is obtained on dividing the square of its ionic velocity by the product of its solution tension and the square root of its atomic mass, namely,  $\frac{V^2}{EW^{\frac{1}{2}}}$ . He admits the difficulty of giving a theoretical justification of this measure, pointing out that when  $E = 0$ , it becomes infinite. When  $E$  becomes negative, he transfers it from the denominator to the numerator. In these circumstances it seems hopeless to seek for a theoretical deduction of  $\frac{V^2}{EW^{\frac{1}{2}}}$  as the proper measure of the stimulating power of an ion. Profiting by the pioneering of Mathews, I have been led to the following quite simple theory of his results according to the principles of physical chemistry.

It is helpful to distinguish two chief ways in which a nerve may be stimulated electrically, — first, by electric force without the passage of external electricity into the nerve, and, second, by the actual passage of electricity into the nerve. It is proposed to show in the following that in the experiments of Mathews negative ions stimulate nerve by giving to it their electric charges, whereas positive ions do not give their electrons to a nerve, but depress it through the action of their electric force. According to this distinction all negative ions of the same valency, having therefore the same electric charge, ought to have the same stimulating action, namely, that of giving that charge to the nerve; but, on the contrary, positive ions of the same valency, though having the same electric charge, ought not to produce

<sup>1</sup> MATHEWS: This journal. 1904, xi, p. 455.



the same depressing action, because, being of different sizes and of different dielectric capacity (specific inductive capacity) they have different electric forces at their surfaces. If  $a$  is the radius of an ion and  $K$  its dielectric capacity,  $\nu$  its valency, and  $e$  the electron charge, the electric force at its surface is  $\frac{\nu e}{K a^2}$ . The charge  $\nu e$  will produce an inductive charge in the nerve proportional to  $\nu e$ ; so the actual force on the nerve will be proportional to  $\frac{\nu^2 e^2}{K a^2}$ . The most striking result obtained by Mathews is contained in his Table IV, which shows that for ten out of sixteen salts of sodium with monovalent acids the minimum strength of a stimulating solution is one-twelfth gram-molecular, or  $\frac{m}{12}$ . For sodium iodide it is  $\frac{m}{13}$ , and for sodium hydrate, the most important exception, it is  $\frac{m}{20}$ . With due allowance for the exceptions, the generalization is clear, that negatively charged monovalent ions have the same stimulating power. When the formic, acetic, and butyric anions stimulate to the same extent as the chlorine and bromine ions, which are so different from them, we have clearly to do with a fundamental relation. In the case of the sodium salts of bivalent acids, with the exception of acid salts and the carbonate borate and bichromate, the minimum stimulating concentration is  $\frac{m}{32}$ . With sodium citrate, the case best representing trivalent acids, the concentration is  $\frac{m}{50}$ . Now, in considering these numbers 12, 32, and 50, we must remember that the stimulation of a cut nerve is essentially a surface phenomenon at the severed end. Hence in the present connection we ought to consider the number of molecules per unit surface and not the number per unit volume. So we ought to compare  $12^{\frac{2}{3}}$ ,  $32^{\frac{2}{3}}$ , and  $50^{\frac{2}{3}}$ , namely, 5.24, 10.08, and 13.57 as measures of the number of molecules of sodium compounds with mono-, di-, and tri-valent acids which just stimulate. But as the acid radicles of these types carry 1, 2, and 3 electrons, we get for the measure of the stimulating power per electron the values 5.24, 1, 10.08, 2, and 13.57, 3, namely, 5.24, 5.04, and 4.52. These numbers are not identical, as they ought to be to confirm sharply the important principle that stimulation by solutions is a surface action; but they agree well enough to help confirm a later calculation involving the principle of surface action as fundamental. Their mean value is 4.9.

With the salts of potassium and ammonium the experimental results are not so clear cut, though on the average they confirm the prin-

principle of surface action satisfactorily. The minimum stimulating concentration for salts with monovalent acids is about 4.5, for divalent acids 14, and for trivalent 20 in the potassium citrate and 30 in the ammonium citrate. If we take 4.5, 14, and 25 as mean values, and raise them to the  $\frac{2}{3}$  power in order to compare effects per unit surface, we get 2.73, 5.81, and 8.55, which upon division by 1, 2, and 3 respectively give 2.73, 2.90, and 2.85. Here the agreement is as good as possible under the conditions. For the lithium salts the data are few, and give 3, 10, and 30 for the minimum stimulating concentrations for the three classes of salts. Taking the  $\frac{2}{3}$  power of these and dividing by 1, 2, and 3, we get 2.08, 2.32, and 3.22. Here the last number is markedly discrepant.

The other result of importance concerning negative ions found by Mathews is the exceptional behavior of OH. The minimum stimulating concentration found for the hydrates is  $\frac{m}{20}$  for Na,  $\frac{m}{20}$  for K, and  $\frac{m}{18}$  for Li, while it is  $\frac{m}{40}$  for Sr. For all hydrates it is about  $\frac{1}{20}$  gram-equivalent per litre. Here the positive ion seems to be of no account. Probably there is a different chemical action between nerve and alkali from that between nerve and neutral salt, the OH concentration at the nerve being so much increased that the positive ion of the hydrate never gets near enough to the nerve to make its field of electric force effective. Amongst negative ions OH is remarkable for the large ionic velocity usually assigned to it. In a paper contributed to the Philosophical Magazine I have sought to show that the OH ion does not really have this exceptional velocity, but that it dissociates a certain amount of water into H and OH ions whose velocities are credited to the original OH ion. This exceptional power of the OH ion is doubtless partly the cause of its exceptional behavior in stimulating nerves. By using the result 20 to measure the stimulating power of an equivalent of hydroxide, we might make an attempt to separate the stimulating and depressing parts of the action of a dissolved salt.

If 20<sup>2</sup> measures the stimulating power of the OH ion, its value, 7.4, may be taken to give the power of all negative ions. Hence, since 4.9 measures the power (of electric origin) of such types as NaCl and  $\frac{1}{2}$  Na<sub>2</sub>SO<sub>4</sub>, we get the depressing power of the Na ion as 2.5. For equivalents of K, Li, and NH<sub>4</sub> salts the stimulating power is about 2.8; hence the depressing powers of K, Li, and NH ions are about 4.6.

Special interest attaches to the H ion of acids. Its action appears

most clearly in the experiments on acid salts. While the minimum stimulating molecular concentration of  $\text{Na}_2\text{SO}_4$  is  $\frac{1}{3^{\frac{1}{2}}}$ , that of  $\text{NaHSO}_4$  is only  $\frac{1}{6}$ . Hence the depressing power of the H ion per unit surface exceeds that of the Na ion by  $32^{\frac{3}{2}} - 6^{\frac{3}{2}}$ , or 6.8. With  $\text{K}_2\text{SO}_4$  and  $\text{KHSO}_4$  the difference in the minimum stimulating molecular concentrations is only that between 5 and 4,  $\text{K}_2\text{SO}_4$  being markedly exceptional.  $\text{NaH}_2\text{PO}_4$  stimulates in concentrations  $\frac{m}{v}$  per litre, so  $6^{\frac{3}{2}}$  measures its stimulating power per unit surface. For  $\text{Na}_3\text{PO}_4$  the normal power would be  $50^{\frac{3}{2}}$ . In this case the depressing power of the H ion exceeds that of the Na ion by  $\frac{(50^{\frac{3}{2}} - 6^{\frac{3}{2}})}{2}$ , or 5.1. On the average the depressing power of the H ion exceeds that of the Na ion by  $\frac{(10.2 + 6.8)}{3}$ , or 5.7. But the depressing power of Na was tentatively estimated above at 2.5, so that the depressing power of H is 8.2. Now the stimulating power of an equivalent of an ion was taken at 7.4. It seems to me to be the safer conclusion to state that the depressing power of H is about equal to the stimulating power of a monovalent negative ion. From this it would appear that the H ion differs from other positive ions in being able to give up its positive charge to nerve like a negative ion. As regards ionic velocity, the H ion is still more exceptional than OH, according to the values hitherto accepted. Just as in the case of OH I have sought to show that the true ionic velocity of H is not exceptional, but that the H ion splits about twice as much  $\text{H}_2\text{O}$  into H and OH as the OH ion does, and is credited with the velocities belonging to these products of its action on water. Now in certain cases, as for instance that of globulin, the action of acids and alkalis on proteids is far more like a definite chemical one than that of neutral salts. On this account the positive charge of the H ion may be given up to nerve in a way not possible to the positive ions of neutral salts. If the depressing action of the H ion is equal to the stimulating action of monovalent negative ions, then acids ought to have neither a stimulating nor a depressing effect on nerve. In his experiments Mathews found the acids to possess a small stimulating power, which he attributes to other causes than those now under investigation. In another way he showed hydrochloric acid to have a depressing power much greater than the stimulating power of sodium chloride or rubidium chloride. It seems to me, however, that it is necessary to distinguish two sorts of depressant action, namely,

that associated with permanent chemical change in the nerve and that not so associated. For example, the salts of heavy metals such as copper and mercury most probably form irreversible precipitates in contact with nerve. This chemical effect complicates the physiological investigation of the purely ionic electrical effect. The clearest fact about the H ion is that it is a powerful depressant. The evidence, on the whole, goes to show that its depressing power is about equal to the stimulating power of a negative ion equivalent.

The next business is to study comparative values of the electric force acting on the nerve at the surface of the different positive ions,  $\frac{v^2 e^2}{K a^2}$ . Values of  $K$ , the dielectric capacity of the stuff of the ion, are taken from "The Dielectric Capacity of Atoms,"<sup>1</sup> as also values of  $B$ , the limiting volume of a gram atom,  $B^{\frac{2}{3}}$  being proportional to  $a^2$ . As we desire to compare the action of equivalents, we must divide  $\frac{v^2 e^2}{K a^2}$  by  $v$  to get the effect to be ascribed to each electron charge  $e$  in the ion. So in the subjoined table are given values of  $\frac{1000 v}{K B^{\frac{2}{3}}}$  to represent those of  $\frac{v e^2}{K a^2}$ , which we wish to study. For Mg, Ca, Sr, and Ba  $v = 2$ , and for the rest  $v = 1$ .

	H	Li	Na	K	Rb	Cs	NH <sub>4</sub>	Mg	Ca	Sr	Ba
K	2.07	6.27	3.24	1.62	1.28	1.08	1.67	6.58	5.16	4.72	3.83
B	8.00	2.00	7.40	18.60	34.40	56.00	18.00	5.60	8.60	10.60	16.60
$\frac{1000 v}{K B^{\frac{2}{3}}}$	120.00	100.00	81.00	88.00	74.00	63.00	87.00	96.00	92.00	88.00	8.00

The value of  $K$  given for H is one calculated in accordance with my contention that the ionic velocity hitherto assigned to H, namely, 318, ought to be replaced by an unexceptional value, 67.5. For H the value of  $\frac{1000 v}{K B^{\frac{2}{3}}}$  is the largest in the list. Hence, even if the H ion does not give up its charge to nerve, as suggested above, it must exercise the largest depressing effect through its electric force. Ac-

<sup>1</sup> SUTHERLAND: Philosophical magazine, 1904 [6], vii, p. 402. or Australian association for the advancement of science, 1904, x, p. 122.

According to the above list Cs ought to have the smallest depressing effect and Rb ought to come next. But Mathews found Rb to have the smallest depressing effect, although expecting Cs to give it. He quotes Grützner as having found Cs to be less depressing than Rb. Evidently the point requires further experimental investigation. Next to Rb in low depressing power and nearly equal come Na and Ba in the above list, as Mathews found them. Then come K and  $\text{NH}_4$  nearly equal to Sr, in accordance with experiment. The list makes Li a rather decidedly stronger depressant than K and  $\text{NH}_4$ , whereas we saw above that it comes out about equal to them in the experiments. Mg and Ca appear to depress a little more than Sr, while experiment makes all three about equal. The striking point about the theoretical measure  $\frac{\nu c^2}{K a^2}$  for the depressing power of a positive ion equivalent is that it contains  $\nu$ , the valency. In view of this fact and the considerable range in the values of K and of B, it seems to me that the theory gives an adequate qualitative account of the electric action of positive ions on nerve. As to a complete quantitative test of the theory we are confronted with the difficulty that we do not know the relation between magnitude of electric force and amount of stimulation. If the depressing powers of Na and K ions are as 2.5 to 4.6, it would seem that depressing power is proportional to excess of electric force over some lower limit which just fails to stimulate.

We can apply a rather searching test to the foregoing theory by using it to calculate the amount of electricity available in the experiments of Mathews with solutions for comparison with the directly determined charge of negative electricity necessary to stimulate nerve. Weiss has shown<sup>1</sup> that to produce stimulation of a nerve a certain minimum quantity of electricity is required. For the sciatic of a frog he found this to be of the order  $10^{-9}$  coulomb, which is equal to 3 C.G.S. electrostatic units. Consider now a solution such as that of sodium chloride which Mathews found to stimulate frog's nerve at concentration 58.4/12 gms. per litre. In a solution of concentration 58.4 gms. per litre there are as many molecules of NaCl as there are molecules of H<sub>2</sub> in 2 gms. of hydrogen, or  $88 \times 10^{22}$ . Thus a solution of NaCl of strength 58.4/12 contains  $7 \times 10^{19}$  ions of Cl per c.c., so that we may take a square centimetre of nerve section immersed in such a solution to be in contact with  $17 \times 10^{12}$  ions of Cl. But the

<sup>1</sup> WEISS: Comptes rendus, 1901, cxxxii, p. 1068.

electron charge of each ion is  $3 \times 10^{-10}$  electrostatic C.G.S. units. So the total charge of the Cl ions in a  $\text{cm}^2$  is  $51 \times 10^2$ . By measurement I have found the sectional area of the sciatic nerve of a medium-sized frog to be  $0.0038 \text{ cm}^2$ , while the sum of the sectional areas of the axis cylinders in it is  $0.002 \text{ cm}^2$ . Hence the total charge of the Cl ions in contact with the cross section of all axis cylinders in frog's sciatic nerve in Mathews' experiments with  $\frac{m}{12}$  solutions is 10 C.G.S. electrostatic units. A fraction of this, namely, about  $\frac{1}{3}$ , is made ineffective through being neutralized by the depressing effect of electric force from the Na ions, so that we deduce 7 units as the stimulating minimum. The agreement in the order of magnitude of the 7 deduced from Mathews' experiments and the approximate 3 determined directly by Weiss is quite satisfactory, and furnishes substantial confirmation of the contention that the stimulation of nerve by solutions must be regarded as a surface action at the cross section of the nerve.

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NOTE UPON THE PRESENCE OF AMIDO ACIDS IN  
THE BLOOD AND LYMPH AS DETERMINED BY  
THE  $\beta$  NAPHTHALINSULPHOCHLORIDE REACTION.

BY W. H. HOWELL.

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SINCE the introduction of the  $\beta$  naphthalinsulphochloride reaction by Fischer and Bergell,<sup>1</sup> it has been used by a number of observers to test for the presence of amido acids in the urine. The result of this work has been to show the probable normal occurrence in the urine of glycocoll at least, partly in a free form and partly combined.<sup>2</sup> Von Bergmann<sup>3</sup> has also demonstrated by the same reaction the probable existence of an amido body of unknown composition in the normal blood of dogs, and in human blood in a case of acute yellow atrophy. In the course of some work upon the composition of blood with special reference to the absorption of the products of proteid digestion, the present author has obtained the  $\beta$  naphthalinsulphochloride reaction from normal blood by a simple method. Instead of removing the proteid incompletely by heat coagulation according to the method used by von Bergmann, the amido bodies were separated by dialysis. For this purpose the convenient collodium membrane was used in place of parchment paper. This membrane, first employed, I believe, in pathological experiments at the Pasteur Institute, can be made very easily and be given any desired form. It dialyzes much more rapidly than parchment paper for all readily dialyzable substances, but is impermeable to the usual proteids of blood unless the dialysis is con-

<sup>1</sup> FISCHER and BERGELL: *Berichte der deutschen chemischen Gesellschaft*, 1902, xxxv, p. 3779.

<sup>2</sup> IGNATOWSKI: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 371; EMBDEN and REESE: *HOFMEISTER'S Beiträge*, 1905, vii, p. 411; FORSSNER: *Zeitschrift für physiologische Chemie*, 1906, xlvii, p. 15; ABDERHALDEN and SCHITTENHELM: *Ibid.*, 1906, xlvii, p. 339, and SAMKELY: *Ibid.*, 1906, xlvii, p. 370.

<sup>3</sup> VON BERGMANN: *HOFMEISTER'S Beiträge*, 1904, vi, p. 40.

tinued for a long period. In the present experiments the membrane was constructed in the form of a flask with a capacity of about 250 c.c. For this purpose one takes a 5 to 6 per cent solution of gun cotton in a mixture of equal parts of absolute alcohol and ether. Some of the solution is poured into an Erlenmeyer flask of the desired capacity, and is then turned out while the flask is kept in slow rotation, so as to form an even layer of the adherent solution over the entire wall. The flask is drained carefully to remove the superfluous collodium, and is rotated slowly in the hands for several minutes until the solution adherent to the walls has hardened superficially. The flask is then filled with water; the membrane of collodium is separated at the mouth of the flask by means of a knife, and is gently detached from the walls of the flask for a distance by means of a blunt glass rod or spatula. After emptying the flask the entire membrane may then be removed conveniently by allowing a stream of water to flow in between the membrane and the glass wall. The membrane thus loosened may be pulled out without danger of rupturing. It presents the form of the Erlenmeyer flask, and may be kept in distilled water until used. In using these flasks to dialyze blood a glass tube 30 to 40 cms. in length was cemented into the mouth with collodium solution and firmly tied. By this means one prevents the overflow of the flask which would otherwise occur in consequence of the permanent osmotic pressure exerted by the blood. The blood was allowed to flow directly from the blood vessel used into the flask, which was then swung in a vessel containing two to four litres of distilled water, and the dialysis was continued for five to twenty-four hours. The dialysate was then concentrated by evaporation to 50 to 100 c.c., and tested directly with the ethereal solution of  $\beta$  naphthalinsulphochloride, made alkaline according to the usual procedure by the addition from time to time of appropriate amounts of a normal solution of potassium hydroxide. The mixture was kept in the shaking apparatus for four to twenty-four hours. A positive reaction for amido acids was obtained in all cases but one in blood taken from fourteen different animals. The experiments were all made upon dogs, and the blood was collected either from the portal vein or from the carotid artery or jugular vein in animals that had been well fed, five to six hours after the meal, or in animals in a fasting condition, twenty-four to fifty hours after a meal.

**Examination of the dialysate.**— After a dialysis of five to six hours the dialysate was usually entirely clear; after a longer dialysis, how-



ever, it might become slightly milky, owing possibly to the diffusion and precipitation of some of the globulin.<sup>1</sup> On concentrating the dialysate on a sand bath a slight precipitate might coagulate out, especially if the dialysis had been continued for a long time. The filtrate from this precipitate was somewhat colored. An effort to clarify the solution by treatment with animal charcoal was unsuccessful, since this substance holds back the amido acids. With regard to the amido bodies in the blood it was found that they are removed practically completely by a dialysis of five to six hours. If after this time the flask is dialyzed against a new bulk of distilled water, the dialysate on concentration and examination in the usual way gives little or no indication of the presence of amido acids, while the first dialysate gives a distinct reaction. The condensed and filtered dialysate contains some sugar, but gives no indication of proteids when tested by the biuret or millon reagent. Inasmuch as preliminary tests with solutions of Witte's peptone showed that the collodium membrane is readily permeable to solutions of peptones and proteoses, the entire absence of a biuret reaction in the dialysate is a strong indication that no proteoses or peptones exist in the blood, even in the portal blood collected during the period of maximal absorption from the intestines. The question of the normal existence of peptones or proteoses in the blood, especially after a proteid meal, has given rise to much discussion (see accompanying paper on the proteids of the blood). The fact that a large proportion of the blood collected from the portal vein during full digestion and dialyzed against a membrane readily permeable to these substances, gives an entirely negative reaction to the biuret test, must be accepted as strong proof that the peptones and proteoses do not exist normally in the blood. When the concentrated dialysate was saturated with zinc sulphate, a small flocculent precipitate was obtained, but that this precipitate did not consist of peptones or proteoses or indeed of proteid of any character was shown by the fact that when filtered off and dissolved the solution failed to give a perceptible reaction with the biuret test.

**The  $\beta$  naphthalinsulphochloride reaction.**—As stated above, this reaction was positive in all cases except one in specimens of blood taken from fourteen different animals. The exceptional case was that of a dog in fasting condition, but it should be stated that in this instance the dialysate was shaken with the reagent for only two

<sup>1</sup> On two occasions hæmoglobin passed into the dialysate, owing to some imperfection in the membrane.

hours. The procedure used in testing was as follows. The concentrated dialysate was shaken with the ethereal solution of the reagent in distinct alkaline reaction for usually eight to nine hours. The ethereal solution was then removed by means of a separating funnel, and the aqueous solution after filtration was acidified with hydrochloric acid. A cloudy white precipitate was obtained, which if shaken out with ether seemed to dissolve completely. The ethereal solution thus obtained was separated and evaporated to dryness, and the residue was dissolved in water to which a trace of ammonia had been added; the excess of ammonia was removed by heating and the solution was filtered and precipitated by acid. The precipitate thus obtained settled to the bottom as a slightly colored layer which on examination under the microscope presented the appearance of minute droplets of an oily character. The precipitate could not be made to crystallize. Repeated solutions in alkaline liquids with precipitation by acid, and long standing at low temperature failed to cause the formation of crystals. Repeated precipitations from hot water or dilute alcohol failed to give a better result. In this respect the naphthalinsulphoamido compound differed from that obtained by other observers from the urine, and it would appear therefore that the amido acid indicated is not glycocoll. The compound obtained by von Bergmann from the blood behaved in a similar way as regards crystallization. If the first precipitate of the naphthalinsulphoamido compound obtained on acidifying the shaken mixture was allowed to stand instead of being shaken out with ether, it settled to the bottom of the beaker after some hours as a yellowish layer which adhered tightly to the bottom in the form of a smear. When the supernatant liquid was poured off and the smear was treated with ether, a portion dissolved. The portion soluble in ether, treated as described above, gave a pure white precipitate on acidifying which, however, could not be obtained in crystalline form. The portion not soluble in ether balled together as a gummy mass when rubbed with a rod and was deeply colored. This portion dissolved readily in dilute solutions of alkali, and on acidifying precipitated in the form of yellowish flocks which deposited finally as clumps of yellowish granules. This portion seemed to contain all of the pigment present in the original precipitate. The failure to obtain the naphthalinsulphoamido compound in crystalline form suggested the possibility that the reaction might be due to one of the more complex polypeptids. An effort was made therefore to hydrolyze this body. The concentrated dialysate was evaporated to

dryness on the water bath, and the residue was boiled with strong hydrochloric acid for eight hours with a reflux condenser. The solution was then evaporated to complete dryness on the water bath, taken up with water and treated with the  $\beta$  naphthalinsulphochloride. The precipitate obtained still deposited in oily drops, which could not be made to crystallize after repeated precipitations and exposure to low temperatures.

**Comparison of portal and jugular blood.** — The blood was examined from dogs in full digestion, five to six hours after a meal, and from others in a fasting condition, twenty-four to fifty hours after a meal, and in some cases the blood was withdrawn from the portal vein directly, in others from the jugular vein or carotid artery. The amount of material obtained as a naphthalinsulphoamido compound was small in all cases, and no weighings have been made as yet, but judged by the appearance of the precipitate, a larger yield was obtained from the portal blood taken during digestion than from the jugular blood, and a larger reaction from the blood of the fed animal than from that of the starved animal. In three cases out of four examined, however, a positive reaction was obtained from both the portal and the jugular blood of animals starved for fifty hours, and this fact would indicate that amido acids are constantly present in the blood. It is hoped later to obtain quantitative measurements. The fact that the amido acids seem to be increased after digestion, while no trace of peptones or proteoses can be dialyzed from the blood, lends support to the modern view that the proteids during digestion and absorption are completely hydrolyzed into their constituent amido acids, and that if any synthesis occurs, the products formed are either not present in the blood or are in a form which does not dialyze. It should be added that the concentrated dialysate from the blood gave always a precipitate with phosphotungstic acid in acid solution. Inasmuch as this reagent yields a precipitate so readily with dilute solutions of potassium or ammonium salts, a reaction of this kind is without significance in regard to the products of digestion unless the presence of organic bases can be detected by other means. An effort to obtain from this precipitate definite reactions for arginin gave negative results.

**Amido acids in the lymph.** — In one animal that had been well fed lymph was collected from the thoracic duct. Fifty cubic centimetres of this lymph were dialyzed against a litre of water for twenty-four hours. The dialysate was concentrated in the usual way, and, as in

the case of the blood, it contained sugar but gave no reaction with the millon or biuret test. Treated with the  $\beta$  naphthalinsulphochloride, it yielded a slight turbidity which settled to the bottom of the beaker as oily droplets which formed an adherent precipitate similar to that obtained from blood. The precipitate in this case was dissolved in barium hydrate, the excess of barium was removed by carbon dioxide; the solution was filtered, boiled, and again filtered. On acidifying a milky precipitate was obtained which on settling showed some tendency to crystallize in needles.

**The osmotic pressure of the portal and the arterial blood.**— In the dialysis as carried out in these experiments the blood showed considerable permanent osmotic pressure. If the blood was first heated to the point of coagulation, however, and then dialyzed, it showed little or no osmotic pressure, the membrane being so permeable to the salts and other readily diffusible constituents of the blood that the difference in concentration due to these substances was very quickly equalized by diffusion. It is evident, therefore, that the permanent osmotic pressure exerted by the blood is due to the contained proteids, or, as Reid supposes, to certain unknown substances, combined or mixed with the proteids, which are altered or precipitated when the proteids are coagulated. In these experiments the osmotic pressure was measured simply by the height to which the liquid rose in the tube attached to the dialyzing flask. It was observed that in the specimens of portal blood the liquid rose more promptly and to a greater height than in the case of the carotid blood. With the portal blood the height of the column of liquid was equal to 270 to 300 mm., while in the carotid blood it was from 200 to 220 mm. Inasmuch as the percentage of proteids in the portal blood is greater than in the carotid blood, this fact alone may explain the difference in permanent osmotic pressure. As measured by the lowering of the freezing-point, the concentration of the portal blood is considerably above that of the carotid blood, and the difference is distinctly increased during the period of digestion, as is shown by the following experiment.

**Dog A.** — Starved for twenty-four hours. Serum obtained from clots of blood, taken from the portal vein and the carotid artery. Serum entirely clear and slightly concentrated from standing.

Zero point for distilled water . . . . .	= 2.989
Freezing-point of carotid serum (3 determinations)	2.370
	2.369
	<u>2.369</u>

2.369

Freezing-point of portal serum (3 determinations) 2.349  
 2.350  
2.352

2.350

$\Delta$  of serum of carotid blood = 0.620°; of portal blood = 0.639°; difference = 0.019°.

**Dog B.** — Well fed; bled five hours after meal. Serum collected from clots was very milky — was centrifugalized.

Freezing-point of carotid serum (2 determinations) 2.360  
2.355

2.357

Freezing-point of portal serum (2 determinations) 2.324  
2.320

2.322

$\Delta$  of serum of arterial blood = 0.632°; of portal blood = 0.667°; difference = 0.035°.

#### SUMMARY.

1. By means of dialysis through collodium membranes a substance or substances may be separated from the blood which give the reaction of amido acids with  $\beta$  naphthalinsulphochloride.

2. In the well-fed animal the amido acids are present in the blood of the portal vein in larger amounts than in the general systemic blood. Even after a starvation of fifty hours, however, a positive reaction may be obtained from the blood.

3. Lymph collected after a meal and treated in the same way gives a positive reaction.

4. Although the collodium membrane is readily permeable to peptones and proteoses, no indication of the existence of these substances can be obtained from dialyzing portal blood collected during the period of maximum absorption.

5. Owing probably to its larger percentage in proteids, the portal blood exhibits a permanent osmotic pressure larger than that shown by blood collected from the general circulation.

THE PROTEIDS OF THE BLOOD WITH ESPECIAL  
REFERENCE TO THE EXISTENCE OF A  
NON-COAGULABLE PROTEID.

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**B**LOOD serum, when made feebly acid and completely coagulated by heat, is found to contain a non-coagulable residue which may be precipitated by saturation with ammonium sulphate, by phosphotungstic acid, and by other reagents. This non-coagulable portion of the serum has been investigated by several observers, and opinions differ as to whether it is a normal constituent of the blood or an artificial product formed during the act of heat coagulation. The investigations of previous observers may be summarized briefly as follows: Chabrié seems to have been the first to call attention to this substance.<sup>1</sup> His method was to neutralize the serum (human) with acetic acid and heat it on the water bath until it was converted to a jelly. This jelly was cut into small pieces and extracted with boiling water. In the opalescent extract was found a proteid which he designated as albumon. It was not coagulated by heat, but was precipitated by alcohol and gave a number of the proteid reactions. Brünner<sup>2</sup> repeated Chabrié's experiments, modifying somewhat the method of preparation. He concluded that Chabrié's albumon does not pre-exist in the serum, but is split off from the serum proteids during the process of heat coagulation. As proof for this belief, he states that pure solutions of paraglobulin and serum albumin yield a similar substance when submitted to heat coagulation. This explanation seems to have been accepted generally by later writers, but it may be pointed out that the experiments given by Brünner are not entirely conclusive, since the methods used by him to prepare

<sup>1</sup> CHABRIÉ: Comptes rendus de l'Académie des sciences, 1891, cxliii.

<sup>2</sup> BRÜNNER: Zur Kenntniss der Eiweisskörper des Blutserums, Inaugural Dissertation, Bern, 1894.

his pure solutions of paraglobulin or serum albumin were such as might have precipitated the albumon, assuming that this proteid pre-exists in the blood. Zanetti<sup>1</sup> has described a similar proteid which he obtained from ox's serum. This proteid after purification by repeated precipitation by alcohol gave many of the usual proteid reactions and in addition yielded a reducing body when treated with dilute acids. Zanetti concluded that the non-coagulable residue in blood serum belongs to the group of ovomucoids. Langstein<sup>2</sup> repeated Zanetti's experiments. He found that the proteid prepared by the latter's method did not yield a reducing body in all cases, and he was inclined, therefore, to regard the reducing body, when present, as an impurity. He was convinced, however, that a non-coagulable proteid exists in the blood serum and assigned it to the group of albumoses, although no special reason for this classification was given other than the non-coagulability by heat. Bergmann and Langstein<sup>3</sup> estimated the residual nitrogen-containing material of serum by the Kjeldahl method, after removing the coagulable proteid by heating in weakly acid reaction and in some cases after the addition of  $\text{NaH}_2\text{PO}_4$  to  $\frac{1}{2}$  per cent. The amount of the residual nitrogen varied according to the conditions from 7.67 per cent to 14.7 per cent of the total nitrogen of the blood; and examination of the substance yielding this nitrogen showed that it gave proteid reactions. They separated this non-coagulable residue into a portion consisting of primary and secondary proteoses and a portion precipitable by phosphotungstic acid. They give no conclusive proof, however, that the proteids in question belong to the group of proteoses. Kraus<sup>4</sup> used a somewhat similar method. He precipitated blood plasma by heat coagulation in acid reaction, and treated the filtrate, after concentration, with  $\frac{1}{10}$  its volume of saturated solution of zinc sulphate and also by saturation with sodium chloride in acid reaction. If the precipitate produced by the last reagent cleared up on heating, he concluded that all coagulable proteid had been removed. The solution was then precipitated by saturation with zinc sulphate or by phosphotungstic acid, and the amount of nitrogen contained in it was determined. The results

<sup>1</sup> ZANETTI: *Annali di Chimica e di Farmalogica*, No. 12, 1897; quoted from *Jahresberichter der Thier-Chemie*, 1897, xxvii, p. 32.

<sup>2</sup> LANGSTEIN: *HOFMEISTER'S Beiträge*, 1902, iii, p. 373.

<sup>3</sup> BERGMANN and LANGSTEIN: *HOFMEISTER'S Beiträge*, 1904, vi, p. 27.

<sup>4</sup> KRAUS: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 52.

obtained were variable, indicating that when the blood contained a total nitrogen of 2.73 to 4.47 per cent, the residual nitrogen of the non-coagulable portion varied from 0.039 to 0.146 per cent. In this as in the paper by Bergmann and Langstein no positive proof is given that the non-coagulable proteid belongs to the group of albumoses, and no proof whatever that the substance in question is not split off from the other proteids of the blood during the act of heat coagulation. So far as the residual nitrogen is concerned, it is evident that, as determined by these authors, it includes such substances as the amido acids, urea, lecithin, etc., which would be left in the serum after removing the coagulable proteid. Regarding the question of the existence of peptones or proteoses in the blood, Hofmeister, Toepfer, Emden and Knoop are quoted as having obtained positive evidence, while Neumeister, Abderhalden and Oppenheimer report negative findings.<sup>1</sup>

As stated in an accompanying paper, the author has shown recently that when an animal is bled from the portal vein during the height of digestion, and the volume of blood obtained (250 c.c.) is dialyzed against distilled water, using a collodium membrane, no trace of peptones or proteoses can be detected in the concentrated dialysate. Since the collodium membrane is readily permeable to peptones and proteoses, and since the other diffusible constituents that exist in the portal blood, such as sugar, amido acids, ammonia salts, can be detected easily in the dialysate, it follows that if this form of proteid exists in the blood in even minute amounts it would be detectable by this means. Whether there is a proteid in the blood not coagulable by heat is, it seems to the author, a different question from whether peptones and proteoses are present. The former question is difficult of solution, but regarding the latter the evidence is so unsatisfactory that one is justified at present in denying their existence normally in the blood in amounts that can have a physiological significance.

**The non-coagulable proteid in serum.** — That the blood serum contains after heat coagulation a residual non-coagulable proteid is a fact beyond question, if by non-coagulable proteid is understood

<sup>1</sup> HOFMEISTER: *Zeitschrift für physiologische Chemie*, 1881, v, p. 127; TOEPFER: *Wiener klinische Wochenschrift*, 1902, No. 11; EMDEN and KNOOP: *HOFMEISTER'S Beiträge*, 1903, iii, p. 120; NEUMEISTER: *Zeitschrift für Biologie*, 1888, xxiv, p. 272; ABDERHALDEN and OPPENHEIMER: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 155.



one not coagulated by heat in feebly acid reaction in the otherwise unaltered serum. The author has attempted to study this proteid using the following method. The serum (dog, beef, horse) was collected usually from a clot, although in some cases oxalate or fluoride plasma was used. The serum was diluted with once or twice its volume of a solution of sodium chloride, 0.7 per cent, and thoroughly centrifugalized. It was then heated on a water bath to  $85^{\circ}$  C. and dilute acetic acid was added, while stirring, until the reaction was permanently but feebly acid. The heavy coagulum obtained was filtered off through cotton cloth, and the serum was again heated to  $85^{\circ}$ , with the addition of more acid if necessary to maintain a feebly acid reaction, and again filtered through filter paper. The clear yellow filtrate gave no further coagulum upon heating to  $85^{\circ}$  or  $100^{\circ}$  C. when beef serum was used. Dog serum, if kept for a number of minutes at  $85^{\circ}$  to  $100^{\circ}$  C., developed an opalescence which gradually took the form of a fine precipitate. This precipitate could not be filtered off successfully in all cases. The beef serum, although it remained entirely clear or developed only a slight opalescence when boiled, also gave a similar fine precipitate on boiling if the reaction was made strongly acid with acetic acid. This precipitate brought down with it all of the pigment present, and was characterized when fresh by a delicate agreeable odor. The non-coagulable proteid left after the heat coagulation of the albumin and globulins of the serum falls, therefore, into two parts, — one which is entirely unaffected by boiling in strong acid reaction, and one which by prolonged heating to  $85^{\circ}$  to  $100^{\circ}$  C., especially in strong acid reaction, is precipitated more or less imperfectly. The qualitative reactions of these proportions were apparently the same, so that in most cases the entire proteid left in the clear filtrate from the coagulum thrown down at  $85^{\circ}$  was treated as one. The actual amount of this material and of its two parts was determined in several analyses, but the figures did not agree very closely, owing, as was discovered later, to the fact that lecithin and other substances extractable by alcohol and ether are present, and the thoroughness of this extraction alters considerably the results. The following figures, however, were obtained. The total non-coagulable proteid determined in three specimens from different animals by precipitation with ammonium sulphate and reckoned in percentages of the weight of serum used, was, respectively, 0.76, 0.85, and 0.91 per cent, an average, therefore, of 0.84 per cent. The portion coagulated at  $100^{\circ}$  C. in strong acid reaction, determined in six specimens, was

0.31, 0.31, 0.21, 0.35, 0.29, and 0.38 per cent of the serum used, giving an average of 0.31 per cent. The proteid left in the blood serum after boiling in strong acid reaction was therefore equal to about 0.5 per cent. This proteid was not a form of acid albumin, since it was not precipitated by neutralization nor by saturation with sodium chloride.

**Qualitative reactions of the non-coagulable proteid.**— These reactions apply to either portion of this proteid as described above.

1. It gives an excellent millon reaction.
2. It gives a blue-purple biuret reaction.
3. It gives a xanthoproteic reaction.
4. It is not precipitated by dialysis, nor by carbon dioxide after dilution.
5. It is precipitated by potassium ferrocyanide and acetic acid if the reaction is made strongly acid.
6. It is precipitated by nitric acid, the precipitate not disappearing on heating.
7. It is not precipitated by complete saturation with sodium chloride.
8. It is incompletely precipitated by saturation with magnesium sulphate.
9. It is completely precipitated by ammonium sulphate added to a saturation of 50 to 70 per cent.
10. It is precipitated by alcohol added to a concentration of 50 per cent or more.
11. It is precipitated in acid solution by phosphotungstic acid or iodine in solution in potassium iodide.
12. After dialysis and the addition of sodium, potassium, or lithium chloride it gives no precipitate on boiling. A coagulum occurs, however, under these conditions upon the addition of ammonium chloride or sulphate or the chloride of magnesium, calcium, or barium.
13. It gives always a strong reaction for phosphorus and a distinct reaction for iron. The sulphur reaction was in some cases negative.

**The content in phosphorus**— It was quickly discovered that the non-coagulable proteid gives a strong reaction for phosphorus, and a number of quantitative determinations of this element were made by the ammonium molybdate method. The proteid was prepared as follows. After complete coagulation at 85° C. the filtrate was precipitated by saturation with ammonium sulphate and was purified

by three successive precipitations. The final solution was in some cases dialyzed in parchment tubes against running water and distilled water until it failed to give a reaction with barium chloride, and was then precipitated by the addition of alcohol. In other cases it was heated for two hours at a temperature of  $105^{\circ}$  to  $110^{\circ}$  C., and the precipitate then washed with boiling water until all sulphate was removed. The figures obtained by the first analyses made were very variable, ranging from 0.144 to 2.9 per cent of the weight of proteid, and it was found that this variation depended on the extent of washing of the precipitate with alcohol and ether. When the precipitate obtained by either method was thoroughly digested with boiling alcohol and then with ether, the phosphorus reaction disappeared entirely or was so small that no weighings could be made. The alcohol and ether extracts were found to contain lecithin and other related bodies, as described below. It is evident from these facts that the non-coagulable proteid does not belong to the group of nucleo-proteids or nucleo-albumins, but when precipitated carries down with it some lecithin in a form of combination such that the lecithin can be extracted by hot alcohol. Hoppe-Seyler long ago called attention to the fact that the globulins of the blood when precipitated carry down some lecithin, and the present author has found this to be the case even with the precipitate of blood serum produced by carbon dioxide. Fuld and Spiro<sup>1</sup> call attention to the fact that pseudoglobulin, obtained by saturating serum to 34 to 46 per cent with ammonium sulphate, always contains phosphorus. Bernert<sup>2</sup> also reports that in a milky ascitic exudate examined by him the portion of the proteid precipitated by one-half saturation with ammonium sulphate gave an abundant reaction for phosphorus, but that all of the phosphorus-containing material could be extracted by hot alcohol. Freund and Joachim<sup>3</sup> claim to have discovered a phosphorus-containing proteid in the blood which they designate as a nucleo-globulin. Since, however, they give no satisfactory evidence of obtaining from this proteid any of the characteristic dissociation products of nuclein or pseudonuclein, it remains doubtful whether the proteid they were dealing with was a nucleo-proteid or nucleo-globulin, or simply a

<sup>1</sup> FULD and SPIRO: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 140.

<sup>2</sup> BERNERT: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlix, p. 48.

<sup>3</sup> FREUND and JOACHIM: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 407.

lecithin-containing proteid from which the phosphorus-containing material might have been extracted by the action of hot alcohol. The author has prepared this so-called nucleo-globulin by the method described by Freund and Joachim, and finds that it contains lecithin and other materials extractable with alcohol and ether. After thorough extraction with boiling alcohol and with ether the residue remaining failed to yield a positive test for phosphorus.

**Examination of the phosphorus extract.** — The non-coagulable proteid prepared as already described was usually extracted first with warm absolute alcohol, then with a mixture of equal parts of alcohol and ether, and finally with boiling absolute alcohol. The extracts taken together were evaporated to dryness at a low temperature, and yielded a yellow waxy material very soluble in ether, but soluble with some difficulty in alcohol. This material contained much lecithin. When mixed with a drop of water and examined under the microscope, it gave beautiful myelin tubes, and when treated according to the usual method with barium hydroxide, carbon dioxide, and platinum chloride, it yielded the orange-colored crystals characteristic of cholin. Two quantitative analyses of the residue after repeated solutions in ether and evaporation at a low temperature yielded 1.25 and 1.77 per cent of phosphorus. In the first analysis the residue was weighed directly after heating for several hours at  $100^{\circ}$ , until an approximately constant weight was obtained. In the second the weight of substance used was determined by difference, that is, the non-coagulable proteid was weighed before and after extraction with the alcohol and ether. Further analyses were not made, as it seemed certain from the low percentage of phosphorus that the residue was not composed entirely of lecithin. This conclusion was verified by a qualitative examination of the residue, according to which the following separate parts could be recognized: —

I. A portion precipitable from ethereal solution by acetone, the precipitate soluble in cold alcohol. This portion contained nearly 3 per cent of phosphorus and was largely lecithin.

II. A portion precipitable from ethereal solution by acetone, but the precipitate not soluble in cold alcohol. Its ethereal solution evaporated to dryness yielded a hard waxy mass which gave an abundant reaction for phosphorus. Under the microscope it showed a granular structure, but gave no myelin figures on the addition of water.

III. A portion precipitable from its ethereal solution by the addition of alcohol. The precipitate was slightly colored and soluble in water.

The amount of this material was very small; it gave a phosphorus reaction, but failed to respond to any of the reactions for jecorin.

IV. A portion not precipitable from its ethereal solutions by acetone, soluble in cold alcohol. The residue on evaporation was oily; it gave a reaction for phosphorus, and under the microscope showed numerous crystals resembling those of fatty acids and with a melting-point at about 60° C. The residue had an acid reaction.

V. A portion not precipitable from its ethereal solutions by acetone, soluble with difficulty in cold alcohol. On evaporation it gave a hard waxy mass which gave only a faint reaction for phosphorus and no myelin figures on the addition of water.

The material available for study did not suffice for further work, but it seemed evident that in the alcohol-ether extract of the non-coagulable proteid there was no substance corresponding to jecorin, while there were present two substances at least (I and II) rich in phosphorus, one of which (I) gave the characteristic myelin figures of lecithin on the addition of water, while the other failed to give this reaction.

The fact that the non-coagulable proteid when precipitated by ammonium sulphate or alcohol yields such a large amount of lecithin and related substances suggests that it may consist of a lecitho-proteid. Several authors, notably Liebermann,<sup>1</sup> have contended for the existence of such a group of proteids. Osborne and Campbell,<sup>2</sup> following Hopper-Seyley, have described a compound of lecithin and nucleoproteid occurring in the yolk of the hen's egg. The assumed compound has the properties of a globulin, and the lecithin in it can be extracted by alcohol. As was stated above, the globulin, particularly the pseudoglobulin, when precipitated by salts or carbon dioxide, carries down with it some phosphorus-containing material, presumably lecithin. But the non-coagulable proteid contains a larger amount of this material. When the blood is coagulated at 80° to 85° C., the heat coagulum contains only a small amount of phosphorus in a form extractable by alcohol, while the non-coagulable proteid is relatively rich in this substance. The portion of the non-coagulable proteid that is thrown down by boiling in strong acid solution contains also much phosphorus material extractable by alcohol. The amount of lecithin or related substances contained in the non-coagu-

<sup>1</sup> LIEBERMANN: *Archiv für die gesammte Physiologie*, 1891, I, pp. 25 and 55.

<sup>2</sup> OSBORNE and CAMPBELL: *Journal of the American Chemical Society*, 1900, xxii, p. 413.

lable proteid is therefore noteworthy. When this proteid is precipitated by salts (ammonium sulphate), the lecithin is carried down; when dissolved, the lecithin passes into solution with it. Alcohol splits off a part of the phosphorus-containing material very easily, but for its complete removal the prolonged action of boiling alcohol is necessary. It is possible, therefore, that the non-coagulable proteid may be a lecitho-proteid compound, whether it pre-exists in the normal blood or is formed during the process of heating. A definite compound between the proteid and the lecithin would be indicated if the amount of lecithin or phosphorus-containing material showed a constant proportion. So far as my analyses go, however, this is not the case. Results of the following character were obtained:—

I. Weight of the non-coagulable proteid precipitated by ammonium sulphate . . . . .	= 0.3959 gm.
Weight of the alcohol ether extract . . . . .	= 0.2480 gm.
Percentage of phosphorus in the alcohol ether extract	= 1.77 per cent.
Percentage of phosphorus in the proteid after extraction	= 0.87 per cent.
Weight of lecithin estimated from the phosphorus . .	= 0.1474 gm.
Percentage of lecithin in the non-coagulable proteid .	= 37 per cent.
II. Weight of the non-coagulable proteid precipitated by ammonium sulphate . . . . . = 0.8495 gm.	
Weight of lecithin estimated from the phosphorus in the alcohol ether extract 0.113, and in the proteid after extraction 0.092 . . . . .	= 0.205 gm.
Percentage of lecithin in the non-coagulable proteid .	= 24 per cent.

Although not constant, it will be seen that the amount of phosphorus-containing material in the precipitated non-coagulable proteid is very large, much greater than in the portion of the serum proteids coagulated by heat. This adherent or combined lecithin modifies the properties of this proteid, and there can be no doubt that the album of Chabrié and the non-coagulable proteid of the blood investigated by other authors consisted of this compound. It was not possible to determine the properties of the proteid combined with the lecithin, since the method of removing completely this latter substance, namely, by prolonged boiling with absolute alcohol, must have altered this proteid constituent. The material left after extraction with the alcohol was only partially soluble in water. The portion dissolved was precipitable by alcohol, but this precipitate in turn was only partially soluble in water. The aqueous solution thus

obtained became opalescent on boiling, and with the biuret reagent gave a redder tint than the original proteid.

**The iron and sulphur contents of the non-coagulable proteid.** — After removal of the phosphorus-containing bodies and other material extractable by alcohol and ether, the remaining material was found to contain sulphur and iron. A single analysis for sulphur gave for 0.7033 gm. of the proteid 0.0229 gm. of barium sulphate, or 0.45 per cent of sulphur. The content in iron was more noteworthy. The proteid material after thorough extraction with alcohol gave always a distinct reaction for iron. The possibility that this iron might have arisen from the slight amount of hæmoglobin usually present in the serum was disproved by a special experiment made with the oxalated plasma of the horse. This plasma was entirely free from hæmoglobin, as far as could be determined by its color when seen in mass, by the spectroscope and by the results of Almen's test. The non-coagulable proteid prepared in the usual way from this plasma gave an excellent iron reaction. The iron was determined quantitatively in three cases by fusing the proteid with sodium carbonate and potassium nitrate and precipitating by ammonium hydroxide. The three analyses gave, respectively, 0.19, 0.17, and 0.29 per cent of the extracted proteid. The maximum amount of iron is so small that it would seem impossible for it to form a constituent part of the proteid molecule, as is the case also with many of the iron-containing proteid compounds, such as the nucleo-albumins, found in the tissues or liquids of the body. The amount of iron is sufficient, however, to make this compound of interest in connection with the iron metabolism of the body.

**The relation of the inorganic salts of the blood to the heat coagulation of the proteids.** — The usual proteids of the blood serum, serum globulin and serum albumin are distinguished from each other by their reaction toward magnesium sulphate or ammonium sulphate. Moreover the two portions of the globulin usually recognized, namely, euglobulin and pseudoglobulin, are likewise differentiated by the degree of saturation with ammonium sulphate necessary for their precipitation. Every one who has worked with these proteids must have recognized the unsatisfactory nature of this classification. The portion of proteid precipitated by half-saturation with ammonium sulphate and designated as serum globulin is not a well-defined chemical substance with uniform properties, and the same may be said of the fractional portions distinguished as euglobulin and pseudo-

globulin. A solution of either one of these latter proteids submitted to dialysis exhibits a portion that precipitates and a portion that remains in solution,<sup>1</sup>—an indication of the presence of two different substances in spite of the fact that they both come down at a certain degree of saturation with ammonium sulphate. The author has been much impressed also with the fact that the blood proteids after precipitation with ammonium sulphate exhibit quite different properties, as regards their precipitation and coagulation especially, from those shown before the action of this salt. This difference may be shown in several ways. If blood serum is dialyzed very completely, a certain amount of precipitate forms which settles to the bottom of the dialyzing flask and leaves a perfectly clear supernatant liquid that may be filtered off. If now this clear liquid is precipitated by ammonium sulphate, filtered, redissolved, and the solution again dialyzed, a further abundant precipitate comes down. The action of the ammonium sulphate has rendered a portion of the proteid susceptible of precipitation by dialysis. Again, if blood serum is precipitated by ammonium sulphate added to one-third or one-half saturation, and the portion thus isolated is redissolved, it will be found that the proteid in solution is precipitable by a less degree of saturation with ammonium sulphate than was the case with the original serum. The difference in the properties of the proteids caused by precipitation with ammonium sulphate is most clearly shown by the effect on heat coagulation. Facts of the following kind may be demonstrated. If blood serum is precipitated by one-half saturation with ammonium sulphate to remove the so-called serum globulin, and the filtrate containing the serum albumin is thoroughly dialyzed until no reaction is given with barium chloride, the solution thus obtained gives no distinct coagulum on heating. If the solution is concentrated, an opalescence will develop on boiling, but no coagulum. Moreover, if sodium chloride, potassium chloride, or lithium chloride be added to a concentration of, say, two per cent, boiling still fails to give a coagulum; in fact, the presence of these salts may even prevent the formation of an opalescence. Addition, however, of a little ammonium sulphate or chloride, or the chlorides of magnesium, calcium, or barium will cause a flocculent precipitate when the solution is heated to 70°–75° C. On the contrary, if the precipitate of serum globulin thrown down by half-saturation with ammonium sulphate is redissolved and thoroughly dialyzed, the solution precipi-

<sup>1</sup> FREUND and JOACHIM: *Loc. cit.*



tates in part, and the precipitate after solution, as well as the portion not precipitated by dialysis, gives a heat coagulum at  $70^{\circ}$ – $75^{\circ}$  C. in the presence of sodium, potassium, or lithium chlorides. These facts would seem to indicate that serum albumin is a proteid whose combinations with salts of sodium, potassium, or lithium are non-coagulable by heat, and in this respect different in a striking way from the serum globulin which coagulates readily in the presence of these salts. This conclusion is not borne out, however, by observations upon blood serum not treated with ammonium sulphate or upon blood serum completely saturated with ammonium sulphate. If, for instance, one takes normal blood serum and dialyzes it thoroughly, a certain small portion is precipitated. If this precipitate is filtered off and the filtrate is tested as to its heat coagulation in the presence of salts, no indication will be found of the existence of two proteids, one of which suffers heat coagulation in the presence of sodium, potassium, and lithium salts, and one of which remains uncoagulated. On the contrary, all of the remaining proteid that can be precipitated by heat comes down at a temperature of  $85^{\circ}$  C. or more in the presence of sodium, potassium, or lithium salts, and at a temperature of  $70^{\circ}$ – $75^{\circ}$  C. in the presence of ammonium salts. On the other hand, if the serum is precipitated by complete saturation instead of by half-saturation with ammonium sulphate, and the precipitate, consisting of both the serum globulin and serum albumin, is redissolved and thoroughly dialyzed, a certain portion of the proteids will be precipitated, the precipitate coming down in two parts, —one, the larger portion, appearing early in the dialysis, and one later, after the salts have been removed more completely. If these precipitates are filtered off and the clear, thoroughly dialyzed solution is heated after the addition of sodium or potassium chloride, all the proteid capable of heat coagulation comes down at  $80^{\circ}$  C. or below. It would seem from these results that so-called serum albumin left after half-saturation with ammonium sulphate is incoagulable by heat if no salts are present except those of sodium or potassium, but if first precipitated by ammonium sulphate, it is rendered coagulable in the presence of sodium and potassium salts also. It would seem evident from these facts that once the proteid of the blood serum or any portion of it is precipitated by ammonium sulphate its properties become altered; one may say in general that its solubility is decreased or its tendency to precipitation is increased. It is possible that this change may be due to the fact that the precipitate

with ammonium sulphate is a compound of the proteid and this salt, and that when redissolved it goes into solution in this form as a proteid compound different from that formerly existing in the blood. Moreover, when a portion of the blood proteid (the globulin) is precipitated by ammonium sulphate, the portion left behind (the albumin) is also altered, as is shown by the difference in reaction toward heat coagulation in the presence of sodium and potassium salts, between serum dialyzed before and after half saturation with ammonium sulphate. The serum albumin in the latter case is not coagulable by heat in the presence of sodium or potassium salts alone, while in the former case it is completely coagulable in the presence of these salts, although with some difficulty. It would seem that the ammonium sulphate in throwing down a portion of the proteid (serum globulin) had rendered it more easily coagulable, but had left the albumin in a less readily coagulable form. All of these facts tend to throw suspicion upon our present system of classification of blood proteids so far as it is based upon the separation by saturation with the sulphates of magnesium, ammonium, or zinc. Precipitation by these salts alters the properties of the portion precipitated as well as of the portion left in solution. This fact, taken with the further fact that the portions of proteid separated by one-third or one-half saturation with ammonium or zinc sulphate fail to exhibit uniform reactions, would indicate that the method of salting out forms artificial lines of demarcation between the different proteids of the blood. To define a globulin as a proteid precipitated by half saturation with ammonium sulphate or by saturation with magnesium sulphate is unsatisfactory, since the portion left behind as serum albumin, if itself precipitated by further saturation with ammonium sulphate, may exhibit the properties of a globulin as tested by this reaction. The group definition that we give to the globulins is that they are insoluble in water free from neutral salts, and in accordance with this definition it would seem to be more appropriate to restrict the term serum globulin to that portion of the serum proteid which is precipitated upon complete dialysis. The proteid so precipitated seems to contain no lecithin or other phosphorus-containing body. When dissolved in dilute solutions of sodium chloride, it coagulates at 68° to 70° C, the precipitation being complete and precise. The proteid left in solution after the dialysis is, according to the usual nomenclature, a mixture of globulins and albumin, but the so-called globulin remains in solution no matter how complete the dialysis

may be. If, however, the remaining proteid is precipitated by ammonium sulphate, redissolved, and again dialyzed, a very large proportion will be precipitated. Evidently this portion is altered in some way by the action of the ammonium sulphate; a proteid that under its normal conditions acted like an albumin now gives the distinctive reaction of a globulin. The proportion of the proteid precipitable by dialysis seems to be greater when the serum is entirely saturated with ammonium sulphate than when it is half saturated with this salt, — a fact which would indicate that when once the proteid or any portion of it has come down in combination with ammonium sulphate, the presence of this salt in combination renders it less soluble and more easily precipitated by dialysis or heat. It would seem to be more consistent to designate as globulin only that portion of the serum proteid which is precipitated by dialysis when the unchanged serum is submitted to this process, and to call the remainder serum albumin, since it remains in solution in spite of the removal of all diffusible salts. Since, moreover, this portion forms a combination of some kind with lecithin or a lecithin-containing complex upon which its properties depend, it might be more appropriate to speak of it as a lecitho-albumin compound. However precipitated, some phosphorus-containing material may be extracted from the precipitate, whereas the proteid thrown down from the original serum by dialysis alone is free from this material.

**Does a non-coagulable proteid exist in the blood?** — From the facts described in the foregoing pages it is evident that the precipitability of the proteids, and especially their property of heat coagulation, depend upon the character and quantity of the salts present. In the presence of ammonium sulphate and a slightly acid reaction all of the proteid comes down upon heating. But in serum containing only its normal salts there is a portion not coagulable by heat even when the reaction is distinctly acid. Some of the properties of this portion of the proteid have been described, and it is evident from these properties that it does not belong to the group of proteoses and peptones. The important question arises as to whether this proteid exists normally in the blood or is an artificial product split off during the process of heating. This question is difficult or impossible to answer in a satisfactory way. As isolated by the process of heating, it is a proteid differing from both the serum globulin and the albumin as ordinarily described. It is characterized by its large content of phosphorus and iron; it differs from the serum albumin in being

more readily precipitated by ammonium sulphate, and in giving, when thoroughly dialyzed, a blue-purple reaction with the biuret test instead of a reddish purple; it differs from the serum globulin, on the other hand, in not being coagulated by heat after complete dialysis when salts of sodium, potassium, or lithium are added, resembling in this regard the so-called albumin. If, following the usual method of separation, solutions of euglobulin and pseudoglobulin are prepared by fractional precipitation of the serum with ammonium sulphate, and are then completely dialyzed to remove the ammonium sulphate, it will be found that on the addition of sodium or potassium chloride to the dialyzed solution the euglobulin is practically completely precipitated by heating in acid reaction, while the pseudoglobulin is incompletely precipitated, and the portion left in solution resembles the non-coagulable proteid left after heating the whole serum. Serum albumin prepared by repeated crystallization or by precipitating the serum with acetic acid after half saturation with ammonium sulphate is, as stated above, not coagulated at all by heating in the presence of sodium or potassium salts, but in the presence of a small amount of ammonium sulphate or chloride is practically completely precipitated upon heating. If therefore the non-coagulable proteid pre-exists in the serum, it is thrown down, in part at least, with the serum globulin as usually prepared. The author, however, while unable to reach any perfectly satisfactory conclusion in this matter, has been convinced that this non-coagulable proteid is in reality an artificial product split off from the complex material present in the normal serum. This conclusion is based partly upon the inconstancy in the amount and the reactions of this material and partly upon the following consideration: When the whole serum is dialyzed, a portion of the proteid is precipitated, the true globulin. If to the balance of the serum one adds sodium chloride or potassium chloride to the strength of 1 to 2 per cent, and boils, practically all of the proteid is precipitated after one or two heatings. Subsequent addition of ammonium or barium chloride followed by heating causes no further precipitate. If the non-coagulable proteid were present, it should be left in solution, to be thrown down upon subsequent heating with barium or ammonium chloride, since its solutions are incoagulable in the presence of sodium or potassium salts alone. While this argument is not conclusive, none of the facts that may be urged in favor of the pre-existence of this proteid is free from the objection that it may be split off during the process of heating in acid solutions.

## SUMMARY.

1. After complete coagulation of blood serum at 80°-85° C. in feebly acid reaction (acetic acid), there remains in solution a proteid, Chabrié's albumon, which is not coagulated by boiling, but which may be incompletely precipitated by prolonged boiling, especially if the reaction is made strongly acid.

2. This non-coagulable proteid is not a peptone or proteose. When precipitated by nitric acid, the precipitate does not clear up on warming: it gives a blue-purple biuret reaction; it is not readily diffusible, and in the presence of salts of ammonia, barium, calcium, or magnesium is coagulated by heating. When isolated from its solutions by precipitation with ammonium sulphate, added to one-half saturation, or by alcohol, it yields considerable phosphorus-containing material (lecithin), which may be removed completely by extraction with boiling alcohol. It contains also a distinct amount of iron.

3. Blood serum from the fed or starved animal when submitted to dialysis in collodium tubes gives no indication of the presence of a perceptible amount of peptone or proteose.

4. Serum albumin isolated in the usual way by half saturation of the serum with ammonium sulphate followed by complete dialysis, or precipitated from its solutions by acetic acid after the removal of the globulins and then redissolved and dialyzed, shows the peculiarity that its solutions when heated in the presence of sodium, potassium, or lithium salts give no heat coagulum. Addition of small amounts of ammonium sulphate or chloride, or barium, calcium, or magnesium chloride, causes the formation of a coagulum on heating. Solutions of the globulins after precipitation by ammonium sulphate, followed by complete dialysis of their solutions, are coagulated by heating in the presence of salts of sodium, potassium, and lithium, as well as those of ammonia, barium, calcium, and magnesium.

5. The separation of the proteids of the serum into several globulins and albumin by means of fractional precipitation with ammonium sulphate is unsatisfactory, inasmuch as the products isolated do not show uniform properties. Evidence is given that after a proteid has been salted out by ammonium sulphate its properties are changed in the direction of a greater susceptibility to precipitation by dialysis or heating. The reactions of the proteids, particularly the heat coagulation, must be considered with reference to the kind as well as the amount of salts present in the solution.

6. It is suggested that the term serum globulin be reserved for the proteid precipitated from normal serum or plasma by complete dialysis. This proteid shows a uniform and precise temperature of heat coagulation in solutions containing sodium chloride. The portion of the serum proteid not precipitated by dialysis shows the essential properties of an albumin modified by combination with lecithin or a lecithin-containing complex.

## A MOLECULAR THEORY OF THE ELECTRIC PROPERTIES OF NERVE.

BY WILLIAM SUTHERLAND.

IN "The Nature of the Propagation of Nerve Impulse,"<sup>1</sup> the theory was proposed that the nerve impulse is propagated by a shear along nerves, and at the same time it was pointed out that, the elastic properties of matter being of electric origin, the electric properties of nerve were implicitly involved in the theory. In the present paper it is proposed to show the connection between the elastic and the electric properties of nerve explicitly by means of our knowledge of the proteid molecule and a theory of the structure of colloidal solids. The matter will be taken in the following order:—

1. The elastic properties of jellies and their structural cause.
2. The electric origin of rigidity in a jelly.
3. Calculation of the charge of electricity required to stimulate a given nerve.
4. Various quantitative electric properties of nerve.

### I. THE ELASTIC PROPERTIES OF JELLIES AND THEIR STRUCTURAL CAUSE.

In jellies and the soft tissues like nerve the proteid material forms a network whose meshes are fine enough to retain the molecules of water in batches. The rigidity of a soft tissue or jelly belongs to its proteid framework. No doubt there are cases in which the proteid molecules attach molecules of water to themselves in a loose chemical combination, and thus associate water in the production of rigidity. On the other hand, water in sufficiency dissolves certain proteids, so destroying their rigidity altogether. The typical general case is that of a framework of proteid supporting an aqueous solution of proteid.

<sup>1</sup> SUTHERLAND: This journal, 1905, xiv, p. 112.

To form a conception of rigidity in a jelly of such a type the simplest way is to study jellies containing various amounts of dry gelatine per unit volume and exhibiting every stage of stiffness from the minimum up to that of dry gelatine. For concentrations below that giving minimum measurable rigidity we get colloidal solutions of the gelatine in which the framework of proteid is no longer continuous, but broken up into the particles of the suspension. At lower concentrations still the suspension may disappear with merging of the colloidal type of solution into the ordinary. Apart from detailed theoretical considerations we should expect that the rigidity of a jelly is proportional to the amount of gelatine available for formation of framework, that is, to the excess of the total amount of gelatine above that which gives minimum rigidity. This proportionality will not hold up to the maximum of pure gelatine, because when the amount of water is greatly reduced the structure of the proteid in the jelly resembles that of an ordinary continuous solid rather than an open framework. If a jelly then contains  $w_0$  gm. of gelatine in a  $\text{cm.}^3$ , when its rigidity is just measurable, the rigidity  $n$  of a jelly containing  $w$  gm. of gelatine will be given by the formula

$$n = c (w - w_0), \quad (1)$$

in which  $c$  is a constant so long as  $w$  has not a value near that for solid gelatine. This proposed relation can be tested by means of published experimental work. The measurements of A. Leick in "Künstlich Doppelbrechung und Elastizität von Gelatineplatten"<sup>1</sup> are suitable. In his experiments he determined Young's modulus  $q$  in grams weight per  $\text{cm.}^2$  for various jellies, for which by the theory of elasticity  $q = 3n$  nearly. For a jelly of 1 part gelatine to 5 parts water Maurer<sup>2</sup> found  $q$  in dynes per  $\text{cm.}^2$  to be 417,000 and  $n$  to be 124,000, in approximate agreement with the theoretical requirement  $q = 3n$  nearly. Leick's first set of measurements on jellies made with hard gelatine I find can be represented by

$$q = 7700 (w - 0.066), \quad (2)$$

as the following comparison shows for jellies in which 1000  $p$  has the values given in the first row, with the experimental and calculated values of  $q$  in grams weight per  $\text{cm.}^2$  in the second and third rows:

<sup>1</sup> LEICK: *Annalen der Physik*, 1904, xiv, p. 139.

<sup>2</sup> MAURER: *WIEDEMANN'S Annalen der Physik*, 1886, xxviii, p. 628.



1000 $p$ . . . . .	100	102	186	189	300	320	450
$q$ experimental . . .	242	266	978	977	1545	2157	2944
$q$ calculated . . . .	254	270	916	939	1795	1948	2950

Here the differences between calculation and experiment lie in such a way as to show that experimental uncertainty is the cause. The variabilities of gelatine jelly are well known, being referable probably to the change of  $\alpha$  gelatine into the  $\beta$  gelatine of M. Traube, which does not jell. Leick's results for jellies made of soft gelatine can be expressed by  $q = 4800 (\omega - 0.065)$  with the following comparison :

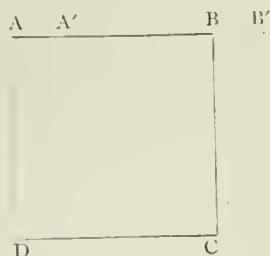
1000 $p$ . . . . .	126	126	127	175	190	208	283	317	456
$q$ experimental . . .	295	323	323	620	578	619	1000	1189	2327
$q$ calculated . . . .	293	293	298	528	600	686	1047	1210	1877

In this series of jellies except the strongest the agreement between calculation and experiment is as good as the experimental conditions allow. In both series of experiments at ordinary temperature the Young's modulus and the rigidity of solutions of gelatine containing less than 0.065 or 0.066 gm. per cm.<sup>3</sup> appear to be negligible, so that the jellies experimented upon may be considered to consist of a fluid solution containing about 0.065 gm. of gelatine per cm.<sup>3</sup> supported by a framework of pure gelatine containing  $(\omega - 0.065)$  gm. per cm.<sup>3</sup> of the jellies. The proposed linear relation between  $q$  and  $\omega$  applies equally well to the results of E. Fraas in "Elastizität von Gelatine-lösungen."<sup>1</sup>

Having thus found the rigidity of a jelly to be a matter of frame structure and proportional to the amount of frame structure present, we proceed to investigate the connection between the rigidity of a jelly and the elastic properties of the material forming the frame structure. The rigidity of a frame is due to the resistance of its ties and struts to extension and compression. Long struts unless laterally supported offer little resistance to shortening. In a jelly we can suppose the contained fluid to give enough lateral support to those proteid threads of the mesh which receive thrust to enable them to act as struts. Thus the rigidity of a jelly depending entirely on the resistance of its ties to lengthening and of its struts to shortening depends directly on the Young's modulus of the proteid threads forming the framework or network. Let  $ABCD$  be a section of a unit cube of jelly which is supposed subjected to a shearing strain pro-

<sup>1</sup> FRAAS: WIEDEMANN'S *Annalen der Physik*, 1894, liii, p 1074.

ducing displacement to  $A'B'CD$ . If  $AA'$  is small,  $AA'/AD$  measures the angle  $ADA'$ , which is the amount of the shear. Now, according to the usual analysis of strains, this shear may be regarded as the elongation of  $DB$  to  $DB'$ , accompanied with the shortening of  $CA$  to  $C'A'$ , lines in the unit cube perpendicular to the plane of the



paper being unaltered. If  $DB'/DB = 1 + \epsilon$ , it follows from the same analysis that  $CA'/CA = 1 - \epsilon$ , and the shear  $AA'/AD = 2\epsilon = s$ , say, so that  $\epsilon = s/2$ . Now, in our unit cube the threads of proteid run at random in all directions, but for purposes of easy approximate calculation we may suppose them equivalent to a distribution in which one-third of their number  $m$  run parallel to  $DB$ ,

one-third parallel to  $CA$ , and one-third perpendicular to the plane of the paper. Hence two-thirds of the proteid material is involved in elongation or contraction of amount  $s/2$ . But the stresses due to these elongations and contractions of the proteid ties and struts in the unit cube are found by multiplying the strain by  $q_0$ , which is Young's modulus for the proteid threads, and by the sectional area  $A$  of each and by their number  $2\frac{m}{3}$ , namely,  $q_0 (\frac{s}{2}) A (2\frac{m}{3}) = q_0 s A \frac{m}{3}$ . Now,  $3 q_0 = n_0$ , the rigidity of the proteid stuff of the threads, so that the stresses are  $n_0 s A m$ , and the rigidity of the jelly is  $n_0 A m$ , and is directly proportional to the rigidity of the proteid material of the threads and to their number per unit volume. This brings us back to the principle discussed above; that the Young's modulus and the rigidity of a jelly are proportional to the amount of gelatine or proteid forming the continuous framework.

We have now to form the simplest molecular conception of the structure of framework in a jelly. Towards that end I shall give a brief summary of a theory of the colloidal state which I have already communicated elsewhere. The salient properties of colloids may be traced to the one fact that their contiguous molecules combine chemically with one another by means of valencies which are often latent. For example, N in  $NH_3$  is trivalent, though in  $NH_4Cl$  it is pentavalent. In extension of the Helmholtz theory of valence I have proposed<sup>1</sup> to express the trivalence of N by associating with it three negative electrons each denoted by  $\ominus$ , and the pentavalence by adding a doublet consisting of one positive electron  $\oplus$  and a negative

<sup>1</sup> SUTHERLAND: Philosophical magazine, 1902 [6], iii, p. 174.

2. In  $\text{NH}_3$  these two opposite electrons keep so close to one another as to neutralize almost completely one another's action, leaving N to act as trivalent. But in  $\text{NH}_4\text{Cl}$  the  $\pm$  of H and the  $\pm$  of Cl cause this latent doublet  $\pm\pm$  of N to open out and become chemically effective. In this way most of the important cases of dual valency in the elements can be interpreted. Now, in a frozen mass of  $\text{NH}_3$ , if the doublet  $\pm\pm$  of each N atom were to open out so that  $\pm$  in one attracted  $\pm$  in a neighbor on one side while  $\pm$  attracted  $\pm$  of a neighbor on the other, the molecules of  $\text{NH}_3$  would become united chemically with one another in chains. The ordinary chemical definition of a molecule would break down. Each of the tangled chains would be a molecule of a polymeric form of  $\text{NH}_3$ . In an extreme case the whole lump of frozen  $\text{NH}_3$  might be one endless chain, a single molecule. This imaginary case with  $\text{NH}_3$  illustrates the proposed theory of the colloidal state, namely, that it is due to the chemical union of neighboring molecules through valencies which have ceased to be latent. In a colloid, then, the usual molecule ceases to be a separate entity; it becomes a pattern which is repeated. Such a pattern I propose to call a *semplar*. A colloid is an indefinite collection of *semplars*. A single file of *semplars* gives us the simplest form of the proteid thread forming the meshes of network or the ties and struts in the framework of a jelly. Each proteid *semplar* must have numerous electrons by which it can join to others, so that the single files can unite with one another chemically where they come in contact, this chemical combination corresponding to the knotting of threads in a net and to the junction of ties and struts in engineering frameworks by pins and rivets. When the water is all driven out of a jelly, the single files of *semplars* form cross connections at so many points, and so much latent valency is called into action, that the proteid becomes practically a different substance, having higher rigidity than that belonging to the *semplars* in single file. That is why our formulæ (1) and (2) for jellies cannot be made to include the case of dry gelatine. I have found the rigidity of dried sciatic nerve of the sheep to be hundreds of times as large as that of the fresh nerve, and the experimental results with coagulated white of egg and with gelatine jellies are similar. Having arrived at a definite mechanical structure for jellies and tissue substance, we can now proceed to connect it with their electrical properties.

## II. THE ELECTRIC ORIGIN OF RIGIDITY IN A JELLY.

In "The Electric Origin of Molecular Attraction" and "The Electric Origin of Rigidity and Consequences"<sup>1</sup> cohesion and rigidity are traced to electric doublets  $\pm 2$  in molecules and atoms. As regards rigidity the essential point in the theory is this: Consider a doublet

$\pm 2$  whose unstrained position of equilibrium is  $AB$ ,  
 $\pm . A$       $A$  while  $A'B$  is the position when  $A$  is sheared to  $A'$ .  
 Work is done in moving  $\pm$  from  $A$  to  $A'$  against the attraction of  $2$ . This is the work of elastic distortion. It is shown that at the absolute zero of temperature the rigidity of a metal is equal to the

electrostatic energy of its molecular doublets in unit volume. Hence we must trace the rigidity of a nerve or a jelly to electric doublets in the proteid semplar. The simplest conception of this doublet, according to current electrical thought, is like the old one of a magnet, which was treated as two opposite poles of strength  $p$  at the ends of its length  $l$  and having a magnetic moment  $pl$ . So the electric doublet in a molecule, atom, or semplar may be regarded as formed of two opposite electron charges of the amount  $e$  associated with ions. These are at a distance  $s$  apart, which is of the order of magnitude of the diameter of the molecule or semplar. For the rigidity  $N$  of a metal at absolute zero the formula established<sup>2</sup> is

$$N = \frac{\pi}{3K} \cdot \frac{e^2 s^2}{\left(\frac{m}{\rho}\right)^2}, \quad (3)$$

in which  $K$  is the dielectric capacity (specific inductive capacity) of the metal,  $m$  the mass of the molecule, and  $\rho$  the density of the metal, so that  $\frac{m}{\rho}$  is the volume of a molecule. At higher temperatures a rather important temperature factor comes in with metals, but in the case of substances like proteids it is probable that the ordinary temperature factor is of small importance, because the movement of the parts of a semplar can have little effect in changing the average effective value of  $s$ , unless with the oncome of so profound a change as that of coagulation. Hence for the rigidity  $n_0$  of a thread of proteid in the framework of a jelly or nerve we write

<sup>1</sup> SUTHERLAND: Philosophical magazine, 1902 [6], iv, p. 625, and 1904, vii, p. 417.

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

$$n_o = \frac{2\pi}{3K} \cdot \frac{e^2 s^2}{\left(\frac{m}{\rho}\right)^2} \quad (4)$$

Treating  $s$  as equal to the diameter of a semplar assumed spherical and therefore having  $\frac{m}{\rho} = \frac{\pi s^3}{6}$ , this gives

$$n_o = \frac{24 e^2}{\pi K s^4} \quad (5)$$

To find  $K$  for proteids we may use Maxwell's relation that  $K$  is equal to the square of the index of refraction. In its solutions gelatine has an index of refraction 1.53; so  $K = 2.3$ . As regards the size of  $s$ , the diameter of a proteid semplar, we shall assume that it is of about the same order of magnitude as the diameter of the molecule of egg albumin when dissolved in water, which I have shown to be about 15 times that of the hydrogen molecule.<sup>1</sup> The limiting volume of a gram molecule  $H_2$  being 8.6, that of the albumin molecule is 27,000. Now, the diameter of the hydrogen molecule is  $2 \times 10^{-8}$  cm., so we get  $s = 30 \times 10^{-8}$ . And  $e$  is known to be  $3 \times 10^{-10}$  electrostatic units. Hence from (5)  $n_o = 4 \times 10^7$  dynes per cm.<sup>2</sup>. But in formula (2) it appears that the proteid threads in a gelatine jelly have a Young's modulus  $q_o = 7700$  gm. weight per cm.<sup>2</sup>, so that experimentally we get  $n_o = 2570$  gm. weight per cm.<sup>2</sup> =  $2570 \times 981$  dynes per cm.<sup>2</sup> =  $25 \times 10^5$ . This is the  $\frac{1}{16}$  part of the value just calculated. If we take  $s$  for the proteid semplar in gelatine twice as large as the  $30 \times 10^{-8}$  used above, we obtain a calculated value of  $n_o$  equal to  $\frac{1}{16}$  of that found above and identical with the experimental value. Thus we have arrived at the important result that the rigidity calculated for the proteid threads in jelly from molecular and electrical considerations is of the right order of magnitude. We can express the velocity of propagation of a shear through the framework or through the jelly, namely,  $\sqrt{\frac{n_o}{\rho}}$  or  $\sqrt{\frac{n}{\rho}}$ , using the appropriate  $\rho$  in each case, in terms of the electrical properties of the proteid semplar by means of our expressions for  $n_o$  and  $n$ . It is possible that in the propagation of nerve impulse the shear is confined to the proteid framework as suggested in my previous paper; it is

<sup>1</sup> SUTHERLAND: Philosophical magazine, 1905 [6], ix, p. 781.

also possible that the shear is such as involves movement of the fluid supported by the framework, in which case the framework would be loaded with the fluid and would propagate a shear more slowly than if it were unloaded. But as we have taken the work of the shear to be that of separating the electrons of the semplar doublets, we have given explicitly an electrical theory of the propagation of nerve impulse. Nerve impulse is propagated by electrical displacements of such a nature that they are accompanied with corresponding material displacements of the semplars, whence the slowness of the propagation of nerve impulse.

### III. CALCULATION OF THE CHARGE OF ELECTRICITY REQUIRED TO STIMULATE A GIVEN NERVE.

A certain electrical polarity having been shown to account for the propagation of shear in a jelly or dead nerve and of nerve impulse in a living nerve, we must consider more closely the difference between dead and living nerve. I take it that the chief difference is this: in dead nerve and jelly the polarities of the proteid semplars are distributed in random directions; in living nerve they are not, but have a preponderating direction which they can maintain or restore while life lasts. The clearest proof of this directed polarity in living nerve is the distribution of potential in an excised length of fresh nerve giving electromotive force between the bounding cylindrical surface and the cut sections at each end. On the average this E. M. F. is about 0.03 volt. Now, if we regard the state of the electrically neutral dead nerve as an arbitrary standard of zero strain, then the E. M. F. can be attributed to a state of strain maintained in the living nerve by a similar process to that by which E. M. F. is caused by strain in a piezoelectric crystal. In the insulating crystal the act of straining produces a definite separation of the two sorts of electricity. If these were allowed to flow as current, the E. M. F. would die away and would be renewed only by further strain. In the excised portion of living nerve there is some provision of energy for keeping up the E. M. F. as long as life lasts. Possibly the action in living nerve is intermittent, the semplars being strained one way while manifesting external E. M. F. and sending current externally, then relaxing and producing the opposite E. M. F. whose current is short circuited internally. Each semplar is thus a source of alternating current, the positive phases of which are

summed externally and the negative internally. This is matter for further investigation, but I propose for the present to associate the E. M. F. of living nerve with the existence of a certain strain in the nerve, that strain consisting of a directed polarity of the doublets in the semplars. This view is considered again in more detail in section 4. Experimentally we know that the strain produces a distribution of negative electricity over the cut ends of the piece of excised nerve and of positive electricity over its bounding cylindrical surface. Now, if a charge of negative electricity is given to the cut end of a nerve, it may be considered to neutralize some of the positive electrons of the terminal semplars, thus destroying part of their polarity. Indeed the whole polarity of the terminal semplars in the cut end of the nerve may be abolished by a large enough negative charge, and the equilibrium of all the chains of semplars in the nerve will be upset. This electric disturbance of the semplar constitutes the central fact of nerve excitation. But we have to distinguish between a disturbance which stimulates and one which depresses. Now, we have seen that the strain in living nerve must be such as liberates negative electricity at the cut ends of excised nerve. Hence negative electricity imparted to the cut end must intensify the strain which is characteristic of the living state. Positive electricity must lower the strain characteristic of the living state. Thus we are led to identify stimulation of a nerve with increase of the strain of vitality, and depression with decrease. As to mechanical stimulation of nerve, we need only remark that mechanical stimulus always consists in straining the nerve substance in some way. When the strain increases the strain characteristic of vitality, it stimulates. If we choose, we can interpret mechanical stimulus as electrical by supposing the strain to produce E. M. F. in the same way as in a piezoelectric crystal. Stimulation of nerve by electrolytic solutions has been shown by Mathews to be electrical in a way for which I have tried to provide a simple theory in a previous paper in this journal. As regards stimulation of nerve by non-electrolytic solutions, Mathews traces it to osmotic pressure, so it becomes virtually a branch of mechanical stimulation. In this way we bring all the types of nerve stimulation under the one heading of a strain consisting of an electrical displacement with an associated material displacement.

To calculate the minimum amount of electricity (negative) for stimulating a nerve we can proceed in the following way. Let us

assume that a single negative electron given to each semplar in the cut end of a nerve constitutes the minimum. Of a vertebrate nerve about one-third is solid matter, of which about one-fourth is proteid, so one-twelfth of a nerve is proteid. Concerning the number and size of the axis cylinders in nerve, I made some observations on sections of the external popliteal of the dog kindly given me by Dr. B. Kilvington from material gathered in his work on nerve regeneration. The mean radius of the axis cylinder is 0.0004 cm., and the mean area of its section is 0.0000005 cm.<sup>2</sup>. In the nerve there were 4200 axis cylinders, so that the sum of their sectional areas was 0.0021 cm.<sup>2</sup>. In sections of the sciatic nerve of the frog kindly prepared for me by my brother Mr. James Sutherland I found the mean radius of the axis cylinders to be 0.00036 cm. and the sum of all their sectional areas to be 0.0021 cm.<sup>2</sup>. This is for a medium-sized frog on which most of the experiments on nerve stimulation have been made. We have now all the data requisite for calculating the amount of electricity necessary to give each semplar in the end of a frog's sciatic a single negative electron. With the formula found for egg albumin,<sup>1</sup> namely, C<sub>1436</sub>H<sub>2364</sub>N<sub>359</sub>O<sub>482</sub>S<sub>15</sub> its molecular mass is 32,814 times that of the hydrogen atom, which is  $2 \times 10^{-24}$  gm. So the mass of the albumin molecule is  $66 \times 10^{-21}$  gm. In a cm.<sup>2</sup> of nerve there is  $\frac{1}{12}$  gm. proteid, and therefore  $\frac{10^{21}}{66 \times 12}$  semplars assumed to have the mass of egg albumin molecules, say,  $10^{18}$ . Hence on a cm.<sup>2</sup> of section of a nerve there are  $10^{12}$  semplars. Each of these receiving the electron charge  $3 \times 10^{-10}$  electrostatic units, the charge per cm.<sup>2</sup> is 300 units. Hence for a nerve in which the sum of the areas of the axis cylinders is 0.0021 cm.<sup>2</sup> the charge will be 0.63 units. Now G. Weiss<sup>2</sup> has found the minimum charge for stimulating frog's sciatic to be of the order  $10^{-9}$  coulomb = 3 electrostatic units. The value just calculated, namely, 0.63, is of the same order of magnitude as the experimental 3. Hence the electrical stimulation of nerve is explicable on the same principles as have been used to account for the propagation of nerve impulse with the same order of velocity as shears through jellies. It has been shown in a previous paper that Mathews' experiments on chemical stimulation lead to about the same measure of the amount of electricity required to stimulate a nerve as the direct determinations of Weiss.

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

<sup>2</sup> WEISS: Comptes rendus, 1901, cxxxii, p. 1068.



IV. VARIOUS QUANTITATIVE ELECTRIC PROPERTIES OF NERVE.

In this section it is proposed to apply the preceding principles to account for certain specific properties of nerve. The most important of these is the discovery of Weiss already considered, namely, that stimulation depends upon quantity of electricity rather than upon quantity of electrical energy. This simple outcome of the classical work on electrophysiology and the more recent researches of Hoorweg, Hermann, Dubois, Waller, and others forcibly recalls Faraday's fundamental law of electrolysis, namely, that the amount of substance electrolyzed is proportional to the quantity of electricity used and not to the electric energy consumed. Weiss finds further<sup>1</sup> that if the quantity of electricity  $q$  required just to produce minimal effective stimulation is communicated to the cut end of a nerve in time  $t$ ,

$$q = a + bt, \tag{6}$$

where  $a$  and  $b$  are constants for a given nerve preparation. This important formula has been extended in an interesting way by M. and Mme. Lapique,<sup>2</sup> who found that in invertebrates, if  $v$  is the voltage applied to a nerve muscle preparation for time  $t$ , then taking  $v t$  as proportional to the  $q$  of Weiss, since the resistance of the preparation is constant,

$$q = a + bt - cv, \tag{7}$$

where  $a$ ,  $b$ , and  $c$  are constants for a given preparation, but vary from one preparation to another. The interpretation of Weiss's formula is this. The quantity  $a$  of electricity is that which gives one negative electron to each semplar in the cut end of the nerve and accentuates the strain in the nerve just enough to give minimal response in the attached muscle. If the electricity  $a$  is not given instantaneously, but in time  $t$ , there is loss by conduction at rate  $b$ , and more than the minimum  $a$  has to be given to make good the loss and bring the amount of electricity on the nerve end at the time  $t$  just up to  $a$ . Hence  $q = a + bt$ . Now, for frog's sciatic in Weiss's experiment  $a$  is of the order  $10^{-9}$  coulomb. The voltages used in such experiments average about 1 volt per cm. of the preparation, and the resistance of frog's sciatic nerve is about  $10^5$  ohms per cm., so the quantity of elec-

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

<sup>2</sup> LAPICQUE: *Comptes rendus*, 1903, cxxxvi, pp. 1147 and 1477.

tricity flowing through is about  $10^{-5}$  coulombs per second, and hence the necessary  $10^{-9}$  coulombs would escape in  $10^4$  second. Therefore, with the second as unit of time,  $\frac{b}{a}$  ought to be about  $10^4$ . According to the Lapiques,<sup>1</sup> for a preparation with the gastrocnemius of a frog (*Rana esculenta*)  $\frac{b}{a} = 3 \times 10^3$ , and for *Rana temporaria*  $1.4 \times 10^2$ , for the gastrocnemius of the toad (*Bufo vulgaris*)  $8 \times 10^2$ , while Cluzet with sciatic preparations of the frog<sup>2</sup> finds  $\frac{b}{a} = 15 \times 10^2$ . These are not far in order of magnitude from the estimated value  $10^4$ . In nerve and muscle of the forceps of a crab (*Carcinus maenas*) the Lapiques find  $\frac{b}{a} = 30$ , and for the mantle of *Aplysia punctata* 10. As to the origin of the term  $-c v$  introduced by the Lapiques into the formula of Weiss, I take it to be the following. A nerve can be stimulated electrically either by receiving a direct charge or by being subjected to electric force without communication of a charge. In applying voltage to a nerve muscle preparation there is stimulation both by charge and by electric force, a certain intensity of electrical force being equivalent to the minimum stimulating charge  $a$ . Suppose a voltage  $v$  applied to the preparation by electrodes at distance  $d$  apart, then the electric force is  $\frac{v}{d}$ , which is equivalent in stimulating power to a charge  $\frac{k v}{d}$ , where  $k$  is a constant for the preparation. Hence, if  $q$  is the amount of electricity given to the nerve end, we must add to it  $\frac{k v}{d}$  to express the total electric cause as a quantity; then, as above, we have to provide a term  $b t$  for loss during time  $t$  and thus obtain  $q + \frac{k v}{d} = a + b t$ , which is equivalent to the formula of the Lapiques, if the  $c$  of their formula varies inversely as  $d$ , a point deserving experimental investigation. Evidently much may be expected from a full investigation of all the terms of (7) for a great variety of typical preparations.

In further illustration of the utility of a physical theory of nerve action we shall estimate the order of magnitude of the stress in nerve

<sup>1</sup> LAPIQUE: *Comptes rendus*, 1905, cxi, p. 537.

<sup>2</sup> CLUZET: *Ibid.*, 1903, cxxxvii, p. 670, and 1905, cxi, p. 1116.

which could produce, or be produced by, the average E. M. F. of 0.03 volt between cross section and surface. In the absence of data concerning the piezoelectric properties of nerve, and remembering that nerve can maintain a current, whereas piezoelectric crystals do not do so, we shall nevertheless calculate the stress required to give an E. M. F. of 0.03 volt between two faces of a quartz thread of square cross section suitably cut from a quartz crystal. Suppose the side of the square to be 0.1 cm. and the length of the thread to be 1.0 cm. Such a thread of quartz in spite of its simplified square section is a fairly good life-size model of a piece of nerve usually studied. It is a small form of the Quartz Piézoélectrique, the standard instrument designed by J. and P. Curie for illustrating the laws of piezoelectricity discovered by them. The thread is supposed cut from a hexagonal prism of quartz so that its length is perpendicular to any one face of the prism, and its breadth parallel to the axis of the prism. A weight of 1 kilogram hung on the lower end of this thread placed vertically causes a negative charge of electricity to appear on one of the two faces of the thread which are separated by the thickness, and an equal positive charge on the opposite face, the amount of each being  $0.063 \frac{l}{c}$  electrostatic units, where  $l$  is the length of the thread and  $c$  its thickness. For our special thread  $\frac{l}{c} = 10$ , and the charges produced by the weight of a kilogram are 0.63. But if  $b$  is the breadth of the thread, the difference of potential between the two charged faces is  $\frac{4\pi c}{lb}$  times the charge. For our special thread  $\frac{c}{lb} = 1$ , and the difference of potential becomes  $4\pi \times 0.63$  electrostatic units = 2.400 volts. Hence, to produce a difference of potential 0.03 volts between the two faces, there would be needed a weight of only 0.03 2.400 kilogram at its lower end, say, 0.01 gm. With this small stress we can obtain in our quartz thread the same difference of potential between opposite faces as appears between the cut end and the cylindrical bounding surface in excised nerve. If nerve were an insulator, we should have a fair amount of prima facie evidence for believing that the observed potential difference in it is of piezoelectric origin and associated with a suitable type of strain. But as nerve is a conductor, though of high resistance, we have to find in it some source of energy capable of maintaining the E. M. F. and its associated current. The expenditure of energy involved is very small;

for 1 cm. of nerve it is of the order of that given by 0.03 volt through  $10^5$  ohms, or nearly  $\frac{1}{10}$  erg per second. This represents a very minute drain on the total amount of chemical energy latent in a cm. of ordinary nerve. The prime distinction between a piezoelectric crystal and nerve is that the crystal cannot supply for itself energy even at this small rate; if it ceases to insulate, it loses the E. M. F. due to strain; whereas nerve in spite of its electrical conductivity maintains its E. M. F. for hours. Nerve showing this similarity to a battery, it might be urged that the origin of nerve E. M. F. should be sought in voltaic rather than in piezoelectric analogies. It seems to me that in nerve we have to do with a novel sort of molecular battery worked on piezoelectric principles. For in the semplars of living nerve we may have motion which is directed because of the semplars being similarly attached to one another. In a single file of semplars we might expect rotation round the direction of the single file to predominate. This would give polarity to the single file, and could produce a separation of the two sorts of electricity just as the impressed polarity of stress in a quartz crystal does. But the energy required to produce this separation of the electricities is derived from the store of rotational energy of the semplars, and not from an external source. If the separated electricities run together as current, the rotating semplars will separate more. In this way they produce current and permanent E. M. F. by drawing upon their stock of rotational energy. In this idea of the existence of directed motion within the semplars we come back to the principle of the gyrostat, which Kelvin in his Baltimore lectures showed to be of such fundamental importance in molecular physics. At any rate, there is an excellent experimental field of inquiry open in the piezoelectric properties of nerve and muscle. The difficulties of the work are increased by the conductivity of these tissues, but sooner or later electrophysiologists will need to attack the inquiry by appropriate methods. Every physical method of investigating polarity in nerve and muscle will have to be brought into operation. For instance, the electric organ of *Malapterurus* shows electrical resistance four times as great longitudinally as transversely. This is but one type of method for measuring directed properties in tissues. The foregoing is an attempt to formulate a theory of the physics of molecular polarity bearing upon the problems of physiology. It will be noticed that the cell, the physiological unit of structure, does not appear in the present theory, which goes back

to the chemical unit, the molecule or semplar. Justification for going behind the cell is to be found in the existence of primitive nerve properties in unicellular organisms. The present theory is not limited to nerve, but applies to any action similar to nervous which goes on in living matter.

MELBOURNE, AUSTRALIA.



PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES  
OF MAGNESIUM SALTS.—IV. THE RELATIONS OF  
THE SALTS TO THE PERISTALSIS OF THE GASTRO-  
INTESTINAL CANAL.

BY S. J. MELTZER AND JOHN AUER.

[From the Rockefeller Institute for Medical Research.]

**T**HE non-stimulating effect of magnesium salts. — In our first paper on the physiological and pharmacological effects of magnesium salts<sup>1</sup> we stated that the basis for our studies was the hypothesis that magnesium favors essentially or exclusively inhibitory processes in the animal body. The facts brought out so far by our experiments are all in harmony with this hypothesis. There is, however, one familiar action of magnesium salts which is interpreted by many writers as due to a stimulating effect of the salts, we mean the cathartic action of sulphate of magnesium. As is well known, many older writers have assumed that the purgative effects of certain salts are due partly or exclusively to an increase of the intestinal peristalsis (Buchheim, Aubert, Thiery, Radziejewsky, and many others). Recently J. Loeb<sup>2</sup> stated that all the salts which give rise to hypersensitiveness of nerve and muscle act as cathartics when introduced into the intestine, and suggested that the saline purgatives facilitate the production of peristalsis by increasing the irritability of the nerves and muscles of the intestine. On the basis of this hypothesis J. B. MacCallum<sup>3</sup> carried out a series of experiments in which certain saline purgatives were injected subcutaneously and intravenously. His results were that "all those salts which act as purgatives when introduced into the stomach or intestine, have the same action when injected subcutaneously or intravenously." He stated explicitly that "magnesium sulphate, when given subcutaneously, is no less active

<sup>1</sup> MELTZER AND AUER: This journal, 1905, xiv, p. 366.

<sup>2</sup> LOEB, J.: Studies in general physiology, 1905, Part II, p. 759.

<sup>3</sup> MACCALLUM, J. B.: This journal, 1903, x, p. 101.

than sodium sulphate." MacCallum arrived at the conclusion that "the essential feature in the action of saline purgatives is not their presence in the lumen of the intestine, but their absorption into the blood, and the production by them of a condition of hypersensitiveness of the nervous system controlling the intestine." Here we have then a pronounced theory supported by experiments that purgatives, magnesium sulphate included, produce peristalsis by increasing the sensitiveness of nerve and muscle of intestines; in other words, here we seem to have an instance in which magnesium salts do not inhibit but stimulate activity.

In taking up the discussion of this question we must state at the outset that we cannot support the statement of MacCallum concerning the purgative effect of magnesium sulphate when given subcutaneously. MacCallum quotes Claude Bernard as holding a similar opinion. Hay,<sup>1</sup> however, has shown that Bernard's opinion was not based upon any experiments which he made himself. On the other hand, Buchheim, Donders, Rabuteau, and many other writers who have experimented with subcutaneous and intravenous injections of magnesium sulphate positively deny the purgative effect of magnesium salts by this method of administration. In the numerous experiments which we made with subcutaneous injections of magnesium salts in dogs, cats, rabbits, etc., we did not meet with a single case in which the subcutaneous injection led to an immediate or late appearance of purgation or even simply to more frequent stools.<sup>2</sup> We may state here, further, that also in the second series of experiments,<sup>3</sup> in which magnesium salts were repeatedly given intravenously and the animal observed for many hours, no purgation took place. We may therefore consider it as an established fact that subcutaneous and intravenous injections of magnesium salts do not produce purgation.

We have, however, to distinguish between the causation of purgation and the production of peristalsis. In the experiments carried out by one of us (A.),<sup>4</sup> with sodium sulphate, sodium citrate, and sodium phosphate, it was found that while subcutaneous and intravenous injections of these salts produced no purgation, they often caused a moderate but definite increase of the peristalsis of some parts of the

<sup>1</sup> HAY: *Journal of anatomy and physiology*, 1882, xvi, p. 245.

<sup>2</sup> MELTZER AND AUER: *This journal*, 1905, xiv, p. 386.

<sup>3</sup> MELTZER AND AUER: *Ibid.*, 1905-1906, xv, p. 387.

<sup>4</sup> AUER: *This journal*, 1906, xvii, p. 15.



intestine. It was therefore necessary for us to establish by direct experiments, in the first place, whether intravenous injections of magnesium salts are capable of starting or increasing intestinal peristalsis, that is, whether magnesium salts are capable of producing some stimulating effect upon the intestines.

We have made a large number of such experiments and we can state our results in a few words: not in one single experiment did the intravenous injection of magnesium salts produce peristalsis in a quiet part or increase it in an already moving part of the gut.

Magnesium salts apparently do not increase the sensitiveness of nerves and muscles of the intestine. Whatever the cause of the purgative action of magnesium salts may be, it is safe to say that the action is not an instance of the stimulating properties of magnesium salts.

**The inhibitory effect upon the peristalsis.**—Knowing now that subcutaneous and intravenous injections of magnesium salts do not bring on intestinal peristalsis, the question arose whether our hypothesis could not be tested just by this phenomenon, that is, whether existing peristalsis could not be inhibited by these salts. In studying this aspect of our question, we were however confronted with the obstacle that spontaneous intestinal peristalsis is an unreliable phenomenon. There would be, in the first place, no peristalsis when we were ready to inhibit it. Furthermore, the cessation of a peristaltic movement after an injection of magnesium salts could not be considered as a safe proof of the inhibitory effect of the salts, since such a cessation frequently enough occurs also spontaneously. To study this side of the question satisfactorily, we had therefore recourse to the previous employment of such means as bring out more or less reliably peristaltic movements or other contractions of the intestinal canal.

The methods of observation of the abdominal viscera employed in this series of experiments were the same which we have used in our studies of the action of ergot upon the gastro-intestinal canal, and we refer to the detailed description given in that article.<sup>1</sup> In the present study, however, the experiments were confined to rabbits. For anaesthesia morphin or ether or both were used.

The following is a brief account of our results:

**The inhibition of the peristalsis brought on by saline purgatives.**—In a previous article by one of us<sup>2</sup> the results of experiments were

<sup>1</sup> MELTZER AND AUER: This journal, 1906, xvii, p. 142.

<sup>2</sup> AUER: This journal, *loc. cit.*

recorded in which sodium sulphate, sodium phosphate, or sodium citrate were administered by subcutaneous or intravenous injections. It was found that these injections brought on moderate peristaltic movements of some parts of the gut, or caused a moderate but definite increase of previously existing peristalsis. Many of these experiments were utilized by us for a study of the inhibitory effect of magnesium upon the peristalsis produced by these salts. The following brief extract from the protocols will illustrate the results.

*Experiment 1.* — Rabbit, morphin. . . . 4.24 P.M., injected subcutaneously 15 c.c. sodium citrate  $\frac{m}{8}$ . 4.55 . . . entire small gut shows peristalsis. . . . 5.01, injected slowly 1 c.c. 25 per cent  $MgSO_4$  into right ear vein . . . peristalsis of gut disappeared almost entirely immediately after injection.

*Experiment 2.* — Rabbit, morphin. . . . 4.07, 15 c.c. sodium citrate  $\frac{m}{8}$  given subcutaneously. . . . 5.10, injected subcutaneously 15 c.c. sodium sulphate  $\frac{m}{8}$ . 5.55. peristalsis fair in upper gut, slight in lower gut. *Injected 1 c.c. magnesium sulphate 25 per cent into left ear vein. Peristalsis stopped almost entirely shortly after injection.* . . .

*Experiment 3.* — Rabbit, morphin. . . . 1.20 P.M., injected subcutaneously 15 c.c. sodium phosphate ( $Na_2HPO_4$ )  $\frac{m}{8}$ . . . . 3.35, entire small gut moves well, ascending colon active, transverse colon and cæcum quiet. 3.42, injected slowly into left ear vein 1 c.c. 25 per cent  $MgSO_4$ . 3.45, small gut shows only slight swaying, definitely diminished, descending colon is perfectly quiet. 3.47, slight movements begin in descending colon and small gut. . . . 3.57, 2 c.c. sodium phosphate  $\frac{m}{8}$  injected into left ear vein. . . . 4.05. small gut shows fair pendular movement, descending colon shows good constrictions. 4.07, injected 1 c.c.  $MgSO_4$  25 per cent into ear vein. *At end of injection small gut practically quiet, descending colon still shows good travelling contractions.* 4.08, descending colon quiet, distended by gas.

In these and other similar experiments with saline purgatives the intravenous injection of a small dose of magnesium sulphate (or magnesium chloride) abolished or at least considerably diminished any peristalsis present. As a rule the peristalsis gradually returned after an interval of rest lasting between six and fifteen minutes. The peristaltic movements after the injection of the saline purgatives was rarely very active, and their inhibition, although very definite, was not a very striking phenomenon.

We have tried to obtain the paradoxical result of inhibiting a peristalsis brought on by a per os administration of magnesium sulphate. We failed, however, to see any peristalsis after administration of even

large doses of these salts. Van Braam Houckgeest<sup>1</sup> and other writers met with similar experience. However, we made only few experiments of this kind, and we shall not dwell on this subject for the present.

**Inhibition of peristalsis produced by ergot.**—In the following we give short abstracts of protocols from experiments made with ergot:

*Experiment 4.*—Rabbit, ether (cord destroyed below fourth dorsal vertebra; ergot intravenously 1 c.c. twice; viscera observed through abdominal muscles, etc.). 2 P. M., abdomen opened under saline. 2.05, small gut distended and shows good pendular movements, descending colon shows good peristalsis, colon shows only slight movements. 2.11, *injected through jugular vein 1 c.c. MgSO<sub>4</sub> 25 per cent.* 2.12, *small intestines and colon stopped moving entirely*, descending colon still moves well (very distended with gas). 2.25, lower small gut begins to move. 2.28, colon shows slight contractions.

*Experiment 5.*—Rabbit. 2 P. M., received 50 c.c. magnesium sulphate per stomach tube. Ether. Skin removed and viscera observed through muscles. . . . 4.06, abdomen opened under saline. Observed until 4.24, no motion anywhere. 4.27 and 4.33, 1 c.c. of ergot in left ear vein 4.35 small gut shows good circular contractions and pendular movements, colon shows travelling peristalsis. 4.39, very strong travelling peristalsis in lower small gut, colon very active. 4.40, 1 c.c. *MgSO<sub>4</sub> 25 per cent in right ear vein.* After injection small gut and colon perfectly quiet, no sign of motion. 4.45, small gut perfectly relaxed, filled partly with yellowish fluid; colon quiet. Respiration good. 4.53, slight movements begin now in small gut; colon quiet.

In the experiments with ergot, in which the intestinal peristalsis was often very active, the inhibitory effect of magnesium was very striking and lasted often from ten to fifteen minutes and even longer.

**Inhibition of peristalsis produced by eserine.**—

*Experiment 6.*—Rabbit, ether. 11.30, abdomen opened in saline bath. 11.37, no movement of gut seen so far. 11.39, 0.2 c.c. of eserine sulphate (0.5 per cent) injected through jugular vein. 11.44, active movements of small gut, colon, stomach, cæcum. 11.47, 0.1 c.c. eserine injected. Powerful contraction of small intestines, colon, and stomach followed the injection. Some convulsions set in; relieved by artificial respiration. 11.49, 1 c.c. of *MgSO<sub>4</sub> 25 per cent injected into the jugular vein.* 11.50, *entire gut relaxed, quiet, no motion.* Artificial respiration stopped, animal

<sup>1</sup> VAN BRAAM HOUCKGEEST: *Archiv für die gesammte Physiologie*, 1872, vi. p. 266

breathes spontaneously. Stomach still shows deep contractions. 11.54, some pendular motion in upper part of small gut. 12.07, lower small gut, ascending colon and cæcum quiet, transverse colon shows fair travelling peristalsis. Injected 0.2 c.c. eserine. Same effect as before. *Injection of 1 c.c. MgSO<sub>4</sub> 25 per cent quieted the animal and relaxed the gut which became perfectly motionless.*

*Experiment 7.*—Rabbit (has diarrhœa), ether. Abdomen opened under saline. Entire intestine shows fair travelling peristalsis. 2.07, injected into jugular 0.2 c.c. eserine (0.5 per cent). Contractions of small gut increased in vigor. . . . 2.30, injected 0.1 c.c. eserine. Tetanic local contractions of gut. Marked tremor of animal. Injected 0.9 c.c. MgSO<sub>4</sub> 25 per cent. Some diminution of tremor and some relaxation of small gut set in, but still some contractions persisted. *Injected a few minutes later again 0.9 c.c. MgSO<sub>4</sub>. The relaxation was now marked and the tremor disappeared.*

In these and other experiments the inhibitory effect upon violent peristaltic or tetanic contractions was beyond doubt.

In these experiments the interesting fact was observed that *magnesium sulphate completely inhibited the general muscular tremor caused by eserine.*

#### **Inhibition of peristalsis produced by barium chloride.—**

*Experiment 8.*—Rabbit, morphine. Abdomen opened under saline. No movements anywhere. 11.12, injected into jugular vein 0.5 c.c.  $\frac{m}{8}$  barium chloride, followed by injection of 1 c.c. saline to wash down contents of cannula. 11.13, powerful travelling constrictions of small gut which repeatedly drove down fluid into cæcum. 11.24, powerful travelling constrictions of cæcum, peristaltic and antiperistaltic waves. Strong waves also in stomach and on part of colon. 11.37, same as before except that small gut shows now only some pendular movements. 11.42, injected 0.5 c.c. BaCl<sub>2</sub>  $\frac{m}{8}$  not followed by saline. At once powerful constrictions swept down the small intestine; colon and small gut blanched, respiration became rapid, and animal shaken by convulsive movements. Artificial respiration given for a few minutes, convulsions disappeared. 11.48, small intestines show good constricting and pendular movements, cæcum and colon show strong peristalsis. 11.52, *injected 1 c.c. magnesium chloride (approximately molecular solution) into jugular vein, not followed by saline. Shortly after movements of entire gut stopped, only very slight swaying of some coil of lower small gut persisted for a while.* 11.54, 1 c.c. of saline injected into cannula of jugular vein to wash down the remaining MgCl<sub>2</sub>. 11.56, no motion anywhere. Respiration became very shallow and artificial respiration given. At 12.20, 0.3 c.c. BaCl<sub>2</sub>

was injected again and followed by saline. This brought on powerful contractions of the intestines which was *completely stopped by injections of magnesium chloride.*

*Experiment 9.* — Rabbit, morphin, abdomen opened under saline. No sign of motion anywhere. 2.32, injected 0.3 c.c.  $\frac{3}{8}$  BaCl<sub>2</sub> into jugular, no saline. Powerful contractions in all parts of the abdominal viscera. About 20 minutes later the vigor of the contractions diminished and the movements were interrupted by periods of rest. 2.56, injected again 0.3 c.c.  $\frac{7}{8}$  BaCl<sub>2</sub>, followed by 1 c.c. of saline. Same powerful effect as before, ascending colon showing much more frequent contractions, cæcum shows powerful travelling constrictions. Small intestines and colon show still good powerful travelling constrictions. 3.24, *injected slowly 1 c.c. MgSO<sub>4</sub> 25 per cent into jugular vein, not followed by saline. Respiration slowed, but artificial respiration not necessary. Movements all over the intestines stopped, small gut relaxed.* 3.27, as before except that ascending colon shows slight travelling ripples. Injected 1 c.c. of saline to *wash down rest of MgSO<sub>4</sub> into circulation. Motion stopped.*

The injection of magnesium sulphate overcame the powerful effects of BaCl<sub>2</sub>, and the inhibition of the intestinal movements took place even when the injection of the magnesium salts was insufficient to embarrass the respiration to any serious degree.

After the above records of the inhibition of such powerful peristaltic movements it is hardly necessary to mention that the intravenous injection of magnesium salts inhibits the normal intestinal movements occurring occasionally spontaneously in the saline bath; or those appearing after the destruction of the cord below the fourth dorsal vertebra. It is perhaps not superfluous to state expressly that the intravenous injection of the magnesium salts inhibits the peristalsis brought on by exposing the coils to the air, since the peristaltic movements here are caused apparently by external, local stimuli of change of temperature, and drying. As to the peristalsis caused by other powerful local stimulation, we cannot make any definite statement as to the effect of an intravenous injection of magnesium salts upon them, since we made no systematic studies on that point. In the few instances in which BaCl<sub>2</sub> or KCl were applied locally to the gut, the intravenous injection of magnesium salts did not abolish the local constrictions. However, it might be only a question of differences in concentrations: the locally applied salts were in molecular solutions, while the magnesium salts reaching the local constrictions by the circulation are obviously greatly diluted by the blood.

We can also report that the post-mortem intestinal peristalsis in animals which received (ante-mortem) intravenously magnesium salts is considerably less in evidence than in normal animals. We have observed it incidentally in studying the effects of intravenous injections of these salts on vital processes, and we have seen it also in a special study in which the post-mortem peristalsis of animals killed by magnesium salts was compared with that of animals killed by calcium salts. The latter subject will be dealt with later in conjunction with other problems in another paper.

Finally, we should mention especially that the intravenous injections of magnesium salts inhibit not only the peristaltic movements of the small intestines, but also the movements of the other parts of the gastro-intestinal canal, when present, namely, the movements of the stomach, the cæcum, and the entire colon. On account of the occasional great distension by gas, the movements of the descending colon are frequently difficult to subdue.

#### SUMMARY.

Subcutaneous and intravenous injections of magnesium salts produce neither purgation nor intestinal peristalsis. The purgative action of these salts is therefore no evidence for their stimulating effect.

The moderate peristalsis produced by exposing the intestines to air, by destroying the dorsal cord or by intravenous or subcutaneous injections of certain saline purgatives, and the powerful peristaltic constrictions produced in all parts of the gastro-intestinal canal by intravenous injections of ergot, eserin, or barium chloride can be completely inhibited by an intravenous injection of magnesium sulphate or chloride in doses insufficient to embarrass the respiration to any serious degree.

The post-mortem peristalsis of animals which received intravenous injections of magnesium salts is considerably less in evidence than that of normal animals.

As an additional fact, which was observed in these experiments, may be appended here that the intravenous injection of magnesium salts inhibits the muscular tremor produced by intravenous injections of physostigmine.

## AN IMPROVED OPERATIVE METHOD OF FORMING AN EXPERIMENTAL ACCESSORY (PAWLOW) STOMACH IN THE DOG.

By JOHN C. HEMMETER.

[*Baltimore.*]

**I**N his article on the Physiological Surgery of the Digestive Tract, J. P. Pawlow,<sup>1</sup> after considering the advantages and disadvantages of previous experiments upon the stomach, gives a description of two new operative methods. The main object of Pawlow's new operation is to secure gastric juice for physiological purposes by preparing an accessory stomach in such a way that the secretory fibres of the organ shall not be injured, that the juice can be obtained in a pure state, that is, without admixture of food, and yet the glandular apparatus be stimulated from the interior surface of the gastric mucosa as it is under normal conditions.

This operation has proved very difficult even in the hands of skilled abdominal surgeons, and when performed under perfect aseptic technique. The animals do not, as a rule, die from infection; they seem to die from the prolonged etherization. The object of the reporter was to devise an operation accomplishing the same purposes as that of Pawlow, and yet capable of a more rapid execution because of greater simplicity in plan. An incision is made almost along the same line as the original incision of Pawlow, but the object of this incision is not to divide the stomach into two parts, for it is only carried through the anterior wall of the stomach. (Pawlow's incision goes through the anterior and posterior wall.) The object of my incision is simply to enable the operator to push the mucosa of the stomach out through the line *AB* (Fig. 1) by invaginating the fundus or greater curvature through it. Next an incision is made only through the mucosa in a semicircular way, from the greater curvature at *C* to the greater curvature at *D*, going about as high as the lower third

<sup>1</sup> PAWLOW: *Ergebnisse der Physiologie*, Erster Jahrgang, 1 Abt. p. 258.

of the stomach, or one-third of the distance between the greater and lesser curvature, along the line *F E G* (Figs. 3 and 4). The

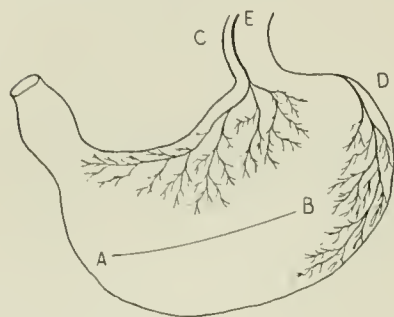


FIGURE 1.—*A B*, line of Pawlow first incision. *C*, vagus and anterior gastric plexus. *D*, vagus and posterior gastric plexus. *E*, esophagus.

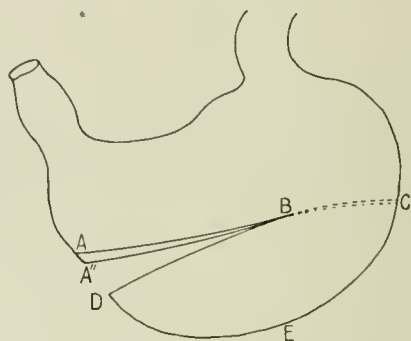


FIGURE 2.—Effect and appearance after first incision according to Pawlow. The lines *AB*, *A'B*, and *DB* are closed by sutures. The accessory stomach is made out of the part enclosed by letters *BC*, *DE*. The stomach is made into two compartments by sewing together two layers of mucosa after they are dissected loose — along the dotted line *BC*.

incision goes through the mucosa only; the mucosa then is very slightly dissected off on either side of the incision not more than is necessary in order to catch hold of it with the forceps, for the pur-

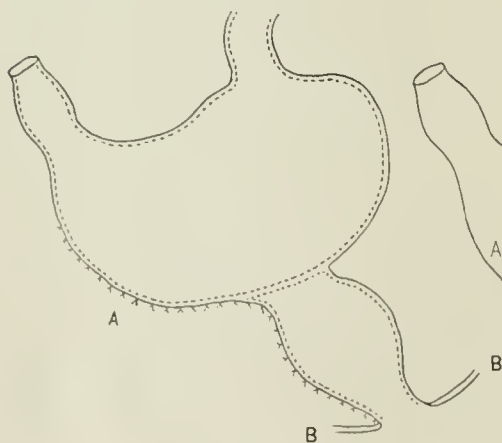


FIGURE 3. Pawlow's origin operation.—*A*, Line of sutures. *B*, abdominal wall. The dotted lines represent mucosa.

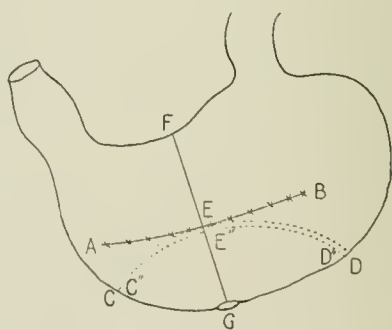


FIGURE 4.



pose of getting sutures through the cut ends of the mucosa. The incision is made both on the anterior and posterior walls of the stomach. As far as possible the incisions must be parallel to each other, so that when the semicircular incision on the anterior wall is approximated to that on the posterior wall of the stomach, they coincide exactly. These two incisions are next united by silk sutures beginning at the point *C* on the greater curvature, and making sure

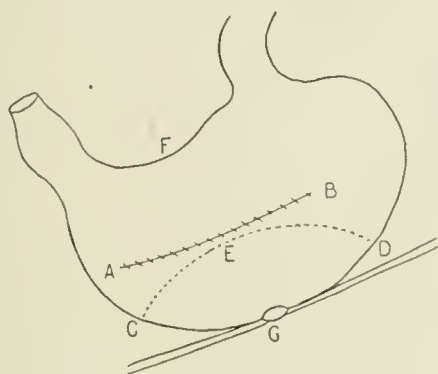


FIGURE 5.—*G*, fistula on anterior abdominal wall.

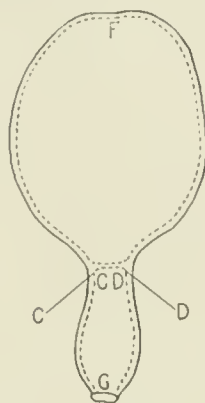


FIGURE 6.

that the angle of the pouch is tightly walled off from the greater part of the stomach by the sutures at this point *C*. The sutures are then carried along the semicircular incision from *C* to *E* to *D*. In this way the anterior and posterior walls are united by an inner row of silk sutures, which are inserted no deeper than the muscularis mucosæ. This has to be done by pushing the part of the greater curvature (*C E D* in Fig. 4) through the incision *A B*. When the anterior and posterior gastric walls are thus united along the lines *C E D* and *C'' E'' D''*, a circular pouch is formed (*C E D G*, in Fig. 4), separating this part of the stomach from the rest. Next the opening in the anterior gastric wall along the line *A B* is closed by sutures, a fistulous opening is made at *G*, and this point attached to the external abdominal wall as in Pawlow's method. On cross section of the stomach along the line *F G* (Fig. 4) the appearance of the main and accessory stomach when viewed from the fundus would be as represented in Fig. 6. The question might be asked, what becomes of that part of the large incision through the mucosa which faces the smaller or accessory stomach along *C* to *E* to *D* on

the inner side of the accessory stomach, because no mention has been made that this is closed by sutures. In most of my animals I have permitted it to take care of itself, for it heals within eight to ten days, as subsequent opening of the gastric cavity at this point has proved. The secretory fibres of the vagus are not injured in this operation, which is simpler of execution, requires less time than the Pawlow operation, and accomplishes all that this operation aims at.

A difficulty met with constantly in all animals thus operated on is the erosion and autodigestion of the skin around the abdominal opening. This untoward complication is due to the proteolytic effect of the gastric juice and to pressure by the rubber tube or cannula used to establish an outlet from the experimental accessory stomach. If the dog is permitted to lie down, there will be still larger erosions, because the abdominal integument comes to rest in a pool of escaped gastric juice.

Two things are of great assistance in this difficulty: one is the support of the animal by two broad bandages passed under the thorax anterior to the fistula and under the abdomen posterior to the same, fixing the dog to an upright holder so that he cannot lie down. The animal becomes reconciled to the holder and bandages in a few days and learns to rest and sleep in this fixture. If other animals are in the same room, the dog operated on must be blindfolded, because the secretion of gastric juice is notably influenced by psychic processes, caused by actions of the experimenter and the behavior of other animals.

Another helpful factor is the dressing of the integument around the abdominal wound during the entire time in which the animal is under actual observation, that is, during the hours when juice is collected and even during the hours of rest. The main object of this dressing is to render the gastric juice inert and at the same time to protect the surface of the skin. After testing a number of substances as dressing powders I finally settled on simple zinc oxide made alkaline with sodium bicarbonate,—about one part of sodium bicarbonate to five parts of zinc oxide. During the hours of rest this powder is applied liberally all around the cannula or rubber discharge tube. But during the hours of collection of juice care must be had lest some of the alkaline powder fall into the collecting bottles and neutralize the juice. It had best be wiped off by a little absorbent cotton before the bottle is applied. While the juice is being collected it rarely spreads to the surrounding integu-

ment and the dressing is not so much needed then. It is in the intervals between the periods of actual collection and study that the oozing of gastric juice causes the cutaneous erosion. In sewing the experimental accessory stomach to the abdominal integument, the gastric juice at times penetrates along the silk sutures into the depths of the skin. All these stitches must therefore be sealed by an alkaline collodion reapplied daily, and no experimental work undertaken until healing is complete. The manner of feeding the animal during this period is described in Pawlow's original publication.<sup>1</sup>

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

# THE RELATION OF OPTICAL STIMULI TO RHEOTAXIS IN THE AMERICAN LOBSTER, HOMARUS AMERICANUS.

By PHILIP B. HADLEY.

[From the Biological Laboratory of Brown University and the Experiment Station of  
the Rhode Island Fish Commission.]

## I. INTRODUCTION.

THE older theories regarding the cause of the orientation of animals in currents of water and air have taken into account only the possible factor of pressure. The most important generalizations upon this subject are from Verworn and Rádl, and are mentioned by Lyon<sup>1</sup> in a paper whose object is to introduce, as a possible cause of the rheotactic reaction in some animals, three factors: a true sense of the unequal pressure in different portions of the environment; a certain variety of contact irritability; and a purely optical stimulus.

The older conception of rheotaxis as given by Rádl may be seen in the following quotation from his work, "Untersuchungen über den Phototropismus der Tiere," mentioned by Lyon. Speaking of the Epheméridæ, Rádl says:

"They react very sharply to a gentle current of air, turning the head toward the current of air, but without leaving the place in which they are floating. The air current acts by exerting pressure on the body surface of the animals, and evidently tends to draw the body into the direction taken by it. On the contrary, the fluttering wings strive to lift the body upward and forward. The points of application of this force, lifting the body, and the force of the air current need be only very slightly separated; and a force couple will result that will rotate the body until the two forces, the muscle force and the force of the air current, draw the body in opposite directions. . . .

. "It is easy to pass from the rheotropism of the Epheméridæ to other

<sup>1</sup> LYON: This journal, 1904, xii, p. 149.

analogous phenomena of rheotropism. It will everywhere be found that the pressure of the air currents acts as one force, and contraction of the muscles as the other."

Some writers, as Verworn, make use of the collective term barotaxis, and include under this head stereotropism, geotropism, and rheotropism, assuming that all the reactions included in these groups are the result of pressure. Rheotaxis, according to Verworn's definition, is that form of barotaxis "in which the stimulus is produced by a gentle current of flowing water." "This," Verworn continues, "is the peculiarity belonging to certain organisms of taking toward flowing water a direction of motion opposed to the direction of the current. Since these organisms thus turn toward a pressure stimulus, rheotaxis is merely a special form of positive barotaxis."

With such a criterion of rheotaxis in mind, this phenomenon has been attributed to the behavior of many different kinds of animals. Wheeler<sup>1</sup> finds, in the orientation of animals flying in the wind, a certain form of rheotropism which he calls anemotropism, and which he discusses as follows:

"It requires but a moment's consideration to see that anemotropism is only a special form of rheotropism. . . . The only difference lies in the fact that the insect reacts to a gaseous, the fish and myxomycete to a liquid current. In both cases the organism naturally assumes the position in which the pressure exerted on its surface is symmetrically distributed, and can be overcome by a perfectly symmetrical action of the musculature of the right and left sides of the body.

Such explanations of rheotropism as these which have been mentioned bring it at once into the field of the motor reflex theory upon which rests the theory of tropisms as it is still held by many investigators. According to this view, certain reactions in animals result from the unequal stimulation of opposite sides of the body, and produce, in consequence, a turning either in one direction or the other. To quote again from Verworn:<sup>2</sup>

"Thus the phenomena of positive and negative chemotaxis, barotaxis, thermotaxis, phototaxis, and galvanotaxis, which are all so interesting and important in all our organic life, follow with mechanical necessity as the simple results of differences in biotonus which are produced by the action of stimuli at two different poles of the animal cell."

<sup>1</sup> WHEELER: *Archiv für Entwicklungsmechanik*, 1899, viii, p. 373.

<sup>2</sup> VERWORN: *General physiology*, 1899, p. 503.

And again :

" They [the tropisms in general] depend, first, upon the specific irritability of certain elements of the body surface; and, second, upon the relation of symmetry of the body. Symmetrical elements at the surface of the body have the same irritability; unsymmetrical elements have a different irritability. . . . These circumstances force an animal to orient itself towards a source of stimulus in such a way that the symmetrical points on the surface of the body are stimulated equally. In this way the animals are led, without will of their own, either toward the source of the stimulus or away from it."

A certain departure from the view of rheotaxis which these theories of the tropisms demand, has been made by Lyon, who suggests that the rheotactic responses of many kinds of animals may be partly due to optical stimuli. His conclusions in the matter may be stated as follows :

1. In rheotropism the chief element of stimulation is the environment, not the current. The current is responsible for the orientation only in so far as it causes a relative motion between the fish and its environment.

2. Contact between fishes and stationary objects in their environment may produce an orientation. This, however, is due to the action of the current in sweeping the fishes past stationary objects.

3. Relative velocities of the medium in different parts of the environment may cause orientation. Here the case is similar to the above; for, if a part of the medium moves while another part is at rest, the sensation to the fish is probably similar to that derived from the contact with solid objects.

Having now examined these sources, we may assume that rheotaxis is a form of behavior found in very many animals, and is, in all cases, in some way connected with the movement of an environment with relation to the position of the animal in that environment. At the present stage of our inquiry we cannot vouch for more than this fact. Thus, without entering into a further discussion of this point, it is the purpose of the writer to introduce the fact that certain of the free-swimming stages of *Homarus* manifest the phenomenon of rheotaxis in a high degree, and to present the records of a few experiments which show to some extent the relation of optical stimuli to this mode of behavior.

## II. PROCEDURE.

**The normal swimming of the lobster.** — It may be of some advantage to consider, first, the methods of swimming of the lobster in its early stages of development. On a basis of morphology and behavior alone, the life of the lobster may be divided into three periods: (a) the first three stage periods (the larval stages); (b) the fourth stage period, which is the characteristic free-swimming period; (c) all later stages. During the first period the swimming apparatus consists of the exopodites of the thoracic appendages which beat the water with short vibratory strokes. If the appendages are extended (forward), the stroke of the exopodites becomes forward and downward, and the resulting motion of the larval lobster is upward and backward. When, however, the thoracic appendages are contracted (directed posteriorly),<sup>1</sup> the resulting motion of the young lobster is forward and upward. The progressive movement of the lobster in the first three stages is almost wholly dependent upon these two methods of swimming, together with their concomitant variations, which are more or less subservient to varying conditions of temperature, light, and other environmental factors. Bodily conditions (physiological states), so far as they influence the manner of voluntary swimming, are not of consequence at this period of the lobster's life; and for this reason no evidence of rheotaxis is observable in the behavior of these stages in the life of *Homarus*.

When, on the other hand, we come to consider the behavior of the fourth stage lobster, the condition is different. The swimming appendages, characteristic of the first three larval stages, have been lost in the recent molt. The swimmerets, however, on the under side of the abdominal segments, are now functional in a high degree, and upon their use is dependent the forward swimming of the lobster in this stage. He now swims with directness and purpose, usually near the surface of the water, — a phenomenon which may be determined by the influence of light, or, more probably, by a food-seeking impulse.<sup>2</sup>

<sup>1</sup> The writer hopes at a later date to consider the relation between the phototactic responses of the larval lobster and the degree of extension or flexion of the thoracic appendages.

<sup>2</sup> In a series of experiments the full results of which will be published at a later date, the writer has learned that, if clam juice be introduced through a glass tube into the bottom of a cylinder where a group of fourth stage lobsters are resting, there is an immediate flocking to the surface, where active swimming is maintained for some moments, in spite of the fact that there is no trace of clam juice or other stimulating agent in this area.

**Preliminary observations on rheotaxis.**—When the writer's attention was first directed to this subject by Dr. A. D. Mead at the Wickford Hatchery of the Rhode Island Fish Commission, it was apparent that a very slight current of water in the large hatching-bags would initiate a marked rheotactic reaction on the part of the fourth stage lobsters, and, further, that this response appeared to be more definite by night than by day. It was further noted that this rheotactic reaction was more strongly evinced in the earlier part of the fourth stage period, and that in the later part of this period and in the succeeding fifth stage the bottom-seeking tendency on the part of the confined lobsters often obscured further manifestation of the rheotactic phenomenon. A favorable opportunity was soon presented at the Wickford Experiment Station to study the rheotactic proclivity of large numbers of lobsters, in all stages, in the large canvas hatching-bags where they were confined. In these bags, which were twelve feet square and about four feet deep, the movement of a paddle, revolving on a shaft at the bottom of the bag, served to create a constant circular current in a clockwise direction. In many of these bags in which the eggs were hatched, the young lobsters were raised to the fourth or sometimes to the fifth stage; and in all these series there could be observed the modification of the swimming process as the young lobsters slowly passed on from stage to stage. In the first three stages the swimming was always uncertain and desultory, though in the third stage lobster there could often be observed a slight tendency to retain better the equilibrium in the water.

By the time that the first few individuals had molted into the fourth stage, however, one could observe distinctly the contrast between the methods of swimming in the third and fourth stage lobsters. While the third stage individuals were borne along unresistingly in the strong circular current, the fourth stage lobsters, on the other hand (even within a minute after molting into this stage), would turn directly, head to the current, and maintain their swimming in this direction so actively that, if the current was not too strong, they would often make some headway against it. This characteristic manner of swimming was evinced in an ever-increasing number of lobsters, until the whole body of them had passed into the fourth stage; and then it was a most interesting sight to observe the young animals, with hardly an exception, heading into the current, and, as one great phalanx, following their circular course,—but, because of the force of the current, backwards.



In this instance, just as in the case of fishes swimming in the current of a stream, we find the lobsters themselves suspended in a body of water which is moving at a certain rate and in a circular course about the central paddle shaft. The question here arises, Is the resulting orientation of the lobsters the result of the pressure of the water? The discussion of this question may for the present be deferred, for we are not now able to state to just what extent water pressure may be responsible for the orientation of the lobsters in the large hatching-bags. We may, however, call further attention to some of the actual conditions of the manifestation of this phenomenon. It can be noted that the rheotactic response is observable both by night and day, but is possibly more definite at night (a fact which, to say the least, does not favor the view that the reaction is due to an optical stimulus). Moreover, when the reaction is temporarily interrupted by the introduction of obstructions, or by creating other currents in the bag, the rheotactic response is manifested again directly, as the cause of the interruption has ceased and the primary current again comes into force. It may be noted, further, that light under certain conditions may modify in a great degree the nature of the rheotactic response. In daylight one could observe that the definiteness of the reaction was greater when, in the course of the revolutions about the central shaft, the lobsters came into a shaded area, as, for instance, the shadow of the gearing or of the framework of planks surrounding the bags. In passing suddenly from such a shaded area into the bright sunlight (and especially if the head be turned in this direction), the reaction might be momentarily lost until the lobsters had passed on into another shadow, or had come into such a position that their heads were no longer directed toward the brightest light.

These observations, made upon the lobsters in daylight, were further supplemented by others made during the twilight and darkness, when, no matter what traces of desultory swimming might have been evinced during the day, the rheotactic reaction was most definitely manifested. It was furthermore determined that, just as the definiteness of the rheotactic reaction during the daytime might be lost temporarily by swimming from shadow into full light, so might the reaction at night be modified or totally annihilated by introducing, at various angles to the current, the intense rays from an acetylene light. No matter at what angle the pencil of rays was introduced, one result followed: the annihilation of the rheotactic reaction as a

result of the effort to swim in the direction of the most intense rays of light.<sup>1</sup>

Somewhat later, as the group of fourth stage lobsters molt into the fifth stage, the definiteness of the rheotactic response is diminished until at last, when the fifth stage has been reached by all the individuals, there are none left to manifest any swimming tendency whatever. Thus there comes and disappears in the life of a lobster the "swimming stage," and with it the very definite rheotactic reaction.

It was these observations that led the writer to make some attempt to discover whether or not an optical stimulus might produce the so-called rheotactic response in the fourth and later stage lobsters.

**Apparatus.** — The apparatus for the experiments consisted of (1) a glass dish for confining the lobsters, and (2) a mechanical device for rotating a visual field, or, as we shall hereafter speak of it, the "environment," around the lobsters, which were placed in the dish men-

<sup>1</sup> Thus, as the writer has explained in another paper (HADLEY: Rhode Island Fish Commission Bulletin No. 25, 1906; also, *Science*, 1905, xxii, p. 675), we may observe the following modifications in the rheotactic reactions of the fourth stage lobsters, due to the direction in which the light rays strike the water-course in which the animals were swimming:

1. If there is no current in the bag, and the pencil of rays is introduced vertically into the water, the lobsters gather in the illuminated area and tend to swim in the direction of the light rays toward the source of illumination. (The fifth or later stage lobsters, and sometimes even the late fourth stage, will not give this or the following reactions.)
2. If the current is in motion, and the pencil of light is introduced at right angles to the direction of the current, the lobsters tend to swim in tangential lines to the current, or so that they move as nearly as possible in the direction of the incident rays of light.
3. If the pencil of light is so introduced that the rays stream opposite to the direction of the current, the definiteness of the rheotactic response is, if possible, accentuated.
4. If the pencil of light is so introduced that the rays stream in the same direction as (or tangent to) the direction of the current, the rheotactic reaction in the illuminated area is broken because of the fact that the lobsters turn in the current, swim with it, and tend to approach the source of illumination; although, in so doing, the force of the current of water quickly sweeps them past the illuminated area.
5. If the source of the rays be gradually withdrawn from the bag containing the lobsters, in a "serpentine curve," in such a manner that the direction of the rays is constantly changing, but at every point is tangent to the said curve, the lobsters may be made to follow in the same "serpentine" path, at the same time quite losing their orientation to the directive influence of the current.

tioned above. In order to keep the lobsters near the margin of the dish, where they might be more readily influenced by the moving environment, a second, smaller dish, filled with sand, was placed within the first, thus forming a ring of the outer portion of the larger receptacle. In this ring the lobsters might travel in either direction, — clockwise or counter-clockwise (Figs. 1 and 2).

The device used for rotating an environment about the lobsters, confined as described above, consisted of a modified kymograph, so arranged that the speed could be regulated at will. For the environment,

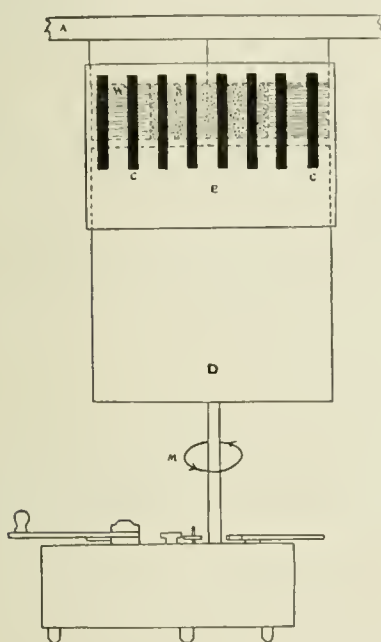


FIGURE 1.—Side view of device used for rotating an environment around the lobsters. *A*, rod from which glass dish is suspended; *H'* represents section of circular path about inner glass dish; *S* represents section of glass dish containing sand, placed within the larger dish; *E* represents the cardboard cylinder placed over the drum (*D*), and containing the "windows" (*C*). *M* represents the direction of rotation of the environment.

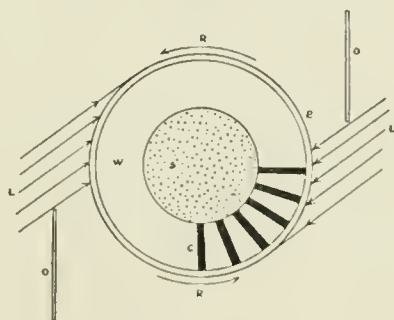


FIGURE 2.—Top view of the device used for rotating an environment around the lobsters. *E* represents the outer cylinder of cardboard (the environment); *H'* represents the circular path around the inner glass dish (*S*) filled with sand; *C* represents the "windows" in the cardboard disk underlying the bottom of the larger glass dish; *L* represents the direction of the incident light rays striking the dish; *O* represents the obstructing objects which determine the direction of the light striking the dish; *A'* represents the direction of the rotation of the environment.

a cylinder of cardboard, containing at the upper end longitudinal slits ("windows"), was placed over the drum so that it would revolve with it; this cylinder also projected two inches beyond the top of the drum so that within this projecting portion might be

suspended the dish containing the lobsters. This dish was so hung that the bottom was one-eighth of an inch above the top of the drum, the projecting cardboard extending a slight distance above the top of the dish (Fig. 2). Furthermore, a disk of cardboard, slitted radially, was placed over the top of the drum and beneath the bottom of the suspended dish, in order to afford a moving environment beneath the lobsters.

By means of this contrivance it is clear that, when the drum was set in motion, the cardboard cylinder surrounding the suspended dish would rotate; and likewise the card disk beneath the dish. Thus, from the standpoint of the lobsters, although the water in which they were swimming was absolutely motionless, the environment on both the side and bottom would be continually moving in the direction of the rotating drum, — in this case, counter-clockwise.

**Method of recording results.** — In the greater number of experiments, the reaction of 5 or 10 individuals was tested at the same time. These lobsters were placed in the circumscribed area of the dish and this dish placed directly over the drum and within the cardboard cylinder. The lobsters were then allowed ten or fifteen minutes to become acquainted with their new surroundings. After this period of time readings were taken to determine whether, when the environment was not in motion, there was any appreciable tendency for the confined lobsters to travel to a greater extent in a clockwise or in a counter-clockwise direction around the central dish. These records were taken at the end of each minute for a period of eight minutes. The first preliminary tests, while no drum was in motion, demonstrated at once that there existed an unexplainable tendency for the lobsters to travel around the central dish in the clockwise direction. Forty-eight readings in six sets of experiments, each set involving the use of from 5 to 10 lobsters, showed that while 137 individuals moved in a counter-clockwise direction, 213 moved clockwise. When it was attempted to discover the reason for this tendency, manifested even before the drum was set in motion, it transpired that the directive influence of the light coming from the windows in the room where the experiments were being performed, was responsible for this behavior. It appeared that the rays of light coming from the two windows on opposite sides of the dish, because of interposed objects, struck the dish in tangential lines on opposite sides (Fig. 2); so that on one side of the circular compartment the rays came from the left, while on the side diametrically opposite, they

came from the right. Thus the direction of the light stimulus resulted in determining the direction of movement of the young lobsters (at all times away from the source of light), and compelled them to travel in a clockwise path about the inner dish. Since it was not possible at the time of experimentation, because of the position of the windows, to produce a uniform lighting on all sides of the lobsters, it was deemed best to allow the conditions to remain as they were in these preliminary tests. For this reason, if an optical stimulus, caused by the rotating environment, should have any directive influence upon the movement of the young lobsters, the resulting reaction would represent not only an overcoming of the natural tendency to remain at rest, but also a reversal of the tendency, already in effect, to travel in the opposite direction from that of the moving environment. These conditions prevailed during the course of the experiments which are now to be described.

### III. EXPERIMENTS.

In the first experiments wherein the rotating environment was brought to bear upon the fourth and fifth stage lobsters, it was demonstrated that, just as the influence of light may have both a kinetic and a directive effect upon the behavior of the young lobster, in a like manner might the rotating environment be effective through both its kinetic and its directive influence. In case the lobsters are at rest at the beginning of the experiment, the first response to the rotation of the environment is a movement in *some* direction,—the result of the kinetic effect. Gradually, however, the directive influence makes itself felt; the young lobsters respond accordingly, and manifest a tendency to move *in the same direction as the moving environment*.

In all, seven sets of lobsters which were used for the experiments are here to be recorded. These are sufficient to demonstrate the general drift of the results. Of these seven sets three were of 10 lobsters each, the others of 5 lobsters each. Each set was given eight successive tests, one minute apart. Besides these there were other experiments in which the immediate physical environment was modified by placing sand on the bottom of the dish where the lobsters were confined; and other experiments in which blinded lobsters were employed. Either 5 or 10 lobsters were generally used for experiment, for so small a number scattered over the field could be taken in at a glance; and, furthermore, this number seemed sufficiently

large to give representative results. In all the sets of experiments, moreover, the first direction of movement, while the drum was not in motion, was tested. The results in all the tests, as will be observed in the following record of experiments, agree with great uniformity.

## FIRST SERIES.

TEN INDIVIDUALS; RAPIDLY MOVING DRUM.			
Drum not in motion.		Drum in motion.	
Counter clockwise.	Clockwise.	Counter clockwise.	Clockwise.
4	6	8	2
4	6	9	1
5	5	6	4
3	7	5	5
3	7	7	3
3	7	5	5
5	5	6	4
4	6	4	6
31	49	50	30

In the series of experiments recorded above, it is evident that the tendency for the lobsters to move in the clockwise direction when the drum is not in motion has, by bringing into effect a rotation of the environment in the opposite direction (counter-clockwise), brought about a reversal in the direction of the progressive movement, so that finally many of the lobsters come to travel in the same direction as that of the moving environment. The remaining individuals may be either at rest or travelling in the opposite direction.

In the second series of experiments the speed of the drum was diminished from 42 revolutions per minute to 30 revolutions. The result was similar to that obtained in the first series, save that a proportionately greater number of lobsters in the second series moved in the direction of the rotating drum, *i. e.*, counter-clockwise. In view of these results, in all the later experiments, with one exception, the slow-speed drum was employed.

SECOND SERIES.

TEN INDIVIDUALS ; SLOWLY MOVING DRUM.			
Drum not in motion.		Drum in motion.	
Counter clockwise.	Clockwise.	Counter clockwise.	Clockwise.
5	5	8	2
4	6	7	3
5	5	6	4
5	5	10	0
3	7	9	1
4	6	7	3
5	5	8	2
6	4	9	1
37	43	64	16

THIRD SERIES.

TEN INDIVIDUALS ; VERY SLOWLY MOVING DRUM.			
Drum not in motion.		Drum in motion.	
Counter clockwise.	Clockwise.	Counter clockwise.	Clockwise.
2	8	4	6
4	6	7	3
2	8	6	4
3	7	8	2
5	5	7	3
4	6	7	3
4	6	6	4
5	5	7	3
29	51	52	28

In this series of experiments the conditions were still further modified by diminishing the speed of the drum to 20 revolutions per minute. The results obtained coincided more closely with those obtained in the case of the rapidly moving drum; *i. e.*, a smaller number of lobsters moved in the direction of the moving environment. In spite of the slight differences which are to be observed in these

## FOURTH SERIES.

FIVE FIFTH STAGE LOBSTERS; SLOWLY MOVING DRUM.			
Drum not in motion.		Drum in motion.	
Counter clockwise.	Clockwise.	Counter clockwise.	Clockwise.
1	4	3	2
4	1	2	3
2	3	3	2
0	5	2	3
0	5	3	2
2	3	3	2
3	2	2	3
1	4	3	2
13	27	21	19

three series, the conclusion which we must draw from the series as a whole is that the fourth stage lobsters may manifest a so-called rheotactic reaction as the result of an optical stimulus.

In the experiments next to be recorded (Series IV, V, VI), instead of using the fourth stage lobsters, individuals which had molted into the fifth stage were selected. It may be appropriate to note at this time that the behavior of the fifth stage lobster differs, in some respects, from that of the fourth stage. While the fourth stage is typically a swimming animal, and only under especially favorable conditions manifests any tendency to burrow, the fifth stage lobster, on the other hand, possesses the hiding and burrowing habit well developed, and instead of swimming actively at the surface of the water, as does the



fourth stage lobster under most circumstances, he retreats to the depths and attempts to hide in some sheltered corner. Quite in agreement with these modes of behavior is the fact that the response of the late fourth stage lobsters to the moving environment was never so definite as the reaction of the early fourth stage lobsters; and like-

FIFTH SERIES.

FIVE FIFTH STAGE LOBSTERS; SLOWLY MOVING DRUM.			
Drum not in motion.		Drum in motion.	
Counter clockwise.	Clockwise.	Counter clockwise.	Clockwise.
2	3	3	2
2	3	1	4
2	3	4	1
2	3	3	2
1	4	3	2
0	5	4	1
1	4	2	3
2	3	4	1
12	28	24	16

wise the fact that the reaction of the fifth stage lobsters was less definite than either, especially when the bottom of the glass dish containing the lobsters was partly covered with sand or gravel. Such a condition appeared to annihilate whatever tendency to wander about remained in the fifth stage lobsters, and to call forth a certain kind of contact irritability which is observable in many phases of the lobster's behavior when he is in the latter part of, or past, the fourth stage.

In the fourth and fifth series of tests, mentioned above, the results show that, to a less degree than in all previous cases, has the effect of the moving environment been able to overcome the natural tendency on the part of the fifth stage lobsters to avoid the light, or to cause them to move in the counter-clockwise direction. For this

reason we cannot interpret the actual degree of the rheotactic response by the ratios, 21:19, 24:16; for in all these cases there was, as has been said, a conflict between the tendency to move with the rotating environment and the tendency to move away from the source of light; and this holds true, not for the fifth series alone, but for all other series as well.

## SIXTH SERIES.

FIVE EARLY FIFTH STAGE LOBSTERS; SLOWLY MOVING DRUM.			
Drum not in motion.		Drum in motion.	
Counter clockwise.	Clockwise.	Counter clockwise.	Clockwise.
3	2	3	2
3	2	1	4
3	2	1	4
3	2	2	3
1	4	2	3
0	5	1	4
0	5	4	1
2	3	2	3
15	25	16	24

Under the conditions of a weak light stimulus, the tendency to keep pace with the moving environment prevailed; but when the intensity of the light (striking the dish in a tangential clockwise direction) was increased, then the directive influence of the light was sufficiently powerful to overcome the possible reaction to the moving environment. Taken together, the fourth and fifth series of experiments demonstrate the fact that, while under the conditions of a stationary environment the tendency to avoid the light and to travel in a clockwise direction is relatively stronger than in the fourth stage, still the tendency to move with the moving environment, though effective in some degree, is less so than in the lobsters of the fourth stage.

In the sixth and seventh series of tests one change was made in the nature of the experiment. While in Series I to V the bottom of the dish in which the lobsters were confined consisted of clear glass over which the lobsters crawled, in Series VI and VII, on the other hand, the bottom of the dish was thinly covered with a layer of sand in such quantity as to furnish footing for the crawling

SEVENTH SERIES.

FIVE LATE FIFTH STAGE LOBSTERS; SLOWLY MOVING DRUM.		
Drum not in motion.	Drum in motion.	
	Counter clockwise.	Clockwise.
The lobsters are resting quietly on the bottom, headed, for the most part, in the clockwise direction; some individuals occasionally turn and face in the opposite direction.	3	2
	3	2
	1	4
	1	4
	2	3
	2	3
	3	2
	3	2
	18	22

lobsters, and at the same time not to inhibit the effect of the moving disk beneath the glass bottom. The results here found are indicative of the fact that there is no marked difference between the behavior of the fifth stage lobsters in a quiet environment and the same lobsters subjected to a moving environment, when in both cases the bottom of the dish has been sanded. In this series of experiments, when the drum was not in motion, the lobsters were for the most part resting on the bottom of the dish or moving slowly in the clockwise direction, — although some of them would occasionally turn and face or travel in the counter-clockwise direction. When the environment was rotated, it was first the kinetic effect that influenced the lobsters; and as a result, they would begin to move in one

direction or the other. At no time, however, did the majority, in any one series of experiments, move in the counter-clockwise direction; and in the series recorded above (seventh series), it appears that the greater number travelled in the clockwise direction. Thus we may assume that when sand is placed on the bottom of the dish, there is produced (always in the case of the fifth stage lobsters and sometimes in the fourth stage) a kind of contact irritability which modifies the otherwise more or less definite tendency to be influenced by the moving environment. Lyon has assumed that, in the case of fishes, under similar experimental conditions, contact between them and points in their immediate and motionless environment may serve to correct the false, visual impression gained from the movement of the outside environment. It would appear that this explanation may also partly account for the fact that the definiteness of the rheotactic reaction is gradually diminished as the lobsters pass on through the fourth, fifth, and later stages; for during these successive periods contact irritability is constantly becoming of more importance as a factor which modifies the behavior of the young lobster. And yet, on the other hand, this contact irritability does not wholly correct the false impression gained from the motion of the environment; for we have seen that in Series VII a certain proportion of the lobsters which formerly were headed in the clockwise direction came either to head or to move in the opposite direction, although the orientation of the majority was not changed.

By the time the lobster has entered the sixth stage the bottom-seeking habit has become so well grounded that the free swimming, so characteristic of the fourth stage lobster, is rarely indulged in; and in consequence, although the lobsters are strongly influenced by the rays of light, the motion of the environment no longer has the power to stimulate a movement in the counter-clockwise direction; and it may, moreover, even give, through its purely kinetic effect, an initiatory stimulus which results in a movement in the clockwise direction. It is perhaps unnecessary to state that in the case of the blinded lobsters no reaction took place, either to the movement of the environment or to the incident rays of light.

#### IV. SUMMARY.

I. Under proper conditions of stimulation, rheotaxis is shown in a marked degree in the behavior of *Homarus*, and is first and most

strongly evinced in the fourth stage in the lobster's development. As contact irritability assumes greater importance in determining the behavior of the lobster beyond the fourth stage, rheotaxis is gradually lost.

2. The rotation of an environment, at an optimum rate, about a dish containing fourth or fifth stage lobsters, may have two effects: (1) the kinetic effect, to stimulate the lobster to some reaction; (2) the directive effect to cause the lobster to move in the direction of the moving environment. In other words, we may say that the so-called rheotactic response in *Homarus* may be initiated as the result of a purely optical stimulus. (This does not, however, preclude the possible effect of pressure or contact irritability, which may, in some cases, be operative in bringing about the final orientation of the lobster.)

3. The manifestation of the rheotactic reaction which would otherwise occur may be prevented, modified, or wholly annihilated by introducing other stimuli such as light, changes in the physical environment, etc. If the light be weak, the tendency to manifest a rheotactic reaction may overcome the tendency to respond to light. If the light be intense, or introduced suddenly, the rheotactic response may give way to the phototactic.

## THE MAINTENANCE OF CEREBRAL ACTIVITY IN MAMMALS BY ARTIFICIAL CIRCULATION.

BY C. C. GUTHRIE, F. H. PIKE, AND G. N. STEWART.

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

**I**N a former paper we<sup>1</sup> endeavored to fix the limits within which the central nervous system of mammals may, after temporary anæmia produced by ligation of the head arteries and consequent loss of function, be restored to activity when the ligatures are removed.

The experiments reported in the present paper were begun as a preliminary series to those already published. One object was to determine whether any solution other than the animal's own blood would sustain any degree of cerebral activity. After working out the limits within which the normal circulation of the animal's own blood was effective, it became an interesting problem to investigate the maintenance of activity or the resuscitation of the brain of a decapitated animal by circulating through it, under the best possible conditions, the blood of another animal of the same species. The best possible conditions were considered to be fulfilled when the cerebral vessels of the decapitated head were anastomosed by suture directly to the corresponding vessels of another animal of the same species.

### PREVIOUS WORK.

Gies<sup>2</sup> investigated the irritability of the brain during anæmia produced by perfusion with solutions of pure sodium chloride, Ringer's solution and modifications of it, Schücking's solution, rabbit and horse serum, and 0.7 per cent sodium chloride solution containing paraxanthin or "chloralbacid" (a chlorine compound with albumin). In toads all reflexes persisted for some time, *c. g.*, six and one-fourth hours for respiration when the animal was perfused with 0.6 per cent

<sup>1</sup> STEWART, GUTHRIE, BURNS, and PIKE: *Journal of experimental medicine*, 1906, viii, p. 289.

<sup>2</sup> GIES: *This journal*, 1903, ix, p. 131.

sodium chloride solution. In cold-blooded animals the various functions ceased in the following order: (1) respiration, (2) skin reflex, (3) lid reflex, (4) nose reflex, and (5) heart beat. In warm-blooded animals, which, however, are only incidentally alluded to, the order of cessation was: (1) lid reflex, (2) respiration, (3) nose reflex, and (4) heart beat.

A number of other attempts, all of which were unsuccessful, have been made to maintain the activity of the reflex nervous centres of animals by perfusion with solutions of inorganic salts of the blood. RIES,<sup>1</sup> the latest investigator in this line, was unable, working with frogs, to find any such solution. Perfusion with rabbit's serum revived the centres after they had lost their irritability following perfusion with the inorganic salt solutions.

Laborde<sup>2</sup> was the first to perfuse the isolated human brain, using the heads of decapitated criminals and the blood of dogs and oxen. In one case, that of the criminal Gagny, he connected by glass tubes the left carotid of the head with the corresponding carotid of a vigorous dog, and into the right carotid of the head he injected defibrinated ox-blood. The head of Gagny was received about the seventh minute after decapitation. Cannulas were adapted to the cerebral arteries in about ten minutes more. The perfusion was begun about eighteen minutes after decapitation. Stimulation of the Rolandic area of the cerebral cortex, which meanwhile had been laid bare, caused movements of the orbicularis palpebrarum muscle, of the eyebrows, the supra-orbital portion of the frontal muscles, and the elevators of the jaw, — phenomena which persisted fifty minutes. Of his fifth perfusion (that of the head of Heurtevent of Caen) Laborde says that the results were absolutely negative as far as they concern the restoration of excitability of the cerebrum. In no case did Laborde get any return of voluntary movements.

Hayem and Barriere<sup>3</sup> decapitated dogs and perfused the head with defibrinated blood in five experiments, and entire blood in seventeen experiments, using bottles filled with blood of horses and dogs for the purpose. The entire blood gave the more satisfactory results. Their conclusions were: (1) The corneal reflex disappears before the last respiratory movement. (2) The head then becomes completely inert and the pupils dilate, with definitive death. (3) Resuscitation occurs

<sup>1</sup> RIES: *Zeitschrift für Biologie*, 1906, N. F. xxix, p. 279. Full literature.

<sup>2</sup> LABORDE, cited by HAYEM and BARRIERE, q. v.

<sup>3</sup> HAYEM and BARRIERE: *Archives de physiologie*, 1887, p. 1.

when the perfusion is made without delay. (4) But, to give satisfactory results, the perfusion should be done with oxygenated blood at suitable temperature and pressure, and should be sufficiently copious and prolonged. (5) Resuscitation was possible only for very brief intervals under any conditions.

References to other work are given in our former paper.

An idea of our own observations will most easily be conveyed by presenting the condensed protocols of three selected experiments.

*Experiment 1.* — With Locke's solution.

Small dog; young. Ether. Tracheotomy. Cannula for injecting warm Locke's solution in jugular vein; connected with pressure bottle. Cannula for bleeding in femoral artery. 400 c.c. to 500 c.c. of blood withdrawn.

About 700 c.c. of Locke's solution injected first time. Then alternate bleeding and injection of Locke's solution until one litre had been injected. Corneal reflex still present when injection was stopped; reflex continued for a period of a few minutes during which no fluid was withdrawn or added.

A second period of bleeding and injection of Locke's solution followed. Corneal reflex disappeared. Then great dyspnoea and deep respiration. Heart stopped in twenty-five minutes from beginning of first bleeding. Fluid escaping from femoral cannula still contained a fair number of red corpuscles.

Locke's solution, even when mixed with a considerable proportion of the animal's own blood, did not maintain the corneal reflex for any length of time.

*Experiment 8.* — With defibrinated blood.

Pup. Ether. Blood obtained from a dog on the previous day had been defibrinated and kept on ice for twenty-four hours. Used for artificial circulation through brain of pup.

11.23 A. M. Tied right subclavian artery and vein. Artificial respiration.

11.26 A. M. Tied aorta and put cannula in central end.

11.28 A. M. Tied inferior vena cava.

11.38 A. M. Put cannula into inferior vena cava toward heart, running it up into auricle. Corneal reflex present.

11.39 A. M. Tied heart in auriculo-ventricular groove, omitting the great veins, and immediately began artificial circulation from a pressure bottle with the defibrinated blood prepared. Strong movements of head, neck, and jaws, with gasping movements like dyspnoea, although defibrinated blood is circulating freely. (The blood in the tubing and cannula may not have been well oxygenated, but that in bottle was.) Tested



corneal reflex repeatedly and found it well marked. There were also voluntary movements of eyes when administration of anæsthetic was reduced. Pupils contracted.

11.47 A.M. Corneal reflex very feeble in right eye; good in left eye. Spontaneous respiratory movements ceased at this time.

11.48 A.M. Corneal reflex absent in both eyes. Pupils strongly contracted. Tried for light reflex several times in last few minutes, but could not be certain of its presence.

Stimulation of vago-sympathetic nerve in neck caused dilation of pupil of right eye until 12.10. of left eye until 1.22. Corneal reflex was maintained for about nine minutes.

*Experiment 12.* — With entire blood and vascular anastomosis.

Two dogs, one somewhat larger than the other, were etherized. Tracheotomy. The peripheral ends of the carotid arteries and internal jugular veins of the smaller dog were anastomosed to the corresponding vessels of the larger dog by Carrel's method.<sup>1</sup> The skin flaps from the neck were then sewed together.

4.35 P.M. Now severed head of small dog from body with exception of backbone.

4.36.30 P.M. Now severed backbone and spinal cord of small dog in lower cervical region, completing decapitation. Respiratory movements ceased. No corneal reflex because of deep anæsthesia.

4.40 P.M. Respiratory movements of nostrils and mouth of transplanted head began; rate about 35 per minute. Good gasping movements and good corneal reflex. Pupils at nearly maximal dilation. Respiratory movements of transplanted head soon fell to 20 per minute. Administration of ether to pumping dog now temporarily stopped.

4.43 P.M. Winking movements of eyes of transplanted head apparently a little in advance of respiratory movements, but of same rhythm.

4.46 P.M. Winking less rapid than respiratory movements of transplanted head; well-marked corneal reflex.

4.47 P.M. Pupils at half maximal dilation in transplanted head. Again gave ether to pumping dog.

4.51 P.M. Tried for light reflex on transplanted head; probably not present, but could not be certain.

4.52 P.M. Respiratory movements stop in transplanted head. Ether to pumping dog stopped, but there is considerable dyspnœa.

4.53 P.M. Carotid artery to transplanted head clamped; respiratory movements started in head. No ether to pumping dog.

4.55 P.M. Gasping movements of mouth, winking, twisting of transplanted head.

<sup>1</sup> CARREL: *American medicine*, 1905, x. p. 284.

- 4.55-30 P. M. Respiratory movements, 20 per minute in both heads.
- 4.57 P. M. Piece of meat pushed down throat of transplanted head swallowed.
- 5.02 P. M. Pupils of transplanted head smaller than at last observation.
- 5.07 P. M. No corneal reflex in transplanted head. Pumping dog breathing very slowly.
- 5.08 P. M. Artificial respiration for pumping dog; no reflexes. Gasps in transplanted head.
- 5.08.45 P. M. Powerful gasp by pumping dog.
- 5.10 P. M. Transplanted head had lost all signs of activity.
- 5.12 P. M. Thorax of pumping dog opened; heart practically stopped.
- 5.13.30 P. M. Massage of heart of pumping dog.
- 5.17 P. M. Movements of tongue of pumping dog.
- 5.19 P. M. Blood circulating in tongue of transplanted head; eye no longer cloudy.
- 5.21 P. M. Experiment concluded. Pumping dog killed with ether.

To summarize the chief results of Experiment 12: Winking movements were observed for about nineteen minutes. Corneal reflex maintained for twenty-seven minutes. Respiratory movements persisted for about thirty minutes and were sometimes synchronous in both dogs. Swallowing movements and winking were well marked, indicating a considerable degree of activity of some at least of the cerebral and medullary centres.

#### GENERAL SUMMARY AND CONCLUSIONS.

We conclude, from a consideration of the above data, (1) that solutions of the inorganic salts of the blood are totally inadequate to sustain the activity of the brain including the medulla oblongata, either as regards reflex or voluntary function. Even when mixed with a considerable proportion of blood, these solutions are inadequate.

(2) Defibrinated blood, circulated by means of a pressure bottle, maintained the activity of the reflex centres and also of the cortical motor centres for short periods, up to eight or nine minutes.

(3) The circulation of properly oxygenated entire blood through the isolated brain under the best possible conditions attainable, *i. e.*, when the isolated head was supplied with blood from the circulation of another animal of the same species, maintained reflex and even voluntary activity of the higher nervous centres for considerable periods. Voluntary movements persisted for about nineteen minutes,

corneal reflex for twenty-seven minutes, and respiration for thirty minutes.

(4) An unbroken connection between the brain and lower part of the spinal cord is, therefore, unnecessary for maintaining at least some of the medullary and cerebral centres. Suitable nutritive conditions are sufficient.

It is uncertain whether any significance should be attached to the synchronism of the respiratory movements of the isolated head with those of the pumping dog, which was sometimes observed. It has been shown<sup>1</sup> that the blood pressure of an animal modifies the rate and character of its respiratory movements. These movements are slower and deeper during low blood pressure than during high pressure. The synchronism observed might be thought to indicate that the condition of the blood and the pressure at the moment were more important factors in determining the rate of discharge of a given respiratory centre than any inherent property of the centre itself. It is possible, of course, that the synchronism was merely a coincidence, and therefore indicates nothing as to the influence of the state of the blood upon the centre.

<sup>1</sup> GUTHRIE and PIKE: *This journal*, 1906, xvi, p. 475.

# THE INFLUENCE OF TEMPERATURE UPON THE RATE OF HEART BEAT IN THE LIGHT OF THE LAW FOR CHEMICAL REACTION VELOCITY. — II.

BY CHARLES D. SNYDER.

[From the American University Table at the Zoölogical Station, Naples.]

COHEN<sup>1</sup> pointed out that the temperature effect upon Hertwig's developing frog eggs obeyed the law for the influence of temperature upon chemical reaction velocities; also that the output of carbon dioxide in Clausen's experiments with germinating seeds followed the same law. Quite recently Peter<sup>2</sup> has shown that the rate of development of echinoderm eggs also follows this law; and Loeb,<sup>3</sup> that the rate of artificial maturation of Lottia eggs is a function of the same logarithmic formula. Jost<sup>4</sup> again shows that this relation holds good for physiological activity in plants.

In a former paper<sup>5</sup> the writer showed that the influence of temperature upon the rate of beat of isolated hearts of the Pacific terrapin (*Clemys marmorata*) obeyed the law for chemical reaction velocities. The heart beats followed the law especially well between 7.5° and 30° temperature. Just lately T. B. Robertson<sup>6</sup> observed the rate of heart beat in a minute *Daphnia* under various temperatures. He finds that the rate of the heart, beating in the living body of the animal, within certain limits of temperature is also in accordance with the law for chemical reactions.

## THE RATE OF HEART BEAT IN PHYLLIRRHÖE AS INFLUENCED BY TEMPERATURE.

In the Gulf of Naples is found a beautifully transparent nudibranch, *Phyllirrhoe*, whose heart can easily be seen through the body tissues.

<sup>1</sup> COHEN: Vorlesungen über physikalische Chemie, 1901, pp. 42-45.

<sup>2</sup> PETER, KARL: Archiv für Entwicklungsmechanik der Organismen, 1905,

xx, p. 130.

<sup>3</sup> Quoted after T. B. ROBERTSON, Biological bulletin, 1906, x, p. 242.

<sup>4</sup> Biologisches Centralblatt, 1906, xxvi, p. 225.

<sup>5</sup> SNYDER, CHARLES D.: University of California publications, Physiology, 1905, ii, p. 125.

<sup>6</sup> ROBERTSON, T. B.: *Loc. cit.*

If one puts a few of these animals in a dish of sea water, they swim around for a little while and then sink down to the bottom of the dish, where they lie on their sides at rest. In this position the heart can easily be seen, and its rate taken, with a stop-watch, by direct observation.

The animals which came into the laboratory were from two to three centimetres long. Four of them were put into separate dishes containing each about a half litre of sea water. The temperature of the water in all the dishes was the same. After a few minutes, while the animals were resting, counts were made of the heart beats and recorded. The animals were later transferred to other dishes of sea water having a higher or a lower temperature. In a few minutes counts of the heart beats again were made and recorded, and so on.

It was at once seen that at room temperature there was little individual variation of heart rate. However, the animals were exposed to each temperature for about half an hour, during which time many counts were made on each specimen.

The average rates of all these observations for the different individuals, at the various temperatures tried, are as follows:

Specimen number.	At 16°.	At 20°.	At 25°.	At 29°.
1	35	40	57	62
2	36	46	66 <sup>1</sup>	80
3	37	52	66	82
4	<u>39</u>	<u>47</u>	<u>66</u>	<u>84</u>
Average rates	36.7	46.5	63.7	77

When calculated, the temperature coefficients for these individual cases are:

Specimen number.	( $\frac{28}{18}$ ) $\frac{1}{4}$ .	( $\frac{25}{16}$ ) $\frac{1}{9}$ .	( $\frac{25}{20}$ ) $\frac{1}{9}$ .	( $\frac{28}{20}$ ) $\frac{1}{4}$ .	( $\frac{29}{20}$ ) $\frac{1}{9}$ .
1	2.8	1.8	2.8	2.7	1.7
2	3.1	2.0	2.8 <sup>1</sup>	3.2 <sup>1</sup>	1.9
3	3.5	2.0	2.5	3.1	1.7
4	3.0	1.8	2.8	3.1	2.0

The coefficients of the average rates:

3.4	1.86	2.7	2.9	1.8
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<sup>1</sup> Specimen No. 2 died before this experiment was made. The rate here is of another individual, No. 5.

The average coefficients are:

3.1                  1.9                  2.8                  3.0                  1.8

From the table of coefficients (quotients) above, it will be seen that the individual variation is from 1.7 to 3.5, and that the variation of the average coefficients for different temperatures is from 1.8 to 3.1. The average coefficient for all temperatures of the experiments is 2.52.

It was concluded, therefore, that the heart of the mollusk, *Phyllirhoe*, is influenced by temperature in the rate of its beat at a velocity similar to that for a chemical reaction.<sup>1</sup>

#### THE RATE OF HEART BEAT IN *MAIA VERRUCOSA* AS INFLUENCED BY TEMPERATURE.

During the past season experiments were made on the influence of temperature upon the isolated hearts of a crustacean, *Maia verrucosa*. Preliminary tests were made to determine the constituency of a salt solution in which the heart of this animal, after removal from the body, would live longest. A solution similar to that employed by Rogers<sup>2</sup> was finally selected. The chief difference in the two solutions is in the concentration. The solution used for *Maia verrucosa* hearts was  $\frac{5}{8}$  molecular. The number of parts by volume, —

NaCl . . .	100.0	MgCl <sub>2</sub> . . .	7.8
KCl . . .	2.2	MgSO <sub>4</sub> . . .	3.8
	CaCl <sub>2</sub> . . .		3.0

Filtered sea water to which was added a slight amount of calcium chloride (1 c.c. to 100 c.c. of sea water) was also found to be equally as good as the artificial solution.

The heart of *Maia verrucosa* is a delicate organ, averaging about 1 c.m. in horizontal diameter. Unless great care is exercised in removing it from the body, the heart comes to a standstill from which it does not recover. In no case could the rhythm be maintained much more than an hour.

On account of the extreme frailty of the tissues, the heart was not

<sup>1</sup> For complete reference to this literature see my former paper, *loc. cit.*, pp. 134 ff.

<sup>2</sup> ROGERS, CHARLES G.: *Journal of experimental zoölogy*, 1904, ii, p. 237. The concentration of Rogers' solution was  $\frac{7}{8}$  mol.

usually attached to a lever, and so few records were taken on the kymograph. The slow rate of the heart beat, however, made it quite easy to count the contractions by direct observation, timing them with a stop-watch. Most of the observations were made by this latter method.

TABLE I.

The rates of heart-beat of *Maia verrucosa* as influenced by temperature, together with the corresponding temperature coefficients.

Number of experiment.	Temperature in degrees C.	Duration of exposure to new temperature, in minutes.	Number of beats per minute.	Average rates at different temperatures.	Calculated coefficients of Rate at $T_{n+10}$ Rate at $T_n$
1. a	17.0	3	34.0	34.0	3.0
	7.0	6	14.0		
	7.0	12	8.0	11.0	
b	18.0	1	13.0	12.0 4.0	3.0
	18.0	2	12.0		
	18.0	6	11.0		
	8.0	3	4.0		
2. a	9.0	3	15.0	15.0	2.0
	19.0	3	30.0		
	19.0	4	30.0	30.0	
b	26.0	4	20.0	19.0 8.0	2.3
	26.0	6	18.0		
	16.0	4	8.0		
	16.0	7	8.0		
3. a	20.0	10 12 13	42.0	42.0	4.0
	14.5		24.0		
	14.5		21.0		
	14.5		18.0	21.0	
b	20.5	3	36.0	36.0	2.2
c	25.0	2	40.0	38.0	1.8
	25.0	3	36.0		
4	16.0	25	9.0	9.0	2.1
	25.0	13	17.0	17.0	
5. a	17.0 (ca.)	3	23.0	23.0	4.0
	12.0	8	11.5	11.5	
b	19.0	2	31.0	31.0	3.3
	10.0	4	10.0	10.0	
6	16.0	5	3.0	3.0	5.3
	24.0	3	11.0		
	24.0	7	15.0	13.0	
7	25.0	?	16.0		2.9
	16.0	?	6.0		

The data obtained were at once inspected for evidence pointing toward chemical reaction velocity. The quotients of rates at  $10^{\circ}$ , or nearly  $10^{\circ}$ , apart are shown in the left-hand column of the table below. Where observations of rates at temperatures less than  $10^{\circ}$  apart were made, the coefficient (as was done above) is roughly calculated from the data at hand for the full  $10^{\circ}$ .

The quotients here are apparently high, the average of all of them being 2.99. But, after all, this is just what one should expect, inasmuch as the rates are for relatively low temperatures. The experiments on the *P. terrapin* heart showed that this quotient grew larger with the lower temperatures and smaller with the higher temperatures. In the present experiments the highest temperature at which the *Maia verrucosa* hearts continued to beat was  $26^{\circ}$ ; <sup>1</sup> while in the former experiments the *P. terrapin* hearts beat at a temperature as high as  $40^{\circ}$  C.

However it will be remembered that this temperature coefficient, even in the case of pure chemical reaction velocities, varies, as van't Hoff<sup>2</sup> has shown, considerably. And so the velocity of heart rate of *Maia verrucosa* falls clearly within the limits of chemical reaction velocities. Therefore we are justified in concluding from the above data that the rate of heart beat in *Maia verrucosa*, within limits of normal variations of temperature, increases with the temperature at a velocity similar to that of a chemical reaction.

#### THE RATE OF HEART BEAT IN MAMMALS AS INFLUENCED BY TEMPERATURE.

It has now been shown that a reptile heart, two very different crustacean hearts, and a molluscan heart are all influenced by temperature, in their rate of beat, at the same velocity as a chemical reaction is influenced by temperature.

The question naturally rises, to what extent does temperature affect the mammalian heart in this way? For an answer we need only turn to the original papers of Newell Martin and of Langendorff.

<sup>1</sup> The isolated heart of *Maia verrucosa*, under different conditions, will probably beat at higher and at lower temperatures than it did in my experiments. Whether the vitality of the animals was impaired by the great amount of volcanic ash which fell into the Gulf of Naples this spring, and from which animals undoubtedly were suffering, or whether other methods must be employed, cannot be stated at this time.

<sup>2</sup> VAN'T HOFF: Vorlesungen über theoretische und physikalische Chemie, 1898, i. p. 224.



These authors published very complete tables of their results, showing the various temperatures and corresponding rates of heart beat. They experimented for the most part upon the isolated hearts of dogs and cats. From their results the temperature coefficients for rates  $10^{\circ}$ , or nearly  $10^{\circ}$ , apart have been calculated. In cases where only rates at less than  $10^{\circ}$  apart are recorded the coefficient is determined by interpolation.

From Martin's first experiments on dogs<sup>1</sup> we have the following:

*Experiment 1.*—Temperatures  $37.8^{\circ}$ ,  $27.8^{\circ}$ , and  $40.5^{\circ}$ ,  $30.5^{\circ}$ ; quotients, **3.3** and **3.0**.

*Experiment 2.*—Temperatures  $38.5^{\circ}$ ,  $28.5^{\circ}$ , and  $42.5^{\circ}$ ,  $32.5^{\circ}$ ; quotients, **3.2** and **1.8**.

*Experiment 3.*—Temperatures  $39.3^{\circ}$ ,  $29.1^{\circ}$ ,  $40.0$ ,  $30.0^{\circ}$ ;  $38.5^{\circ}$ ,  $28.9^{\circ}$ ; quotients, **2.3**, **2.1**, **2.3**.

*Experiments 4, 5, and 6.*—Temperatures  $30.5^{\circ}$ ,  $39.6^{\circ}$ ;  $36.4^{\circ}$ ,  $40.4^{\circ}$ ;  $37.5^{\circ}$ ;  $32.5^{\circ}$ ,  $30.5^{\circ}$ ; quotients, **2.3**, **2.7**, **3.1**, and **3.3**.

Martin and Applegarth later on experimented upon "the temperature limits of the vitality" of cat hearts, the hearts being completely isolated from the body. From their tables of observations<sup>2</sup> we have the following coefficients:

*Experiment 1.*—Temperatures  $34^{\circ}$ ,  $24^{\circ}$ ;  $33.7^{\circ}$ ,  $23.5^{\circ}$ ;  $32.7^{\circ}$ ,  $22.3^{\circ}$ ; quotients, **3**, **3.2**, **3.1**.

*Experiments 1 and 2.*—Temperatures  $35.2^{\circ}$ ,  $25.7^{\circ}$ ;  $36.7^{\circ}$ ,  $26.7^{\circ}$ ;  $38.5^{\circ}$ ,  $28.5^{\circ}$ ;  $40.0^{\circ}$ ,  $29.9^{\circ}$ ; quotients, **2.5**, **2.3**, **1.9**, **1.3**.

Langendorff<sup>4</sup> succeeded in maintaining the rhythm of the isolated hearts of cats at widely different temperatures, the lowest being about  $7^{\circ}$ , the highest about  $46^{\circ}$  C. The records of 15 hearts are tabulated. From these tables the writer determined the average rate for each degree of temperature. Coefficients for the rates  $10^{\circ}$  apart were calculated. These are set down in the right-hand column of the following table:

<sup>1</sup> MARTIN, H. N.: Philosophical transactions, 1883, clxxiv, pp. 679-685.

<sup>2</sup> The last three temperatures and their corresponding coefficients, 3.1 and 3.3, are taken from observations during the first half-hour only.

<sup>3</sup> MARTIN and APPLGARTH: Studies from the Biological Laboratory, Johns Hopkins University, 1890, iv, pp. 282 ff.

<sup>4</sup> LANGENDORFF, O.: Archiv für die gesammte Physiologie, 1897, lxvi, p. 355.

TABLE II.

Table of average rates of isolated cat hearts at temperatures between 10° and 47° C., (after Langendorff), together with the quotients of the  $\frac{\text{rate at } T_{n+10}}{\text{rate at } T_n}$ .

Temperature, C.	Rate of beat per minute.	Quotient.	Temperature, C.	Rate of beat per minute.	Quotient.
10	10	$\frac{5.4}{1.0} = 5.4$	31	116	$\frac{11.6}{4.8} = 2.4$
14	15	$\frac{6.7}{1.5} = 4.4$	32	134	$\frac{13.4}{6.5} = 2.0$
15	21	$\frac{8.5}{2.1} = 4.0$	33	166	$\frac{16.6}{5.7} = 2.9$
16	26	$\frac{9.7}{2.5} = 3.7$	34	183	$\frac{18.3}{6.7} = 2.7$
17	37	$\frac{10.5}{3.7} = 2.8$	35	195	$\frac{19.5}{8.5} = 2.3$
18	38	$\frac{9.3}{3.8} = 2.4$	36	178	$\frac{17.8}{9.7} = 1.8$
19	43	$\frac{12.2}{4.3} = 2.8$	37	226	$\frac{22.6}{10.5} = 2.1$
20	54	$\frac{11.6}{5.4} = 2.1$	38	214	$\frac{21.4}{9.3} = 2.3$
21	48	$\frac{11.9}{4.8} = 2.4$	39	214	$\frac{21.4}{12.4} = 1.8$
22	65	$\frac{13.4}{6.5} = 2.3$	40	156 <sup>1</sup>	$\frac{15.6}{11.6} = 1.3^1$
23	57	$\frac{16.6}{5.7} = 2.9$	41	215	$\frac{21.5}{11.5} = 1.8$
24	67	$\frac{18.3}{6.7} = 2.7$	42	229	$\frac{22.9}{13.4} = 1.7$
25	85	$\frac{19.5}{8.5} = 2.3$	43	229	$\frac{22.9}{13.8} = 1.3$
26	97	$\frac{9.7}{2.6} = 3.7$	44	226	$\frac{22.6}{18.8} = 1.2$
27	105	$\frac{10.5}{3.7} = 2.8$	45	258	$\frac{25.8}{19.5} = 1.3$
28	93	$\frac{9.3}{3.8} = 2.4$	46	249	$\frac{24.9}{14.8} = 1.4$
29	122	$\frac{12.2}{4.3} = 2.8$	47	245	$\frac{24.5}{24.5} = 1.0$
30	116	$\frac{11.6}{5.4} = 2.1$			

<sup>1</sup> One observation only at this temperature (40°), and this on a heart which had already been subjected twice to very low temperatures; the heart already showed signs of exhaustion.

The temperature coefficients in the above tables deserve special examination. Neither Martin nor Langendorff, when they performed their experiments, had any idea of comparing their results with temperature velocity of chemical reactions. Indeed, the formula for such a reaction velocity was then not yet determined. The methods of work and the accuracy of observation of these investigators, how-

ever, are models of thoroughness and excellence. Their data are abundant and conclusive. We need only apply our new knowledge to this body of facts to answer the question, — to what extent, when influenced by temperature, does the velocity of the heart rate of the mammal correspond to that of a chemical reaction velocity?

Let us remember that the chemical velocity constant not only varies generally between 2 and 3 for 10° difference of temperature, but that occasionally it may even be slightly less than 2 and considerably more than 3. In our tables we notice that the constant (coefficient) for the middle ranges of temperature is, with two exceptions, between 2 and 3, and that those numbers which are more or less than 2 or 3 are for the extreme temperatures.

It is worth while noting here, also, that the higher constants, for the most part, are for the low temperatures and that the lower constants are for the high temperatures. It will be remembered that this was also the case, not only with my *P. terrapin* hearts,<sup>1</sup> but also with the *Maia verrucosa* hearts, as shown on page 353 of this paper. Jost's results<sup>2</sup> show that this same variation of the constant obtains also in plants.

A paper recently published by Langendorff and Lehman<sup>3</sup> contains a table of the rates of heart beats of the rabbit at temperatures between 37° and 48° C. The hearts were isolated from the body and the sinus removed. They were fed with physiological salt solution. The table was given to show that removal of the sinus does not affect the influence of temperature upon the rate of heart beat. The quotients are calculated from the rates of temperatures farthest apart. The calculation for quotients corresponding to temperatures fully 10° apart is in each case done by interpolation. They are:

2.3      2.4      3.0      2.5      2.5      2.3

¶ For practical reasons it would be of special interest at this point to know the temperature coefficient of the human heart.

From Liebermeister's<sup>4</sup> table of fever temperatures, which was compiled from more than 4000 observations on 280 different patients, we get the following coefficients, when calculating for differences of 10° from the data at hand:

3.0      3.7      4.5      4.5      3.5

<sup>1</sup> *Loc. cit.*, p. 146.

<sup>2</sup> JOST: *Biologisches Centralblatt*, 1906, xxvi, p. 233.

<sup>3</sup> LANGENDORFF and LEHMAN: *Archiv für die gesammte Physiologie*, 1906, cxii, p. 352.

<sup>4</sup> Quoted from LANGENDORFF, *loc. cit.*, p. 393.

These constants are remarkably high, and stand in curious contrast to those obtained at the higher temperatures of isolated mammalian and terrapin hearts. The data, being from pathological cases, may have some special significance.

Davy<sup>1</sup> recorded the temperature and pulse rate of his own body about three times per day for a period of eight consecutive months. These observations do not include those made "under the influence of accidental disturbing circumstances, as active exercise, etc.," and so may be considered as the record of the heart rate and temperature of the body as nearly normal as it is possible to obtain. This mass of data is of special significance at this point in contrast to that of Liebermeister's. It gives us the data of one individual in normal health for a prolonged period of time. Davy made 502 separate observations in this series. When the total averages of temperatures and pulse rates are determined for morning, noon, and night, and their corresponding coefficients are calculated, we find them to be as follows:

Temperatures . . .	36.62°	36.94°	37.07°
Pulse rates . . .	54.68	55.2	57.2
Coefficients . . .	2.3	3.1	2.3

These temperature coefficients of the heart rate can no longer be looked upon as mere coincidence; for it has now been shown that they approach the same constant, not only in two important invertebrate groups, crustacea and mollusca, but also in reptilia and mammalia, including man himself.

From these results we need no longer vaguely say that within certain limits an increase of temperature increases, and a decrease of temperature diminishes, the heart rate. But rather may we boldly say that a change of temperature influences the rate of heart beat at a mathematical velocity; that this velocity is the same as that for known chemical reactions as influenced by temperature, and is expressed by the formula of Arrhenius:<sup>2</sup>

$$\log k = -\frac{A}{T} + \text{constant},$$

in which  $A$  is a constant, and  $k$  is the velocity (rate) of heart beat at any absolute temperature,  $T$ , which falls within the limits indicated by experiment.

<sup>1</sup> DAVY, JOHN: *Philosophical Transactions* of the Royal Society, London, 1845, p. 319.

<sup>2</sup> ARRHENIUS: *Zeitschrift für physikalische Chemie*, 1899, iv, p. 226.

THEORETICAL CONSIDERATIONS.

A fact so far-reaching as this demands further consideration; for it must sooner or later throw light on the nature of the heart rhythm itself.

It is most probable that this identity, which we have shown to exist between the temperature coefficients of a chemical reaction and of the heart rate, is due to the fact, as has often been suggested,<sup>1</sup> that chemical reaction is profoundly involved in the production of the intrinsic cardiac stimulus, or in the production of both the intrinsic cardiac stimulus and the cardiac contraction.

If constantly repeated chemical reaction causes each contraction of the heart muscles, at what point in the cardiac cycle does the reaction begin, during what phase is it in progress, and at what point of the cycle does it end? For the reaction is, most probably, a rhythmical process which exhausts itself or comes to an end point once during each heart beat.

For a partial answer to this question we again need only turn to the great body of experimentation which has already been done upon the heart. Burdon-Sanderson<sup>2</sup> showed that an increase of temperature diminished, and a decrease of temperature increased, the refractory period of the frog's heart. Inspection of the results of this experiment reveals at once the remarkable fact that the velocity of this increase, or decrease, of "diminished excitability" varies with the velocity of a chemical reaction similarly influenced by temperature. In the following table are shown the temperature coefficients which correspond to the various temperatures and periods of "diminished excitability" of the experiment:

Temperature.	Time of "diminished excitability."	Temperature coefficients for 10°.
12° C.	2.0''	1.8
15	1.8	2.2
18	1.5	2.0
21	1.2	2.5
24	0.9	2.2
27	0.8	2.0

<sup>1</sup> For literature and discussion of this subject, see HOWELL, W. H.: This journal, 1898, ii, p. 47; also LANGENDORFF, O.: *Ergebnisse der Physiologie*, 1905, iv, pp. 764 ff.; ENGELMANN, Ueber den Ursprung der Muskelkraft, Leipzig, 1893.

<sup>2</sup> BURDON-SANDERSON: *Journal of physiology*, 1880, ii, p. 384.

From this table of coefficients it would appear that the chemical reaction which has to do with the preparation of the heart for the next contraction is already under way during systole.

We are now in a position to explain more fully than has yet been done the rhythmical nature of the heart-beat and also its refractory period.

Let us assume (1) that the excitability of the heart tissues depends upon a certain relation of chemical substances; (2) that this relation is brought about by a chemical reaction, or reactions, approaching a state of equilibrium; (3) that the cardiac contraction is caused by (directly or indirectly), and begins with, the end point of this reaction.

The mechanical act of this contraction itself, however, displaces and removes the end products of the reaction, and thus disturbs the equilibrium. During the systole, therefore, the same chemical reaction, just completed, is started up again. But it does not advance far enough during this phase of the cardiac cycle to produce the state of excitability in the tissues necessary for another contraction. On the other hand, during diastole the reaction has greatly advanced and rapidly approaches an end point (equilibrium) which leads up to, and causes, the next systolic movement.

If this assumption be correct in fact (as the burden of this paper would indicate it is), then an increase of temperature ought to hasten the chemical reaction, and bring the system the sooner into equilibrium; therefore the heart tissues the sooner into a state of excitability, and, finally, into systole. A decrease of temperature, contrary-wise, ought to delay the chemical reaction, thus prolonging the state of inexcitability and retarding the systole. The final and general effect would be an increase and decrease of heart rate, and an increase and decrease of the length of the refractory period, with increase and decrease of temperature, — and this at a velocity identical with that of a chemical reaction velocity.

There is still one other phenomenon which may be considered here, namely, the latent period which follows an electrical stimulation of the heart.

The later an electric shock is applied to the heart during diastole, the shorter are both the latent period which precedes the contraction and also the compensatory period which follows it. This fact agrees fully with our hypothesis. In the first place we have no reason to believe that an electrical stimulus, such as is sent into the heart, would hasten, or affect appreciably, the chemical reaction which we

have supposed leads up to the heart systole. On the other hand the electrical stimulus may do for the heart tissues just what the end point of the chemical reaction (possibly through its newly acquired physical properties) does for the heart automatically, namely, produce the stimulus to contraction. The great length of the latent period in the early stages of diastole suggests that the chemical reaction has not proceeded far enough for any sort of stimulus to bring about a contraction. The fact, however, that a contraction can be produced by artificial stimulus, considerably before the normal period, suggests that the chemical preparation of materials under normal conditions is nearly complete some appreciable time before, let us say, the new physical properties, which it brings about, result in automatic stimulation.

New and additional experiments are being carried out to test further the validity of the views expressed in this paper; also to determine, if possible, what substances are involved in the chemical reaction, or reactions, which we have supposed underlie the rhythmicity of the heart.

The writer wishes here to express his heartiest thanks to the leader and staff of assistants of this institution for the many courtesies they have shown him, and the assistance they have rendered in the prosecution of this work.

# METABOLISM EXPERIMENTS UPON A WOMAN WITH A PERMANENT BILIARY FISTULA.

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Medical College, New York.]

## INTRODUCTION.

**P**RACTICALLY all of the positive knowledge we have of the physiological function of the bile is based upon the observation that fats are poorly absorbed from the intestine in the absence of bile. Aside from this undisputed fact we know little of the part which bile plays in the complex process of metabolism. The opportunity being presented for conducting such experiments upon a patient with a permanent biliary fistula, it seemed desirable to determine, first, whether a person with a permanent biliary fistula and consequently having little or no bile in the intestine, although otherwise in fairly good health, exhibited a metabolism in any respect different from the normal, and, secondly, if such a difference existed, whether a return to the normal could be accomplished by different forms of bile medication.

The greater part of the time spent in this investigation has been used in the study of the urine in the belief that any abnormality of metabolism caused by the absence of bile from the intestine would be thus detected.

The subject of this experiment is a woman with a permanent biliary fistula. Dr. M. Belle Brown, to whom I am indebted for the opportunity of studying the case, kindly gives me the following history.

**Mrs. C. B. D., age 61.** — The patient was of robust physique and unusually good health until the winter and spring of 1894, when she had almost daily attacks of severe pain in the hypogastric region. The condition was diagnosed as flatulent dyspepsia. In May, 1894, a warm bath was followed by a chill and high temperature and pain in the region of the gall bladder.



In three days intense jaundice appeared. Physical examination revealed an enlarged liver extending about two inches below the ribs. Stools were "clay colored" and the urine about the color of port wine. The chills with the accompanying fever continued from May to October. In the fall the temperature was normal, but the stools were light colored and the urine quite dark. Some icterus persisted, with general pruritis. The condition lasted for ten years, during which the patient had frequent attacks of pain and gastric disturbances. Her net weight fell from 160 to 110 pounds.

On Feb. 22, 1905, the patient was operated upon for gall stones. The gall bladder was found one-third smaller than normal, and closely adherent to a mass of inspissated bile, of the size of an English walnut. The gall bladder was opened and a drainage tube inserted. The common bile duct was not opened; no stones were felt in it. Following the operation the jaundice disappeared to some extent, but the stools remained very light and the urine very dark. The drainage tube was left for six weeks, during which time the largest amount of bile passed in twenty-four hours was 4 ounces. On the removal of the tube the fistula closed in a few days, and almost immediately the symptoms of skin irritation and increased jaundice returned.

Three months after the first operation a second was performed. The gall bladder was opened and stitched forward, and the common duct opened. A sound was passed into both hepatic ducts, and blades of long dressing forceps were passed through the common duct into the duodenum. No stones or other obstructions were found. A drainage tube was placed in the fundus of the gall bladder, and another in the common duct. Bile flowed freely from the tube draining the common duct, one and one-half to two pints being passed daily. The irritation of the skin disappeared, and the urine became lighter in color. The stools remained light in color, and probably all the bile passed through the drainage tube. After six weeks the fistula from the common duct healed, forcing the tube out. The bile began at once to flow from the tube draining the gall bladder, and still continues to do so. Recovery from the operation was uneventful.

During the first six months after the second operation there was some diarrhœa, — 8 to 10 movements in twenty-four hours. The stools were soft and of very offensive odor. The use of ichthoform after meals, as well as desiccated ox-bile, gave some improvement. Improvement in this respect was also noted after the injection through the drainage tube of large quantities of the patient's bile. A No. 43 piston syringe was used, and from 10 to 16 ounces of bile and other liquids were occasionally injected at one time. Only slight pressure was necessary.

At the present, more than one year after the second operation, the patient has no itching of the skin, eats and sleeps well, and otherwise

enjoys life. Very nearly if not quite all of the bile flows through the tube into a rubber bag which the patient wears.

This case had been diagnosed as cancer of the liver, catarrhal jaundice, and gall stones. The diagnosis at present accepted by the patient's physician is that the cicatricial tissue of an old duodenal ulcer is contractive at the orifice of the common duct. The patient had an attack of dysentery previous to her illness in 1894, at which time the ulceration perhaps occurred.

When the patient came under the writer's observation on October 15, 1905, she was apparently in fairly good health. Although considerably undernourished as a result of her long illness, she was gaining in weight, had a good appetite, slept well, and complained of no unusual symptoms except occasional attacks of diarrhœa.

Just what the anatomical condition of the bile ducts is in this case can only be surmised. As stated above, no obstruction of any of the ducts was found at the operation, in spite of careful search. But that there is some obstruction is certain, because when the fistula closed following the first operation the jaundice reappeared, and because when the permanent fistula has been artificially closed the jaundice has again appeared only to quickly disappear on opening the fistula and allowing the bile to escape in that way. On the other hand, the ducts are not completely occluded, because large quantities of bile or other fluids have been repeatedly and rapidly injected through the fistula. As much as 400 c.c. of bile has been injected in this way at one time, and it is impossible to believe that it does not ultimately flow, in great part at least, into the duodenum. Furthermore, following the injection of bile through the fistula, as well as following the plugging of the fistula, hydrobilirubin appears in considerable quantities in the feces. At the close of our experiments we proved the patency of the common duct in still another way. A diluted suspension of India ink was injected through the fistula, and in about fifteen hours the India ink appeared in the feces. We know, therefore, that there is a canal leading from the gall bladder into the intestine, and that the bile under certain circumstances flows through this canal. However, so long as the fistula is not closed, only a very small part of the bile reaches the intestine, for the feces showed under this condition only the slightest trace of hydrobilirubin, which could be detected only with some precaution, to be mentioned later.

We at first hoped to be able to conduct experiments on our patient in the same manner as did Schiff<sup>1</sup> on his dogs, with amphibolic fistulæ; but unfortunately, when the canal in the fistula was plugged, the patient after about twelve hours began to complain of a return of the itching, etc., and a little later a slight coloring of the conjunctivæ was noticed, and it became

<sup>1</sup> SCHIFF: *Archiv für die gesammte Physiologie*, 1870, cxi, pp. 598-613.

necessary to open the tube after it had been closed for about forty hours. During this time a large amount of bile found its way into the intestine, as shown by a strong reaction for hydrobilirubin in the faeces for the two or three days following. Although the bile flowed into the intestine, jaundice began to appear. The reason for this is probably that the pressure necessary to overcome the obstruction of the common duct was so great as to cause a rapid reabsorption of a part of the bile through the radicles of the biliary ducts.

While we were unable to force the bile into the duodenum by merely closing the fistula, we have on a number of occasions, as stated above, injected the bile through the fistula in large quantities. The occasional injection of bile through the fistula is obviously not so satisfactory a procedure as would have been the mere closing of the fistula, in which case the bile flows into the intestine at the normal rate of speed. However, the latter ideal condition was impossible, and we were compelled to be content with the less satisfactory procedure of injecting the patient's own bile through the fistula.

#### METHODS.

The analytical methods used for the urine are as follows:

Kjeldahl method for total nitrogen; Folin method for urea,<sup>1</sup> kreatinin, and kreatin;<sup>2</sup> Boussingault-Shaffer method for ammonia;<sup>3</sup> Folin-Shaffer method for uric acid;<sup>4</sup> Folin's new methods for inorganic, ethereal, and neutral sulphur;<sup>5</sup> phosphates were titrated by standard uranium acetate, with powdered potassium ferrocyanide as indicator; indican was determined by the crude but simple method described by Folin.<sup>6</sup>

No discussion of these methods is necessary. All have proved satisfactory. Paton<sup>7</sup> has recently recorded an objection to Folin's urea method,—the difficulty of knowing when the urea is completely decomposed. I have not experienced this difficulty. The method demands considerable care and attention, but if the melted magnesium chloride is kept hot (155° C.) for one full hour and one-half from the time the "popping" becomes strong, all of the urea (as much as is

<sup>1</sup> FOLIN: *Zeitschrift für physiologische Chemie*, 1903, xxxvii, p. 548.

<sup>2</sup> FOLIN: *Ibid.*, 1904, xli, p. 223.

<sup>3</sup> SHAEFFER: *This journal*, 1903, viii, p. 331.

<sup>4</sup> FOLIN and SHAEFFER: *Zeitschrift für physiologische Chemie*, 1901, xxxii, p. 552.

<sup>5</sup> FOLIN: *Journal of biological chemistry*, 1906, i, p. 131.

<sup>6</sup> FOLIN: *This journal*, 1905, xiii, p. 53.

<sup>7</sup> PATON: *Journal of physiology*, 1906, xxxiii, p. 1.

the equivalent of 60 c.c.  $\frac{n}{10}$ ) is invariably decomposed. Low results are usually obtained if the temperature is not maintained for the time stated.

The fæces were dried on the water bath with the occasional addition of methyl alcohol containing 1 per cent hydrochloric acid, and finally in an air bath at 100°-110° C. The total fat content was determined by extraction with ether for about twenty hours, as described by Schmidt and Strassburger.<sup>1</sup> Total nitrogen was determined in the dried fæces by the Kjeldahl method. Hydrobilirubin was tested for either in the fresh or dried fæces as follows: 3 to 5 gm. of the dried fæces, or 10 to 15 gm. of the fresh fæces, were boiled on the water bath with about 25 c.c. alcohol containing 2 per cent sulphuric acid. Without filtering, ammoniacal alcohol was added to alkaline reaction, and then a few drops of a zinc chloride solution. This was filtered into a dry test tube. When hydrobilirubin was present the clear solution showed the green fluorescence. It is important to have a clear solution, otherwise when only traces are present the fluorescence may be masked by the turbidity or precipitate.

In order to judge whether the metabolism in different pathological conditions is abnormal, it will usually if not always be necessary to have a standard of normal metabolism for comparison. The importance of this fact follows logically from the work of Folin on normal metabolism. The results of Folin<sup>2</sup> furnish perhaps the most accurate knowledge we have of the approximately complete composition of normal urine on different standard diets. More data from normal individuals on various diets is highly desirable for the construction of representative standards for the composition of normal urines, but until we have such representative standards, we cannot do better than use the standards of Folin. I have therefore placed at the bottom of the first two tables of this paper the averages of Folin's normal results on his high proteid liquid food and on his low proteid starch-cream diet.

Three diets were used in my experiments in the following order. The first, which was continued for seven days, was a high proteid non-purin diet containing about 21 gm. of nitrogen, and which, though not exactly the same, is quite comparable to Folin's high proteid liquid food. The second diet, which followed immediately after the first for a period of seven days, was a low proteid diet of

<sup>1</sup> SCHMIDT and STRASSBURGER: *Die Faeces des Menschen*, Berlin, 1905, p. 151.

<sup>2</sup> FOLIN: *This journal*, 1905, xiii, pp. 45, 66.

rice, butter, rich cream, sugar and salt, with about 200 gm. potato. The last diet, used for a fifteen-day period was a mixed though fairly constant diet containing some purins and 10-11 gm. of nitrogen.

DIETS.

First experiment (Table I)	}	1 dozen eggs 3 pints skimmed milk Sugar and salt
Second experiment (Table II)	}	200 gm. grain rice (boiled) 100 gm. butter 150 gm. sugar 250 c.c. cream (15% fat) 200 gm. potatoes (boiled or baked)
Third experiment (Table III)	}	Mixed diet of fish, beef, lamb, potatoes, oatmeal, rolls, butter, sugar, salt, rich cream. Patient ate as nearly as possi- ble the same amount of food each day. This was not strictly constant, however.

The analytical results of these experiments will be found in Tables I, II, and III.<sup>1</sup>

<sup>1</sup> Some brief explanation seems desirable of the manner in which the analytical results of these experiments are here expressed. Instead of writing so many grams of urea, kreatinin, etc., as is at present customary, I have chosen to state in the tables only the amount of nitrogen represented by the several nitrogenous substances. Total sulphur, inorganic sulphates, ethereal sulphates, and neutral sulphur are expressed only in grams of sulphur; phosphates and chlorides are expressed in grams of phosphorus and chlorine respectively. The above appears to me to be the most rational and consistent plan of presenting the results of metabolism experiments or even of isolated urine analyses.

The advantages gained from such a plan are brevity, clearness, and convenience. Both on account of the difficulty in examining tables containing many columns of closely printed figures, and on account of the considerable cost to journals of printing large tables, it is desirable that we omit from our tables all unnecessary figures. The absolute amounts of the nitrogenous substances, urea, etc., are unnecessary, because they can be quickly calculated by multiplying the amount of nitrogen (urea-nitrogen, for instance) by the proper factor (2.14 for urea). For the convenience of the reader, such factors are given below. The tables will be found, I think, less confusing on account of the fewer figures. The convenience is found in the original calculation of results, and in the ease with which one can see the distribution of the total nitrogen among the various nitrogenous constituents. For sulphates, phosphates, and chlorides I have merely adopted the custom growing in analytical chemistry, of stating the results as here given, instead of using the rather

TABLE I.  
HIGH PROTEID DIET.

Date.	Volume of urine.	Total nitrogen.	Gm. nitrogen as					Per cent of total nitrogen.					Remarks.
			Urea.	Ammonia.	Kreatinin.	Uric acid.	Undet. residue.	Urea.	Ammonia.	Kreatinin.	Uric acid.	Undet. residue.	
	c.c.	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.		
Oct. 25	960	12.30	9.62	1.18	0.27	0.15	1.08	78.2	9.6	2.2	1.2	88	Bile absent from intestine.
26	975	15.55	12.73	1.42	0.28	0.15	0.97	81.9	9.1	1.8	1.0	6.2	Bile injection.
27	1275	19.70	16.40	1.75	0.27	0.11	1.17	83.2	8.9	1.4	0.6	5.9	"
28	1660	18.70	15.70	1.70	0.26	0.12	0.92	83.9	9.1	1.4	0.6	5.0	Bile absent from intestine.
29	1290	19.20	16.24	1.41	0.25	0.14	1.16	84.9	7.3	1.3	0.7	5.8	Bile absent from intestine.
BILE ABSENT FROM INTESTINE (25, 28, and 29).													
Average . . .		16.73	13.85	1.43	0.26	0.14	1.05	82.7	8.5	1.6	0.8	6.3	
BILE INJECTION (26 and 27).													
Average . . .		17.62	14.56	1.58	0.275	0.13	1.07	82.5	9.0	1.6	0.8	6.1	
NORMAL (FOLIN).													
Average . . .		16.51	14.09	0.84	0.69	0.17	0.75	85.4	5.07	4.2	1.0	4.5	

TABLE I (continued),  
HIGH PROTEID DIET.

Date.	Indican.	Total phosphorus.	Total sulphur.	Inorganic sulphur.	Etheral sulphur.	Neutral sulphur.	Per cent of total sulphur			Remarks.
							Inorganic.	Etheral.	Neutral.	
		gm.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	
Oct. 25	150	1.40	0.94	0.78	0.08	0.08	83.0	8.5	8.5	Bile absent from intestine.
26	100	1.72	1.21	1.03	0.09	0.09	85.2	7.4	7.4	Bile injection.
27	100	1.85	1.50	1.29	0.08	0.13	86.0	5.3	8.7	" "
28	150	1.99	1.43	1.16	0.13	0.14	81.1	8.9	10.0	Bile absent from intestine.
29	150	1.98	1.48	1.23	0.11	0.14	83.2	7.4	9.4	Bile absent from intestine.
BILE ABSENT FROM INTESTINE (25, 28, and 29).										
Average . . .	150	1.79	1.29	1.06	0.11	0.12	82.2	8.5	9.3	
BILE INJECTION (26 and 27).										
Average . . .	100	1.78	1.35	1.16	0.085	0.11	85.8	6.3	8.0	
NORMAL (FOLIN).										
Average . . .	70		1.32 (1.40)	1.17 (1.24)	0.09 (0.57)	0.07 (0.102)	87.8 (88.6)	6.8 (4.0)	5.1 (7.3)	

TABLE II.  
LOW PROTEIN, NON-PURIN DIET.

Date.	Volume. c.c.	Total nitrogen.		Grams nitrogen as						Per cent of total nitrogen.				Remarks.
		gm.	per cent.	Urea.	Ammon.	Kreatinin.	Uric Ac.	Rest.	Urea.	Ammon.	Kreatinin.	Uric Ac.	Rest.	
Oct. 30	1300	11.74	9.78	0.87	0.26	0.14	0.69	83.3	7.4	2.2	1.2	5.9		
31	740	7.56	5.82	0.77	0.26	0.14	0.57	77.0	10.2	3.4	1.9	8.5		
Nov. 1	910	6.44	4.30	1.02	0.25	0.15	0.72	66.7	15.9	3.9	2.3	11.2	Bile absent from intestine.	
2	530	4.32	3.19	0.41	0.20	0.08	0.44	73.9	9.5	4.6	1.9	10.1	350 c.c. bile injected.	
3	785	4.86	3.23	0.75	0.24	0.07	0.57	66.6	15.4	4.9	1.4	11.7	350 c.c. bile injected.	
4	710	3.63	2.52	0.65	0.18	0.07	0.21	68.8	17.9	5.0	1.9	6.4	Bile absent from intestine.	
5	560	4.56	2.87	0.94	0.20	0.08	0.47	62.7	20.6	4.4	1.8	10.3	Bile absent from intestine.	
BILE ABSENT FROM INTESTINE (1, 4, and 5).														
Average . . .		4.88	3.23	0.87	0.21	0.10	0.47	66.3	17.8	4.3	2.05	9.6		
BILE INJECTION (2 and 3).														
Average . . .		4.59	3.21	0.58	0.22	0.075	0.50	70.0	12.6	4.8	1.6	10.9		
NORMAL (FOLIN).														
Average . . .		4.35	2.91	0.38	0.51	0.11	0.42	66.9	8.7	11.7	2.5	9.7		



TABLE II (Continued).  
LOW PROTEID DIET.

Date.	Indican.	Total phosphorus.	Total sulphur.	Inorganic sulphur.	Etheral sulphur.	Neutral sulphur.	Per cent of total sulphur.			Remarks.
							Inorganic.	Etheral.	Neutral.	
		gm.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	
Oct. 30	100	1.26	0.64	0.46	0.08	0.10	62.2	12.8	15.0	
31	100	0.98	0.42	0.26	0.08	0.09	61.2	18.1	20.7	
Nov. 1	100	0.94	0.37	0.21	0.09	0.07	56.8	24.3	18.9	Bile absent from intestine.
2	100	0.69	0.35	0.21	0.07	0.07	60.0	20.0	20.0	350 c.c. bile injected.
3	75	0.79	0.39	0.24	0.06	0.09	61.6	15.4	23.0	350 c.c. bile injected.
4	75	0.60	0.32	0.20	0.05	0.07	62.6	15.6	21.8	Bile absent from intestine.
5	100	0.55	0.34	0.22	0.05	0.08	61.8	14.7	23.5	Bile absent from intestine.
BILE ABSENT FROM INTESTINE (1, 4, and 5).										
Av. . . .	90	0.70	0.34	0.21	0.06	0.07	61.8	17.6	20.6	
BILE INJECTION (2 and 3).										
Av. . . .	87	0.74	0.37	0.22	0.065	0.08	59.5	17.6	22.0	
NORMAL (FOLIN).										
Av. . . .	0	0.47	0.30	0.19	0.036	0.07	63.3	12.0	23.3	

TABLE III.  
 CONSTANT MIXED DIET.

Date.	Volume of urine c.c.	Total nitro- gen. gm.	Grams nitrogen as					Per cent of total nitrogen							
			Urea.	Ammonia.	Kreatinin.	Kreatin.	Uric acid.	Ureide.	Urea.	Ammonia.	Urea + Ammonia.	Kreatinin.	Kreatin.	Uric acid.	Ureide.
			gm.	gm.	gm.	gm.	gm.	gm.	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Nov.															
16	1310	9.73	6.94	1.60	0.33	0.06	0.21	0.59	71.2	16.5	87.7	3.4	0.6	2.2	6.1
17	1045	8.66	6.15	1.25	0.32	0.08	0.22	0.64	71.1	14.4	85.5	3.7	0.9	2.5	7.4
18	1250	11.38	8.36	1.40	0.34	0.19	0.23	0.86	73.5	12.3	85.8	3.0	1.6	2.0	7.6
19	1215	11.18	8.31	1.32	0.36	0.13	0.20	0.86	74.4	11.8	86.2	3.2	1.2	1.8	7.0
20	1665	10.00	7.40	1.21	0.36	0.13	0.20	0.70	74.0	12.1	86.1	3.6	1.3	2.0	7.0
21	1320	9.23	6.90	1.10	0.31	0.07	0.17	0.68	74.8	11.9	86.7	3.4	0.8	1.8	7.3
22	1180 <sup>1</sup>	7.97	5.69	1.02	0.26	0.08	0.17	0.75	71.5	12.8	84.3	3.3	1.0	2.1	9.3
23	1330	10.50	8.01	1.00	0.35	0.12	0.20	0.82	76.4	9.5	85.9	3.3	1.1	1.9	8.8
24	860	8.84	6.51	0.83	0.28	0.15	0.20	0.87	73.7	9.4	83.1	3.2	1.7	2.2	9.8
25	900	9.25	6.87	0.87	0.30	0.15	0.21	0.85	74.3	9.4	83.7	3.2	1.6	2.3	9.2
26	1175	8.77	6.21	1.18	0.35	0.13	0.20	0.70	70.9	13.4	84.3	4.0	1.5	2.3	7.9
27	1090	8.09	8.09	1.41	0.30	0.16	0.21	0.73	74.3	12.9	87.2	2.8	1.5	1.9	6.6
28	970	12.48	8.57	1.85	0.47	0.15	0.26	1.18	68.9	14.8	83.7	3.8	1.2	2.1	9.2
30															
CONTROL (16, 17, 20, 21, 26, 27, and 29).															
Average		9.50	6.94	1.23	0.32	0.11	0.20	0.70	73.0	13.0	86.0	3.4	1.2	2.1	7.3
BILE INJECTION PERIOD (18 and 19).															
Average		11.28	8.34	1.36	0.35	0.16	0.22	0.86	73.8	12.05	85.9	3.1	1.4	1.6	7.7
OX BILE PERIOD (23, 24, and 25).															
Average		9.48	7.04	0.97	0.31	0.12	0.19	0.85	74.3	10.3	84.6	3.3	1.3	2.0	8.8

<sup>1</sup> Loss.

TABLE III (Continued).

Date	Indican	Total phosphates P <sub>1</sub>	Total sulphur S <sub>1</sub>	Inorganic S <sub>1</sub>		Etheral S <sub>1</sub>		Neutral S <sub>1</sub>		Per cent of total sulphur.			Remarks.
				gm.	gm.	gm.	gm.	gm.	per cent	per cent	per cent	Etheral	
Nov.													
16	50	0.66	0.72	0.48	0.09	0.15	12.7	20.8					
17	100	0.53	0.68	0.46	0.08	0.13	11.8	19.1					
18	100	0.70	0.73	0.51	0.10	0.13	13.2	69.1					
19	75	0.77	0.81	0.58	0.09	0.15	11.2	18.5					
20	25	0.68	0.70	0.46	0.08	0.16	10.7	23.1					
21	100	0.69	0.74	0.54	0.10	0.11	12.9	14.9					
22	100	0.60	0.62	0.40	0.07	0.15	63.7	11.8					
23	25	0.78	0.66	0.40	0.12	0.14	60.2	18.7					
24	75	0.88	0.75	0.45	0.11	0.19	60.0	14.9					
25	100	0.75	0.66	0.38	0.11	0.17	57.7	16.0					
26	100	0.70	0.68	0.42	0.09	0.17	61.9	13.2					
27	100	0.73	0.66	0.36	0.08	0.21	55.5	11.8					
28	150												
29	150	0.87	0.85	0.58	0.10	0.17	68.2	11.5					
30	100	1.03	0.87	0.54	0.14	0.18	62.8	16.1					
CONTROL (16, 17, 20, 21, 26, 27, and 29).													
Average	97	0.68	0.72	0.47	0.09	0.15	65.3	12.5				22.2	0.132
BILE INJECTION PERIOD (18 and 19).													
Average	87	0.735	0.77	0.545	0.095	0.14	70.3	11.6				18.1	0.147
OX BILE PERIOD (23, 24, and 25).													
Average	66	0.80	0.69	0.41	0.11	0.17	59.4	16.0				24.7	0.137

350 c.c. bile injected.  
 " " "  
 " " "  
 Loss.  
 5 gm. ox bile.  
 7 " " "  
 8 " " "

Total N  
 Total S.

As stated in the tables, varying amounts of bile were injected through the fistula on two days during each period. This was done in order to determine what effect the presence of bile in the intestines would have on the general metabolism as indicated by the composition of the urine. The days on each diet when no bile was injected were intended, first, to show any deviation from the normal metabolism which might be referable to the absence of bile from the intestine, and the consequent lack of its reabsorption and of the other processes which may be involved; and, second, to serve as control for the periods of bile injection.

The urine constituents which are of interest in this case are neutral sulphur, ethereal sulphur and indican, total nitrogen, urea and ammonia, and kreatinin.

#### NEUTRAL SULPHUR.

The belief appears to be generally held that the neutral sulphur of the urine is to a considerable extent derived from the taurin of the bile.<sup>1</sup> This is based upon the experiments of Kunkel,<sup>2</sup> who found a lowered excretion of neutral sulphur in dogs when bile was diverted from the intestine; and upon the experiments of Salkowski,<sup>3</sup> which showed that after taurin ingestion by man and dogs, it was to a great extent excreted in the urine unchanged, or as taurocarbominic acid, thus increasing the amount of neutral sulphur. If the above belief is correct, we should expect that the neutral sulphur excreted by a

inconsistent and antiquated  $\text{SO}_3$  and  $\text{P}_2\text{O}_5$ , or  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$ , or  $\text{NaCl}$ . The only objection to the plan of presenting results here used is that it differs in some particulars from that used in past papers on metabolism, both in America and abroad, and therefore may cause some temporary difficulty in the comparison of results of different authors. But this difficulty will at worst be no greater than is now usually encountered when one attempts to compare different results; and obviously, should this or a similar plan of presenting results of metabolism experiments be generally adopted, the difficulty would soon disappear or be reduced to a minimum.

Several investigators active in metabolism have agreed with the writer to present their results according to the above suggestions, and it is hoped that still other workers in this line will decide to do so.

Urea factor = 2.145  
Ammonia factor = 1.214

Kreatinin factor = 2.695  
Uric acid factor = 3.00

<sup>1</sup> HOPKINS: SCHAEFFER'S Text-book of physiology, i, p. 632.

<sup>2</sup> KUNKEL: Archiv für die gesammte Physiologie, 1877, xiv, p. 344.

<sup>3</sup> SALKOWSKI: VIRCHOW'S Archiv für pathologische Anatomie, 1873, lviii, p. 460.

patient with a permanent biliary fistula would be low, since no bile containing taurocholic acid would be present in the intestine to be there reabsorbed. Furthermore, if this belief is correct, we should expect the neutral sulphur excretion in such a patient to be increased following the injection of bile into the intestine, or ox bile medication. Regarding these points let us inspect the results in the tables, paying especial attention to the averages representing the several periods. Two rather surprising facts are apparent: first, in Tables I and II, from the high proteid and low proteid diets respectively, the neutral sulphur excretion without bile in the intestine is decidedly just as high as Folin's published results from the normal. Folin's average is 1.32 gm. total sulphur with 0.07 gm. neutral sulphur; the neutral sulphur is thus 5.1 per cent of the total. The average excretion of my patient on practically the same diet, without bile in the intestines is 1.29 gm. total sulphur and 0.12 gm. neutral sulphur, the latter being 9.3 per cent of the total. On this high proteid diet the excretion of neutral sulphur, while the influence of bile was excluded, was even higher than Folin's average, though it is not abnormally high. On comparison with Folin's average for the normal given above, I at first concluded that the neutral sulphur excretion from this case was abnormally high. However, in a personal letter from Dr. Folin regarding his sulphur results on the high proteid diet, he makes the following statement, which with his permission I take pleasure in quoting:

"Renewed investigations of the sulphur metabolism, by the help of the improved analytical technique, have shown that the absolute amount of neutral sulphur eliminated is, after all, to some extent dependent on the sulphur of the food. The variations in the neutral sulphur elimination on nitrogen rich and nitrogen free diets are nearly if not quite as great as the corresponding variations in the elimination of uric acid. Even when I wrote my paper on the laws governing the composition of urine, it seemed to me rather remarkable, not to say suspicious, that such a miscellaneous group of substances as the neutral sulphur must be supposed to represent should exhibit such a constancy. With the technique then in use I was, however, unable to show that the neutral sulphur of the urine was to any extent immediately derived from the sulphur of the food."

In view of Folin's recent results I have used his corrected average<sup>1</sup> for comparison with the figures from this patient while on the high proteid diet, and with his corrected averages my results agree.

<sup>1</sup> Given in parenthesis in Table I, p. 269.

On the low proteid diet, without bile in the gut, the neutral sulphur also agrees well with Folin's average. The absolute amount of neutral sulphur is practically the same as Folin's average, and the difference between the neutral sulphur percentages is well within the normal variation.

For the mixed diet (Table III) I have no normal standard for comparison, but the neutral sulphur is perhaps high even when bile was absent from the intestine.<sup>1</sup> In these experiments, therefore, we certainly find no indication of a lowered excretion of neutral sulphur when bile is diverted from the intestine.

The question whether the neutral sulphur excretion is increased when bile is again introduced into the intestine is likewise answered by these experiments, in the negative. The amount of bile injected during the first experiment (100 c.c.) was too small, and contained only 0.023 gm. sulphur each day. This is perhaps hardly enough to make us expect any increase of neutral sulphur in the urine. At 10 P. M. on November 1 and 2, 350 c.c. of the patient's bile containing 6.4 gm. of solids and 0.054 gm. sulphur were injected each day. The average neutral sulphur in the urine from these two days is 0.08 gm., and the average of the three control days is 0.07 gm. The increase is too slight to justify the conclusion that the bile is responsible. During the third experiment at 10 P. M. on November 17, and again on the 18th, 350 c.c. bile was again injected. This bile contained 7.1 gm. solids and 0.057 gm. sulphur. The neutral sulphur on the days following this injection is not higher than on the control days, when bile was absent from the intestine. The average (0.14 gm.) is even lower than the control average (0.16 gm.).

During November 23, 24, and 25, the patient took by mouth 20 gm. of dried ox bile.<sup>2</sup> The average neutral sulphur for these days is 0.17 gm. I consider the slight increase over the control average quite incidental.

It may be possible that the sulphur contained in the ox bile and in

<sup>1</sup> On the mixed diet the neutral sulphur is decidedly higher than on either the high or low proteid diets. To what is this increase due? During the third experiment the patient walked two miles each day. Is the increased neutral sulphur due to this increased muscular activity, or is it due to some sulphur compounds of the meat eaten? H. BENEDICT (*Zeitschrift für klinische Medizin*, 1899, xxxvi, p. 281) found that the amount of neutral sulphur was not increased by muscular work, or indeed by an increased consumption of body proteid.

<sup>2</sup> Unfortunately, an analysis of this bile was not made. Another sample of the same brand purchased later contained in 20 gm. 0.246 gm. sulphur.

the human bile was not sufficient to cause any great increase in the neutral sulphur of the urine, but this is not probable. Had all the sulphur of the injected bile been excreted as neutral sulphur in the urine, the latter would have been increased 75 per cent on the low proteid diet and about 40 per cent on the mixed diet. On the low proteid diet the increase was only 14 per cent, while on the mixed diet there was a decrease of neutral sulphur equivalent to 12 per cent. The increase following ox bile medication is only 6 per cent. These variations are no greater than are encountered in normal subjects. A stronger argument that the sulphur of the bile is not the source of the neutral sulphur of the urine is, however, our finding that the neutral sulphur from this subject is not lower than normal when bile is excluded from the intestine and is flowing through the fistula.<sup>1</sup>

*I can find no evidence in these experiments that the sulphur of the bile is to any considerable degree the source of the neutral sulphur of the urine.*

This conclusion is, of course, quite contradictory to the common belief stated above. Kunkel claimed that the excretion of neutral sulphur in its relation to total sulphur was decreased in dogs with biliary fistula; and further showing his confidence in the dependence of the neutral sulphur upon the sulphur of the bile, he claimed that the sum of the sulphur of the bile passed through the fistula and the remaining neutral sulphur of the urine equalled the amount of neutral sulphur excreted under normal conditions. In other words, he evidently believed that all of the sulphur of the bile regularly appears each day as neutral sulphur in the urine. Were this true, we should have found the neutral sulphur excretion in this case lower than the normal when the bile was flowing through the fistula, and increased by the amount of the sulphur contained in the bile, after the injection of the patient's own bile back through the fistula. This we did not find.

If after further experiments it should prove to be a fact, as my results indicate, that the neutral sulphur of the urine is *not*, to any considerable extent, derived from the taurin of the bile, we have yet to explain the results recorded in the literature, that the neutral sulphur excretion in cases of chronic icterus, and in similar conditions experimentally produced in animals by ligating the common duct or otherwise, are higher than normal.

<sup>1</sup> SPIRO (Archiv für Physiologie, Leipzig, suppl. Bd., 1880, p. 50) found in the urine of dogs with biliary fistula only from 50 to 81 per cent of the total sulphur as simple sulphates, even when all bile was absent from the intestine.

Fr. Müller,<sup>1</sup> in his series of experiments on cases of icterus, found in a case of cholelithiasis (Experiment 5) on a milk diet containing 48.85 gm. N in three days, or 16.28 gm. N per day, the following amounts of sulphur in the urine:

	Total N	Total S	Total Sulphates	Ethereal S	Neutral S
Total for three days . . .	47.63	4.04	3.38	0.264	0.659
Average per day . . .	15.88	1.35	1.13	0.088	0.220
Per cent of total S . . .			83.7	6.5	17.0

The same patient on a meat diet containing an average of 22.5 gm. N per day excreted in the urine:

	Total N	Total S	Total Sulphates	Ethereal S	Neutral S
Total for two days . . .		3.07	2.52	0.32	0.55
Average per day . . .	17.18	1.53	1.26	0.16	0.275
Per cent of total S . . .			82.4	10.4	17.6

Müller's results were expressed as  $H_2SO_4$ . I have recalculated them as sulphur.

Müller distinctly states in his paper that the neutral sulphur in these cases is not higher than normal, though the correctness of his conclusion is open to some doubt. Certainly the neutral sulphur in the two cases quoted is decidedly higher than Folin's average from normal individuals (0.102 gm.).

Lépine<sup>2</sup> and his coworkers have also shown, in cases of chronic icterus and in dogs with the common bile duct ligated, that the neutral sulphur excretion is relatively higher than normal. While these results are lacking in a satisfactory basis for comparison with the normal, it may fairly be concluded that the neutral sulphur excretion in such conditions is high.

The reason for this high neutral sulphur is perhaps to be found in the fact that when the bile passages are occluded, the bile, instead of flowing into the intestine or being excreted through a fistula, as in the subject of my experiments, was being absorbed from the bile passages by the lymph and blood, causing icterus, and thus being diverted from the normal circulation of the bile ("Kreislaufe

<sup>1</sup> MÜLLER: Zeitschrift für klinische Medizin, 1887, xii, p. 45

<sup>2</sup> LÉPINE: Revue de médecine, 1881, pp. 27, 911. MALY'S Jahresbericht über die Fortschritte der Thierchemie, 1882, xi, p. 327. LÉPINE and GUÉRIN: Comptes rendus de la société de biologie, xcvi, p. 1074.



der Galle"). Benedict<sup>1</sup> thinks that the high neutral sulphur cannot be explained by such direct absorption into the blood, because, he says, the absolute amount of neutral sulphur of the urine coming from the bile would be the same, whether absorbed from the intestine as normally, or absorbed from the bile passages. With this latter conclusion it is difficult to agree.

It seems to me much more reasonable to believe that when the bile is diverted from its normal circulation by means of an obstruction in the bile passages, and is there directly reabsorbed without passing first to the liver, the sulphur compounds of the bile may be more or less rapidly excreted from the blood by the kidneys, thus causing an increase of the neutral sulphur of the urine; whereas normally the sulphur components of the bile are absorbed from the intestine and go by way of the portal vein direct to the liver, there to be retained and again excreted in fresh bile.

According to this conception the taurin of the bile is not normally a source of the neutral sulphur of the urine, but becomes such when the bile is under pathological conditions reabsorbed directly into the circulation from the bile passages without passing first through the liver.

There are few observations in the literature throwing any light on the correctness of this idea. Salkowski,<sup>2</sup> on feeding taurin to man and dogs, found the greater part excreted in the urine as taurocarbominic acid. This result is not necessarily contradictory to the above idea. It is quite conceivable that when a great excess of taurin without a corresponding excess of other bile constituents (cholalic acid) is absorbed from the gut and carried to the liver, the taurin is not converted into taurocholic acid and excreted in the bile, because of the lack of the necessary cholalic acid, but is circulated in the blood and removed therefrom by the kidneys. This explanation was suggested by Salkowski in the paper above cited.

Bergmann<sup>3</sup> has recently shown that cystin alone fed to dogs with biliary fistulæ does not increase the taurin of the bile, but when sodium cholate is fed with cystin, the taurin is much increased. Whether this result may be applied to man is open to doubt, since, according to Loewy and Neuberg,<sup>4</sup> the sulphur of cystin fed to a

<sup>1</sup> BENEDICT, H.: *Zeitschrift für klinische Medizin*, 1898, xxxvi, p. 286.

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

<sup>3</sup> BERGMANN: HOFMEISTER'S Beiträge, 1904, iv, p. 192.

<sup>4</sup> LOEWY and NEUBERG: *Zeitschrift für physiologische Chemie*, 1904, liiii, p. 342.

normal man was excreted in the urine as sulphates and thiosulphates; but in the absence of further evidence we may accept Bergmann's result as supporting Salkowski's suggestion.

#### ETHEREAL SULPHATES AND INDICAN.

The amount of ethereal sulphates and indican excreted by this patient is of interest in connection with the supposed preventive action of bile on intestinal putrefaction. Whether bile has such an action, and the manner of it, has been under dispute. The more recent belief appears to be that the presence of bile in the gut retards putrefaction only indirectly by assisting the absorption of fats and thus allowing the digestive juices to come into contact with the proteids, and preventing their accumulation in the intestine.<sup>1</sup>

As regards the value of the amount of ethereal sulphates or indican as an indication of the degree of intestinal putrefaction, there has been much controversy.<sup>2</sup> Folin's conclusions,<sup>3</sup> stated below, seem to me correct, and are largely confirmed by some of my own unpublished results.

"(1) The urinary indican is not to any extent a product of the general protein metabolism, is therefore probably, as is generally supposed, a product of intestinal putrefaction, and may consequently be assumed to indicate approximately the degree of putrefaction in the intestinal tract.

"(2) The ethereal sulphates can only in part be due to intestinal putrefaction, and neither their absolute nor their relative amount can be accepted as an index of the extent to which putrefaction is taking place in the intestines."

On inspecting the results shown in Tables I, II, and III, we find that on the high proteid diet and without bile in the intestine, the patient excreted an average of 0.10 gm. sulphur as ethereal sulphates as compared with Folin's normal average of 0.09 gm. On the low proteid diet there was a decrease to an average of 0.063 gm. as compared with Folin's average of 0.036 gm. The slight decrease from the amount excreted on the high proteid diet confirms in some measure the conclusion of Folin that the ethereal sulphur decreases with the total sulphur. The increase over Folin's average on the

<sup>1</sup> HAMMERSTEN: *Physiological chemistry*, New York, 1904, p. 339.

<sup>2</sup> HAMMERSTEN: *Ibid.*, pp. 503, 504.

<sup>3</sup> FOLIN: *This journal*, 1905, xiii, p. 99.

low proteid diet, on the other hand, taken in conjunction with the high indican, as compared with Folin's zero, is probably indicative of an increased intestinal putrefaction caused by the absence of bile.

What effect did the injection of bile and ox bile medication have on the ethereal sulphur and indican? There appears to have been no effect upon the ethereal sulphur. This is certainly true for the high proteid and mixed diets (Tables I and III); on the low proteid diet the conclusion is not so clear. The decrease there noted is probably to be ascribed to the normal decrease with the diminution of total sulphur as found by Folin, and not to an effect on the bile injection.<sup>1</sup>

Regarding indican, the bile did effect a slight though distinct decrease; and this decrease is probably to be explained by a diminished intestinal putrefaction. As was to have been expected, the indican was on the whole quite high, and even following bile injection hardly reached the normal.

#### TOTAL NITROGEN.

On the high proteid diet the patient's absorption of proteids was good, shown by the average of the total nitrogen of the urine for the last three days of this period, 19.2 gm., which is 92.3 per cent of the nitrogen of the food. There is some indication that the absorption of proteid was better following bile injection, though I do not feel justified in positively concluding from these experiments that this is so. The increased amount of nitrogen in the first experiment, following bile injection for instance, might well be the result of a slow attainment of nitrogen equilibrium.

Joslin,<sup>2</sup> in experiments upon a case very similar to the subject of these experiments, found an absorption of 93 per cent of the proteid of the food after ox bile medication (30 gm. Fel. taurin in four days) as compared with 81 per cent before and 88.7 per cent following the bile period. The same patient some months later, after a second operation when the bile ducts had been made patent, absorbed 88 per cent of the proteid in the food. The difference in the absorption of proteid with and without bile in the intestine was therefore very slight. There is no satisfactory evidence that bile is concerned in the absorption of proteid.

<sup>1</sup> Except in so far as the decrease of indican above mentioned was accompanied by the decrease of sulphur in the indoxyl-sulphate.

<sup>2</sup> JOSLIN: *Journal of experimental medicine*, 1901, v, p. 513.

## UREA AND AMMONIA.

Based on his experiments upon normal persons, already frequently referred to, Folin has put forward the following "laws": "Urea [is] the only nitrogenous substance [of the urine] which suffers a relative as well as an absolute diminution in the total protein-metabolism."<sup>1</sup> Ammonia: "with pronounced diminution in the protein-metabolism (as shown by the total nitrogen in the urine), there is usually but not always, and therefore not necessarily, a decrease in the absolute quantity of ammonia eliminated. A pronounced reduction of the total nitrogen is however always accompanied by a relative increase of the ammonia nitrogen, provided that the food is not such as to yield an alkaline ash."<sup>2</sup>

Both these statements are confirmed by the results in Tables I and II; the absolute amount of urea nitrogen and likewise its percentage of the total nitrogen are decreased on changing from a high to a low proteid diet. The figures for urea compare well on both diets with Folin's average.

Ammonia decreases in absolute amount and its percentage increases, as Folin found; but on both diets the absolute amount of ammonia nitrogen is considerably higher than the normal. On the high proteid diet this patient excreted an average of 1.43 gm. and 1.58 gm. ammonia nitrogen as compared with Folin's 84 gm. On the low proteid diet without bile in the intestine, we find 0.87 gm. ammonia nitrogen, while Folin's average is 0.38 gm. On the mixed diet without bile in the intestine, we find an average of 1.23 gm. ammonia nitrogen. The percentages of the total nitrogen likewise show that the ammonia excreted by this case is higher than the normal upon the same diets. The reason for this I do not know. It can scarcely be ascribed to the condition of acidosis, since no acetone substances appeared in the urine. Schilling<sup>3</sup> recently confirmed the earlier observations that an increased ammonia excretion followed the injection of large amounts of fats. And Brugsch<sup>4</sup> has shown that acetone substances appeared in the urine of a fasting person who had considerable adipose tissue; while these substances were not excreted

<sup>1</sup> FOLIN: This journal, 1905, xiii, p. 94.

<sup>2</sup> FOLIN: *Loc. cit.*, p. 92.

<sup>3</sup> SCHILLING: *Archiv für klinische Medizin*, 1905, lxxiv, p. 327.

<sup>4</sup> BRUGSCH: *Zeitschrift für experimentelle Pathologie und Therapie*, 1905, i, p. 419.

by a lean and emaciated patient who had taken no food for twelve days. Brugsch concludes that these substances are excreted only when there is a marked consumption of fatty tissue. Neither of these explanations can properly be applied to this case, because the fats of the food were poorly absorbed, and because the patient had only very little adipose tissue.

What effect had bile injection on the ammonia excretion?

The result is negative on the high proteid diet, perhaps because the amount of bile injected was too small (100 c.c. each day). On the low proteid and mixed diets the averages are as follows:

		Total N	NH <sub>3</sub> -N	Per cent of total N
Low proteid diet	Bile absent from intestine	4.88	0.87	17.8
	Bile injection	4.59	0.58	12.6
Mixed diet	Bile absent from intestine	9.50	1.23	13.0
	Bile injection	11.28	1.36	12.05
	Ox bile medication	9.48	0.97	10.3

On the low proteid diet, when more bile (350 c.c. each day) was injected than during the high proteid diet, there is a fairly marked decrease of ammonia nitrogen, both absolute and in per cent, of the total nitrogen following bile injection. On the mixed diet the ammonia nitrogen was not decreased after bile injection, thus throwing some doubt upon the result with the low proteid diet. After ox bile medication, however, there is a decrease of ammonia nitrogen. The results are not conclusive on this point.

Another possible and perhaps probable cause of the high ammonia may have been an excessive formation of organic acids. Unfortunately I did not make separate determinations of the "mineral acidity" and the organic acids, and therefore have no data bearing on this possibility.

#### KREATININ.

The kreatinin excretion in this case is uniformly low as compared with Folin's normal, but it is by no means likely that this is in any way connected with the function of bile in metabolism. There was neither increase nor decrease following bile injection or ox bile medication, and we may safely conclude that the bile plays no part in kreatinin formation. This low kreatinin excretion will be discussed

in connection with other more recent results in a future paper from this laboratory.

#### FÆCES.

The analyses of the fæces are given in Table IV. The per cent of dried substance varied between 12 and 22 per cent. The amount of nitrogen in the fæces is even lower than figures given for the normal<sup>1</sup> and justifies the rather generally accepted statements that the proteid absorption is neither prevented nor retarded by the absence of bile. There is no decrease of nitrogen in the fæces following bile injection or ox bile medication. Joslin,<sup>2</sup> as already stated, found a decrease of nitrogen of fæces during ox bile medication. However, the need for improving the absorption of proteid in such cases is small, as shown by the small amount of nitrogen excreted in the fæces. The large amount of fat in the stools of this case confirms this common observation, but the amount of fat does not seem to have been diminished by bile injection or ox bile medication. This is not in accord with the results of Joslin, who found a decided decrease of fat in the stools during ox bile medication. Our patient took 20 gm. of dried ox bile in three days, while Joslin's patient was given 30 gm. in four days. I cannot explain the difference in results. It might be that there are other factors necessary besides the bile, and such factors may have been present in Joslin's case and absent in ours. At any rate, in view of the clinician's evidence and of Joslin's experiment, ox bile may be of therapeutic value in such conditions.<sup>3</sup>

#### BILE.

Analyses of the bile passed through the fistula during the three experiments are given in Table V. The results do not indicate the amount of bile normally excreted into the intestine by a healthy individual, because, as has been pointed out, fistula bile is always less than the amount normally secreted because of the lack of the bile normally reabsorbed from the intestine. These results are of interest regarding other points, however. By comparing the bile excreted on the high proteid diet with that excreted on the low proteid diet, we should

<sup>1</sup> SCHMIDT and STRASSBURGER: *Die Faeces des Menschen*, Berlin, 1905, pp. 119-124.

<sup>2</sup> JOSLIN: *Journal of experimental medicine*, 1901, v, p. 513.

<sup>3</sup> Dr. BROWN tells me that she has noted some general improvement in this case, especially of the diarrhœa, from the use of ox bile.

TABLE IV.

FÆCES.

Date.	Total dried.	Nitrogen.		Total fat.		Hydrobilirubin.
		gm.	per cent.	gm.	per cent.	
Oct. 24	71.5	2.28	1.6	....	....	trace
25	50.0	1.96	1.0	40.0	20.0	"
26	65.5	1.63	1.1	59.5	39.0	"
27	59.5	1.62	1.0	40.0	23.8	"
28	56.0	1.64	0.9	47.2	26.5	"
29	53.5	1.66	0.9	47.1	25.2	"
30	82.5	1.62	1.3	31.2	25.7	"
31	No movement.		....	....	....	....
Nov. 1	58.0	2.46	1.4	34.4	20.0	trace
2	58.5	2.62	1.5	59.0	34.5	+
3	55.5	2.02	1.1	....	....	+
4	40.0	2.14	0.8	57.8	23.1	+
5	46.3	2.13	1.0	63.8	29.5	+
Av. 7-9	55.0	2.31	1.3	....	....	++
14	....	1.94	....	68.8	....	+
15	....	2.37	....	....	....	+
16	42.5	2.32	1.0	61.2	26.0	+
17	50.2	2.40	1.2	61.6	31.0	+
18	52.0	2.76	1.4	61.2	31.8	++
19	37.3	2.54	0.95	61.3	22.9	++
20	72.3	2.43	1.7	62.7	45.4	++
21	50.0	2.59	1.3	56.7	28.3	+
22	59.0	2.24	1.3	58.6	34.5	trace
23	33.6	2.49	0.8	....	....	"
24	65.0	2.66	1.7	....	....	very slight
25	66.5	2.45	1.6	64.0	42.5	"
26	58.5	2.88	1.7	57.0	33.3	+
27	57.5	2.77	1.6	61.0	35.0	++
28	39.0	2.96	1.15	....	....	+
29	47.0	2.14	1.0	62.2	29.2	+
30	65.0	2.88	1.9	....	....	trace

TABLE V.  
ANALYSES OF BILE.

Date.	Volume, <sup>1</sup> c.c.	Specific gravity.	Average per day.						Nitrogen in urine. gm.	Sulphur in urine. gm.	Remarks.
			Solids.		Nitrogen.		Sulphur.				
			per cent.	gm.	per cent.	gm.	per cent.	gm.			
Oct. 23	200	1.0095									High proteid diet.
24	200	1.010	1.79	3.76	0.074	0.155	0.015	0.032	12.3	0.94	
25	231	1.010							15.55	1.21	Bile injection. "
26	?	1.015	4.02						19.70	1.50	
27	213	1.012							18.70	1.43	Bile injection. "
28	240	1.012	2.87	6.50	0.163	0.368	0.023	0.052	19.20	1.48	
29	Lost.								11.74	0.64	
30	213	1.0105							7.56	0.42	Low proteid diet.
31	240	1.0085	1.825	4.29	0.096	0.225	0.015	0.035	6.44	0.37	
Nov. 1	250	1.0095							4.32	0.35	Bile injection. "
2	?								4.86	0.39	
3	?	1.0105	2.07	5.8	0.111	0.310	0.018	0.050	3.63	0.32	
4	280	1.010							4.56	0.34	
5	240		2.14	5.13	0.11	0.264	0.024	0.058			





be able to see the effect of food on bile secretion. The absence of any decrease of either solids, nitrogen, or sulphur of the bile on passing from a diet containing more than 20 gm. of nitrogen to one containing about 4 gm. is very striking. As a matter of fact, there was an increase instead of a decrease; but to this increase I attach no significance. It is certainly quite remarkable that the liver, which is so active in the formation of nitrogenous and perhaps of sulphur products of metabolism and digestion, secretes little if any more nitrogen and sulphur in the bile when the urine contains 15-19 gm. of nitrogen than it does when the urine contains only 4-6 gm. Yet this fact seems to be fairly well established. Spiro<sup>1</sup> in experiments on dogs with biliary fistula, found relatively only a slight increase of sulphur (0.059 to 0.173 gm.) and nitrogen (0.195 to 0.604 gm.) in the bile when the dogs were changed from complete fast to 1000 gm. meat. The nitrogen in the urine varied between 3.12 and 27.49 gm.

Kunkel,<sup>2</sup> in similar experiments on dogs, found that the per cent of the food sulphur appearing in the bile decreased very considerably with the increase of food sulphur. On a high proteid diet 8 per cent of the food sulphur appeared in the bile, while 30 per cent was excreted in the bile on low proteid diet. Both these authors concluded that processes forming taurin are quite independent of those forming the sulphates of the urine, and that there is only very little relation between the amount of proteid in the food and the nitrogen and sulphur excreted in the bile.<sup>3</sup>

But Wohlgemuth<sup>4</sup> and Bergmann<sup>5</sup> have concluded that the taurin of the bile is derived from proteid. Bergmann's result that feeding cystin without sodium cholate does not lead to increased taurin, together with the fact that increase of food proteid leads to little or no increase of taurin, suggests the question whether the cholic acid is derived from food proteid, food carbohydrate, or is itself a product of metabolism in the strictest sense. If the cholic acid is directly derived from food proteids, as these authors believe to be the case for taurin, it is not clear why increased food proteid did not lead to an

<sup>1</sup> SPIRO: *Archiv für Physiologie*, Leipzig, suppl. Bd., 1880, p. 50.

<sup>2</sup> KUNKEL: *Archiv für die gesammte Physiologie*, 1877, xiv, p. 344.

<sup>3</sup> BARBIERA has also found that the amount of nitrogen in the bile is independent of the nitrogen in the food, the former being about the same after a diet of fat as it is after a high proteid diet. (*Annali di chimica e farmacologia*, Dec., 1894; MALY'S *Jahresbericht*, 1894, xxiv, p. 381.)

<sup>4</sup> WOHLGEMUTH: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 82.

<sup>5</sup> BERGMANN: *HOFMEISTER'S Beiträge*, 1904, iv, p. 192.

increase of sulphur as taurocholic acid in my experiment, or to a still greater increase of bile sulphur in the experiments of Kunkel and Spiro. Further experiments must decide the source of cholic acid. There are doubtless differences characteristic of animal species which should be considered in applying results from experiments on dogs to omnivorous or herbivorous animals.

What effect had bile injection and ox bile medication on the amount of solids, nitrogen, and sulphur in the bile? After each injection of bile through the fistula, as well as after ox bile medication, the amounts of solids, nitrogen, and sulphur were increased. This increase, although not great, is quite considerable, and agrees with the results of Stadelmann, Pfaff and Balch, and others. The experiments of Pfaff and Balch<sup>1</sup> are particularly effective in proving that ox bile or human bile medication increases the amount and composition of the bile. The manner of this action would appear, however, to be not a stimulation of the liver to form fresh bile from the ordinary sources, but merely in furnishing the liver with the ready-made bile constituents, which it separates from the other material absorbed from the intestines, and excretes through the proper channels. If bile as a cholagogue has a stimulating effect on the liver, we should properly expect the amount of bile excreted after its administration to be rather more than the sum of bile administered and the average amount passed before bile medication. This is not the case. In my experiments, for instance, on November 1 and 2, bile containing 6.4 gm. solids was injected each day, which amount added to 4.29 (the average bile solids for October 30, 31, and November 1) gives 10.69 gm., the minimum to be expected if the bile injected caused more fresh bile to be secreted. The average solids following this injection was 5.8 gm. Similarly, if the 20 gm. of dried ox bile be divided between the four days the bile for which was analyzed for the "ox bile period," we have 5 gm. + 4.86 gm. (average of control days) = 9.86 gm. per day. We found an average of 7.95 gm. of bile solids excreted.

Pfaff and Balch's results show the same thing,<sup>2</sup> namely, that after

<sup>1</sup> PFAFF and BALCH: *Journal of experimental medicine*, 1897, ii, p. 49.

<sup>2</sup> In PFAFF and BALCH's experiments during thirty-seven days, without medication, an average of 4.64 gm. bile solids, exclusive of ash, was excreted per day. During twelve days of human bile medication an average of 350 c.c. of the patient's bile (dried), containing 2.8 gm. solids exclusive of ash, was given per day. The average bile solids exclusive of ash excreted during this period was 5.795 gm. per day, decidedly less than the sum of 4.64 and 2.8, or 7.44 gm. About the same relation is seen in the averages of ox bile medication: an average of 7.85 bile

bile medication more bile is excreted, but the amount of solids is not so great as the sum of that excreted before medication and that administered. This is admittedly a faulty line of reasoning, because, instead of absolute amounts of bile salts, the substances in question, I have used the amounts of total solids, including inorganic salts. The amount of bile salts has not been determined in these experiments. But the same conclusion is reached from a consideration of either the total nitrogen or the total sulphur of the bile, and this conclusion, I believe, will be found to be correct: that while bile is a colagogue, it is not an hepatic stimulant.

#### SUMMARY.

1. The patient's metabolism appeared to be normal, except for the poor absorption of fats, the high excretion of indican, doubtless caused by an increased intestinal putrefaction, the high excretion of ammonia, and the low excretion of kreatinin. The kreatinin excretion was not affected by the absence of bile from the intestine, and a discussion of it is left to a future paper. The cause of the high ammonia in this urine is not understood.

2. The relation between the neutral sulphur of the urine and the taurin of the bile is discussed and the conclusion reached, that normally the taurin of the bile is not to any considerable extent the source of the neutral sulphur of the urine. The suggestion is made that in icterus, however, the increase of neutral sulphur may be derived from taurin which has been absorbed from the bile passages directly into the circulation, and has thus been diverted from the normal "Kreislaufe der Galle." The neutral sulphur of the urine from this patient was not lower than the normal, and was not increased when bile was injected through the fistula, or following ox bile medication.

solids exclusive of ash was given as 11.09 exclusive of ash excreted. Had even all of the bile taken been excreted again as bile, we should have an average of  $4.64 + 7.85 = 12.49$ . That so much was not excreted is doubtless to be explained by an incomplete absorption from the intestine. Only during bile salt medication is there indication that more bile salts were excreted than the sum of that given and the average without medication. According to their analyses 34.52 per cent of the bile solids excreted during the period of bile salt medication represents bile salts. This amounts to an average of 4.04 gm. per day, while the average bile salts excreted without medication was 2.11 gm. per day. An average of 1.41 gm. bile salts was given each day. Whether there was here an actual stimulation of the liver to form new bile cannot be decided without further experiment.

3. The amount of fat lost in the fæces was high, and was not decreased by bile injection or ox bile medication. The absorption of proteids was good, even without bile in the intestine.

4. Analyses of the amount of solids, nitrogen, and sulphur in the bile are given, and their relation to the amount of proteid in the food is discussed. The results show no relation between the composition of the bile and the amount of food proteid.

5. The excretion of bile was increased after bile injection and ox bile medication. The method of bile's action as a colagogue appears to be merely in furnishing the liver with ready-made bile constituents which it excretes by the proper channel, the bile passages. Bile does not appear to cause the liver to secrete strictly new bile, and therefore is probably not a liver stimulant.

6. Some of Folin's results regarding normal metabolism are confirmed.

# STUDIES OF THE PERISTALSIS OF THE URETER OF DOGS BY THE GRAPHIC METHOD.

BY D. R. LUCAS.

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## INTRODUCTORY.

OUR present knowledge of the movements of the ureter dates back to the classical studies of Engelmann,<sup>1</sup> published about thirty-seven years ago. His careful studies were made by simple ocular observations of the movements of the ureter in the dog, cat, rabbit, and rat. Engelmann made the statement that in the dog the movements might be studied by the graphic method; he, however, did not carry out such experiments. The studies by Sokoloff and Luchsinger,<sup>2</sup> which were published about a dozen years later, were also made by simple inspection. These observers removed the ureter from the animal and studied the influence of the intraureteral pressure upon the rate of contractions, the ureter being immersed in a saline bath. The next experimental studies, made directly upon the ureter, were reported by Fagge<sup>3</sup> only a few years ago. They were carried out on dogs, and were conducted by the graphic method. L. Stern<sup>4</sup> studied recently by the graphic method the movements of the ureter of guinea pigs. The ureter was removed from the body and kept in warm solution of 0.8 per cent sodium chloride.

Besides these direct investigations of the behavior of the ureter, we find incidental remarks on the contractions of this organ in numerous papers upon the so-called ureter pressure. In these latter experiments, which aimed chiefly at a study of the factors in renal secretion, the ureter was connected with a mercury manometer.

<sup>1</sup> ENGELMANN: *Archiv für die gesammte Physiologie*, 1869, ii, p. 243.

<sup>2</sup> SOKOLOFF and LUCHSINGER: *Ibid.*, 1881, xxvi, p. 464.

<sup>3</sup> FAGGE, C. H.: *Journal of physiology*, 1902, xxvii, p. 304.

<sup>4</sup> STERN, L.: *Thèse de Geneva*, 1903.

Some observers took notice of certain undulations of the mercury which appeared to be due to the contractions of the ureter. Henderson<sup>1</sup> gave a condensed account of the changes in the ureter pressure so far as they were due to the contractions of the ureter. He published no tracings. Frey<sup>2</sup> recently observed frequent fine undulations of the mercury meniscus in the manometer while the pressure was gradually increasing. A curve of these fine, hardly visible undulations was reproduced. These rhythmic waves were less frequent than the respirations, and Frey ascribed them to the contractions of the ureter.

Fagge, who was, as stated above, the first one to obtain graphic presentation of the ureter contractions, gave only a short account of his observations. His method consisted in introducing a very fine silver cannula from the interior of the bladder into the lumen of one ureter. Over the perforated end of the cannula was fixed a small rubber capsule, and in one or two cases the balloon was introduced through a longitudinal slit about the middle of the ureter. It may be remarked here that such a balloon in the narrow lumen of the ureter probably offers considerable resistance to the flow of the urine, which is surely not without influence upon the rate and character of the ureteral waves. Fagge stated that the ureter in many cases was found to be motionless or to present slight contractions recurring at intervals of thirty to sixty seconds. Stimulation of the hypogastric nerve was always followed by a motor effect upon the ureter which consisted either of a quickening of the normal rhythm of contraction or of a production of groups of contractions in a previously motionless ureter. Fagge published two short tracings illustrating these effects.

As a study of the literature shows, the functional activities of the ureter have received very little attention.<sup>3</sup> Consequently, this field seemed to me to offer favorable opportunities for experimental research. For the past two years or more I have been engaged in the investigation of several problems connected with the physiology and pharmacology of the ureter.<sup>4</sup> The work was carried on in the labora-

<sup>1</sup> HENDERSON, V. E.: *Journal of physiology*, 1905-1906, xxxiii, p. 175.

<sup>2</sup> FREY, ERNST: *Archiv für die gesammte Physiologie*, 1906, cxii, p. 71.

<sup>3</sup> The so-called ureter pressure which was the subject of many studies is, as already pointed out by Henderson, a misnomer. In the investigations referred to, it was not the pressure of the ureter that was studied, but the pressure of the kidney secretion as observed in a manometer tied in the ureter.

<sup>4</sup> A number of observations made by me on the physiology and pharmacology

tory of Physiological Chemistry of Columbia University at the College of Physicians and Surgeons. In the present paper, however, I shall confine my statements to a report of the results of a further study of the normal peristalsis of the ureter of the dog by the graphic method, chiefly carried out last summer under the direction of Dr. S. J. Meltzer in the Rockefeller Institute for Medical Research.

#### EXPERIMENTAL.

**Methods.**—The earlier experiments which led up to those herein described were made on dogs. As a rule, large animals were used. Different methods of anesthesia were employed: with ether, chloroform, morphin and atropin, decerebration, intraspinal cocainization, subcutaneous injection of magnesium sulphate, etc. The results which are communicated in this paper, however, were obtained exclusively under morphin anesthesia. Chloroform or ether were employed only for the purpose of studying their effects upon the ureteral peristalsis.

The animal having been anesthetized and tracheotomized, the ureter was exposed by an incision along the linea alba from symphysis pubis to ensiform cartilage. The abdominal walls were then retracted, the intestines were drawn to one side, and the viscera as well as the rest of the animal were covered with warm towels and cotton. When the trocar method (to be described later) was to be employed, the kidney was exposed by another incision, along the lower border of the last rib, or by a small longitudinal incision directly over the kidney. The left kidney was used when this method was employed, on account of its lower and more accessible position.

The principle I have employed in obtaining a graphic presentation of the ureteral movements consists in connecting the lumen of the ureter or the renal pelvis with a water manometer, the undulations of the column of water being then transmitted by means of a float and style to a revolving drum. The connections with the ureter were made by three methods:

of the ureter are omitted from this paper, because further study of the several problems involved is desirable and will be made in the Department of Physiological Chemistry before their detailed publication. I have already presented a summary of some of them in a brief preliminary communication. See the Proceedings of the Society for Experimental Biology and Medicine, 1905, ii, p. 61; also *Science*, xxi, p. 741; *American medicine*, ix, p. 744; *Medical news*, lxxxvii, p. 87. The experiments performed before June 1 of the present year were described in a thesis offered, from the Department of Physiological Chemistry, in partial fulfillment of the requirements for the degree of Master of Arts at Columbia University.



1. By introducing a T cannula into the ureter through a longitudinal slit. For brevity's sake, this will be termed the T cannula method.

2. By introducing into the ureter a cannula which is a modification of the Ludwig-Spengler artery cannula. In this modified cannula the two portions between which the vessel wall is clamped are made of half cylinders, the perpendicular portions of these being set nearer one end of each than the other. With this cannula a much smaller incision is required; no ligation being necessary, the propagation of the muscular wave of the ureter is only slightly interfered with, the vessels of the ureter can be avoided, and the nutrition of the ureter is only slightly impaired. The lumen of the ureter is also much less constricted than by the T cannula. This method will be referred to as the L cannula method.

3. By introducing a trocar through the kidney into the renal pelvis. A trocar with a blunt obturator was pushed through the cortex and medulla of the kidney so that it just entered the renal pelvis. It was retained in place by a purse string suture around the point of puncture of the capsule of the kidney. This also helped to stop bleeding, which, however, was surprisingly slight. A small quantity of warm salt solution or urine aspirated fresh from the bladder was injected through the needle; thus the patency of the cannula and ureter was ascertained. This will be referred to as the trocar method.

In connecting the cannula with the water manometer by means of narrow glass and rubber tubing, the low column of urine was separated from the water in the manometer by a column of air. Any movement of the urine caused an undulation in the manometer. These undulations were recorded on a drum by means of an Emerson float.<sup>1</sup>

The lumen of the ureter remained patent in all methods. The T cannula, however, narrowed the lumen considerably, and the resistance which it offered to the flow of urine through the ureter was much greater than when the L method was employed.

In most of the experiments of the present series the ureter remained in normal connection with the bladder. In some experiments, however, the ureter was severed near the bladder, the urine escaping into the abdomen or being carried out of the body by a glass tube connected with the cannula in the ureter. This cannula narrowed, of course, the lumen of the ureter, and thus afforded some resistance

<sup>1</sup> EMERSON, H: Proceedings of the Society for Experimental Biology and Medicine, 1904-1905, ii, p. 38.

to the flow of urine out of the ureter. In some of the experiments the urine was caused to drop on a pan connected with a Marey tambour, by means of which the flow of urine was recorded.

In some cases the outlet cannula was connected with a measured, vertical glass tube of small calibre, enabling one to study the effect of the resistance to the flow of urine upon the peristalsis of the ureter.

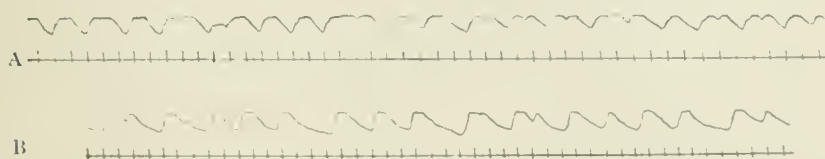
The dose of morphin varied from 0.06 to 0.12, depending on the size of the animal. This was given subcutaneously sixty to ninety minutes previous to the operation. All experiments were commenced in the morning; the animals had not been fed since the previous evening, but they had free access to water.

**The peristalsis of the ureter of dogs.** — Engelmann's statement that in "fresh" animals 3 to 6 spontaneous contractions occur in the ureter per minute (every ten to twenty seconds) refers only to rabbits. For dogs I find nowhere any definite data on this point. The above-mentioned observations of Fagge to the effect that in many cases the ureter was found to be motionless or that the contractions recurred at intervals of thirty to sixty seconds can hardly be accepted as an expression of what really takes place in a normal animal. (I find, however, from one of the protocols of an experiment on a dog by Luchsinger and Sokoloff, that the ureter before its excision was contracting ten times per minute. In a dog in which the ureter was cut in the middle, they observed that the upper part was contracting ten times and the lower part four per minute.) As to my own experiments I have to state at the outset that in the observations made by simple inspection I have never seen a motionless ureter. In a few instances the graphic method has failed to reveal tracings of movements of the ureter. In these very few exceptions, however, the failure was due either to the use of chloroform or to some accidental fault in the technique.

According to Engelmann, the spontaneous contractions of the ureter are less frequent the less "fresh" the animal. In my experiments the animals, when they were ready for observations, were not "fresh" any longer. Moreover, unlike the conditions of Engelmann's simple method of observation, the ureter itself had to be handled in my experiments, in order to insert the cannulas. Furthermore, the deprivation of the renal pelvis and ureter of urine by the opening of the ureter or pelvis is another factor working against the appearance of the peristaltic movements in the ureter. Nevertheless, as a rule,

within a few minutes after the connections of the ureter with the graphic apparatus had been perfected, the ureter was causing curves to be traced on the drum, of one kind or another.

Another point of interest is the fact that when the contractions had become well established, if not purposely interfered with, they



FIGURES 1 *a* and 1 *b*. — Ureter peristalsis. Middle of ureter connected by means of an L. cannula with a water manometer, the style of which traced the undulations. Lower end of ureter connected with a vertical glass tube containing 11 cm. of urine. Figure 1 *a* obtained soon after beginning of experiment; Fig. 1 *b* obtained 3 hours later. Time markings, 6 seconds. Straight line the base line for the ureter pressure.

continued in about the same frequency and character for three to four hours or even longer, — in fact, sometimes as long as the experiment lasted. Figs. 1 *a* and 1 *b* are from the same continuous experiment.

1 *a* was obtained at 11.30 A. M., and 1 *b* was obtained at 2.30 P. M. The tracings differ very little in the two figures, either in frequency

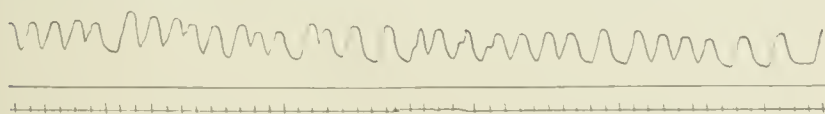


FIGURE 2. — Peristalsis of middle part of ureter obtained by means of an L. cannula. Urine discharged into bladder. Time, 6 seconds. Straight line base line.

or in extent. This is especially noteworthy because during the long interval the animal was exposed to several tests, as, for instance, to the influence of chloroform or ether, and to which I shall refer later.

The above figures also show that in this experiment the contractions of the ureter occurred about 4 to 6 times per minute. This rate is quicker than the one given by Engelmann for rabbits, and more rapid by far than the rates observed by Fagge in his experiments on dogs. However, I have to state that the rate was by no means a constant factor in my experiments. Not only did the number of contractions vary for different animals, but also in the same animal; it varied with different influences, and sometimes changes in rate occurred suddenly without any apparent cause. Figs. 2 and 3 illustrate this. They are from different animals. In Fig. 2 the intervals between the contractions varied between twelve to twenty seconds; in

Fig. 3 the intervals varied between twenty-four and fifty-four seconds and, exceptionally, an interval of even eighty or ninety seconds occurred. This was the case during the first half-hour of the experiment; later, the normal intervals were rarely longer than twenty seconds and were more regular.

The contractions, or rather the markings of contractions of which I am speaking here, were obtained from about the middle of the ureter, the place which probably was mostly under observation by previous experimenters.

While the rate of contraction of the middle part of the ureter varied sometimes, and although the intervals between the contrac-



FIGURE 3.—Peristalsis of middle part of ureter obtained at the beginning of experiment by means of an L cannula. Urine discharged into bladder. Time, 6 seconds.

tions were occasionally very long, I can state nevertheless in a general way that the contractions of the ureter of a dog anesthetized by morphin occur fairly regularly, the length of the period between the contractions varying in different animals as a rule from six to twenty seconds. The variation in different animals as well as in one and the same animal depends largely on external influences, chief among which are to be mentioned the amount of urinary secretion and the anesthetics employed. The significance of these factors I shall discuss later.

So far the experiments by the graphic method confirmed in a general way the statements made by other observers who studied the movements of the ureter by simple ocular inspection. I have, however, made some other observations for which I have found practically no analogy in previous statements. In the curves of Fig. 3 it may be seen that each large elevation is superimposed by a number of fine undulations. This is more pronounced in Fig. 4, which was obtained in a later phase of the same experiment. These tracings were obtained from an "L cannula" in the middle of the ureter, the latter not being interfered with otherwise. I have, however, made a series of experiments in which two cannulas were inserted in the same ureter, one in the pelvis and another at about the middle of the ureter, and here I made the surprising observation that each cannula pre-

sented a radically different curve. While the curves from the middle of the ureter were large and infrequent, the curves from the renal pelvis appeared in the form of very frequent and small oscillations, each "period" lasting only from one second to three.

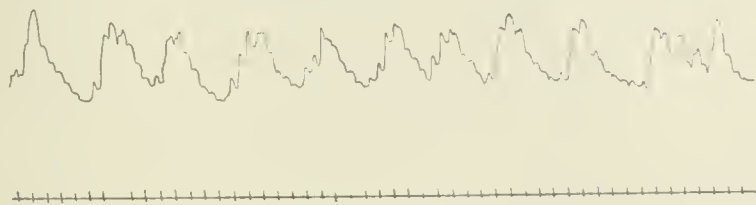


FIGURE 4.— Same as Fig. 3. Obtained about half an hour later.

Fig. 5 presents two such synchronously written curves. The upper curve was obtained from a T cannula which was inserted at the junction of the lower and middle third of the ureter. The lower curve

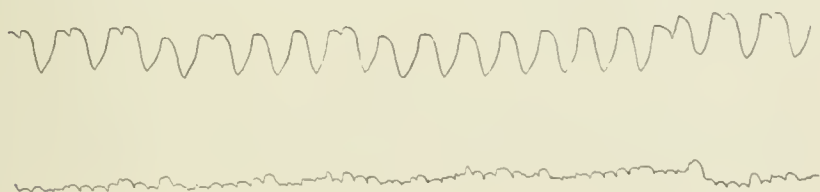


FIGURE 5.— Upper tracing from T cannula in ureter at junction of middle and lower third. Lower tracing from renal pelvis by means of an L cannula. Urine discharging into bladder. Time, 6 seconds.

was obtained from an L cannula inserted in the renal pelvis. The pelvic oscillations were sometimes even more rapid than those shown on the portion of the curve reproduced. The experiment on this animal lasted over six hours, the cannulas were continually writing throughout the entire experiment, and the character of each curve remained the same until the experiment was terminated.

The oscillations appear small and frequent also when obtained from the uppermost part of the ureter (see Fig. 6). It appears, however, that the ureter oscillations are a trifle larger than the pelvic oscillations.

From an analysis of the eleven experiments in which the renal pelvis was connected in one way or another with the recording apparatus, it appears that in four experiments in which the connections were made by means of an L cannula, all have shown the frequent short oscillations throughout nearly the entire experiment. In seven other experiments the connections were made by means of a trocar. Of these in three experiments all of the contractions

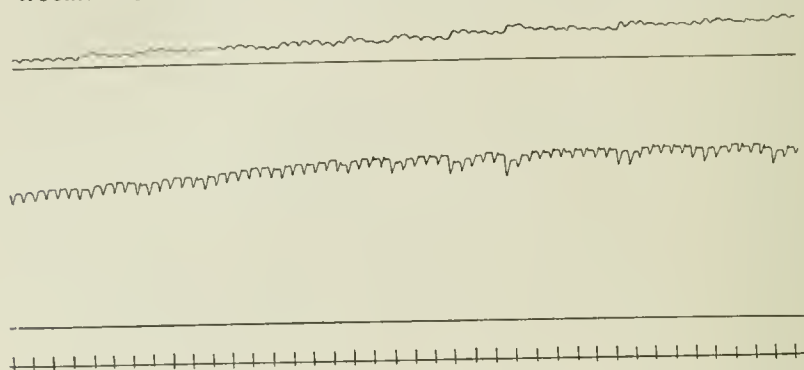


FIGURE 6.—Upper tracing from renal pelvis by means of trocar. Lower tracing from L cannula in ureter very close to pelvis. Both straight lines are base lines for the intraureteral pressures. Urine discharged into bladder. Time, 6 seconds.

appeared in the form of small frequent oscillations. In one (4th) experiment there was at first no movement at all, but later small oscillations developed, apparently spontaneously. In another experiment (5th) there were no spontaneous motions of any kind during the greater part of the experiment, but an intravenous injection of caffeine brought out some very small and very frequent oscillations. In one experiment (6th) the manometer connected with the renal pelvis wrote a straight line during the entire time of observation. Finally, in one experiment (7th), in which it was necessary to give the animal chloroform to keep it in anesthesia, very fine oscillations appeared once in a while, but were interrupted at irregular long intervals by slightly larger curves, which frequently continued without smaller oscillations. Apparently the connection of the renal pelvis with the recording instrument by means of the trocar method was less satisfactory than the L cannula. Very fine movements of the pelvis were probably not transmitted by the needle, but a rise of pressure in the pelvis brought them out. A venous infusion of saline, or an injection of caffeine, and sometimes, also, a resistance to the flow

of urine in the ureter, induced by clamping of the ureter, favored the appearance of the frequent oscillations in the renal pelvis.

I believe these experiments justify the general statement that the renal pelvis and the uppermost end of the ureter of dogs under the above conditions show as a rule very small contractions, which recur for the most part regularly at intervals of one to three seconds. Larger contractions, recurring at much longer intervals, appear sometimes also in tracings obtained from that section of the ureter, but, on the whole, they are rare, they appear only for short periods, are not large, and are always superimposed by the small frequent oscillations. The conditions under which they appear have not as yet been ascertained. Retarded flow in the lower part of the ureter seems to favor their occurrence, and in one case at least, their appearance corresponded to the relaxations of the tonus of the middle part of the ureter. Therefore it is more probable that the elevations were due to reflux of urine to the pelvis than to a contraction of the pelvis (see Fig. 7).

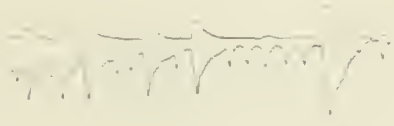


FIGURE 7. — Two-fifths the original size. Upper tracing by trocar from pelvis, lower tracing by T cannula from middle part of ureter. A rise in the pelvis curve corresponds to a drop in the ureter curve. Oscillations in pelvis curve hardly visible. Urine discharged into bladder. Time, 6 seconds.

From the results of two experiments it would appear that the upper end of the ureter shows transitional contractions, that is, the contractions are a little larger and a little less frequent than the ones from the pelvis, but they appear more frequently, and are distinctly smaller than the curves from the middle of the ureter.

That the rapid oscillations were not due to heart beats and respiratory movements has been frequently ascertained by a comparison of the number of each of these movements and also by the graphic method. It was proved especially that the course and rhythm of the renal oscillations were in no way changed by deep respirations<sup>1</sup> or during apnoea (see Fig. 8).

<sup>1</sup> The animals were not curarized. Moderate movements made no impression upon the pelvis and ureter curves. Struggles of the animal, however, drove up the intraureteral pressure and changed temporarily the rhythm of the movements. The effect was probably due to an increased outflow of urine into the pelvis and ureter.

With regard to the contractions of the middle part of the ureter, it has been stated above that the curves representing them were as a rule comparatively infrequent and fairly large. (This applies in

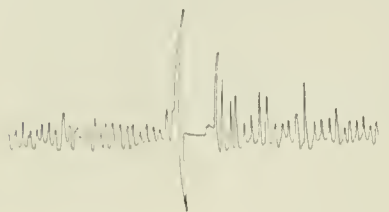


FIGURE 8. — Two-thirds the original size. Upper tracing (respiration) by means of a Marey cardiograph. Lower tracing from ureter close to pelvis by means of an L cannula. The deep respiration as well as the following pause made no impression upon the oscillation of the ureter curve. Time, 6 seconds.

general also to the curves obtained by means of the T cannula, which, by the way, were far less satisfactory than those obtained by means of the L cannula.) Of the fourteen experiments in which the middle part of the ureter was marking contractions, there was none in which the characters of these contractions were essentially the same as those observed in the pelvic tracings. In one experiment the urine was escaping from the lower end of the ureter through a vertical tube against a pressure of 20 cm. of water. In this experiment the contractions showed an inclination to become comparatively small and recur as rapidly as once every three or four seconds. In other experiments in which the urine escaped through a cannula in the lower end of the ureter against a moderate pressure, the curves were of the type represented in Fig. 1. They had

a tendency frequently to appear bigeminal, and exhibited a slight drop at the crest of the curve.

In experiments in which the urine was normally discharged into the bladder, the curves were generally large and appeared less frequent. Among these latter experiments there were three in which most of the curves presenting the large contractions of the ureter were superimposed by numerous finer movements (see Figs. 4 and 9). In the course of the experiments such curves developed the tendency to appear frequently in long lasting elevations, the tops of which would present a shorter or longer line of slight oscillations. Moreover, near the end of a long experiment the large elevations tended



to disappear entirely, and the tracing then consisted only of frequent smaller movements similar to those obtained from the renal pelvis (see Fig. 10).



FIGURE 9. — Peristalsis by an L cannula from middle of ureter showing fairly regular, large contractions, superimposed by smaller oscillations ("groups" of some authors). Urine discharged into bladder. Time, 6 seconds.

Such tracings make the impression that fatigue affects primarily the larger contractions. On the other hand, an intravenous injection of a large quantity of saline in the course of the experiment abolished



FIGURE 10. — Upper tracing by a T cannula at junction between middle and lower third of ureter. Lower tracing by an L cannula from pelvis. About 5 hours after beginning of experiment. Upper tracing shows tendency to lose the larger contraction, but not the finer oscillations. Time, 6 seconds.

for a while the small oscillations and increased the frequency of the larger movements (see Fig. 11). Increased diuresis, and with it increased intraureteral pressure, favors apparently the development of the larger contractions in the middle part of the ureter. It should be stated again that in a large percentage of the curves obtained from the middle part of the ureter no fine frequent oscillations ever appeared either superimposed or alone during a long experiment, although

here too the tracings sometimes exhibited bigeminal or trigeminal crests.

Only two successful experiments were made in which the lower end of the ureter, about one inch away from the bladder, was connected with the recording apparatus. In one experiment the movements were slight, and appeared at intervals of four to eight seconds, and only after considerable intraureteral pressure. For a short time at the beginning of the second experiment there were moderately large

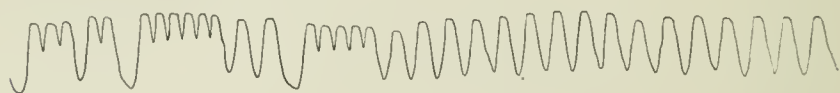


FIGURE 11. — Peristalsis by an L cannula from middle of ureter, showing the effect of an intravenous injection of 50 c.c. of saline. Time, 6 seconds.

contractions at intervals of six to ten seconds, but the contractions soon became frequent and small, and could not be distinguished from the oscillations which were simultaneously obtained from the renal pelvis. Although the number of these experiments is too small to permit of any positive conclusions, I have gained the impression that the contractions of the lower end of the ureter are generally more frequent and not nearly so large as those invariably exhibited at the middle part of the ureter.

These graphic studies of the movements of the ureter brought out, in the first place, the fact that, besides the larger and infrequent contractions which were hitherto known, there are contractions of another kind which are small, but which appear with much greater frequency than the large ones, and which have not been described by other observers. These frequent oscillations appear especially in the renal pelvis and in the upper part of the ureter. Possibly these oscillations appear frequently also in the lowermost part of the ureter. In the middle part of the ureter these frequent small waves appear sometimes as superimposed oscillations of the larger contractions. These large contractions occur for the most part about the middle of the ureter, and occur rarely if ever in the renal pelvis and the adjacent part of the ureter. Possibly they occur occasionally in the lower part of the ureter. Fatigue of the middle part seems to affect primarily the large contractions, and favors the appearance of the frequent

oscillations in cases where they were superimposed upon the large contractions.

The fact just referred to might explain the above-mentioned observation of Frey, namely, that during the rise of the ureter pressure the mercury in the manometer showed very small rapid oscillations. The manometer was surely not connected with the uppermost or the lowermost part of the ureter. These rapid oscillations, therefore,



FIGURE 12. — From the same experiment as Fig. 1. Shows the effect of chloroform upon the ureteral peristalsis.

might have been of the same kind that we have seen in the middle part when the latter was fatigued. Frey says expressly that he saw them only in a few of his experiments.

The middle part of the ureter acts then differently from the upper and lower ends. May this phenomenon not be connected in some way with the fact that the middle part of the ureter has no nerve cells (Engelmann), or a lesser number than both ends (Dogiel and others)?

I shall not enter for the present into a further discussion of the merits of this theory.

**Effect of anesthetics.** — I have yet to report on one or two more findings which are of theoretical and practical interest. I have stated above that in this series of experiments morphin was used as an anesthetic. The reason for not employing such efficient anesthetics as chloroform or ether was because they affect profoundly the behavior of the peristaltic movements of the ureter. Of this point I have made a special study, the essential results of which I shall mention here very briefly.

Both chloroform and ether reduce considerably the size as well as the frequency of the large contractions of the middle part of the ureter, and if continued abolish them entirely, the intraureteral pressure being reduced in all parts of the ureter (see Fig. 12). When the administration of the chloroform or the ether was only brief, sometimes the deteriorating effect did not set in until a little while after the use of the anesthetic was discontinued. In a few cases, a little while after recovery from the evil effect of the anesthetic, another period of deterioration set in as a second after effect. There are some differences between the effects of chloroform and

ether. I shall, however, omit here the minor details, and shall state that in general the effect of chloroform is much more profound than that of ether.

These observations give the probable key to the above-mentioned remarkable experience of Fagge, namely, that in his cases the ureter

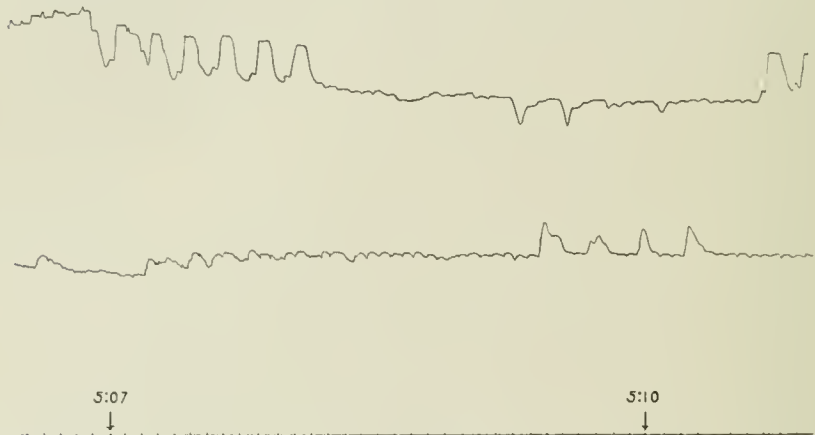


FIGURE 13.—Upper tracing by T cannula at junction of middle and lower third of ureter. Lower tracing by an L cannula from pelvis. Shows the effect of chloroform; the large contractions are abolished, but not the finer oscillations. (The larger elevations on the pelvic tracing correspond to sudden relaxations in the ureter tracing. See Fig. 7.) Time, 6 seconds.

was either motionless or that movement appeared only once in thirty or sixty seconds. He used as an anesthetic, besides a preliminary injection of morphin, the A. C. E. mixture (alcohol, chloroform, and ether).

Of special interest are the following observations. When the anesthetic was administered in cases in which the large contractions of the middle portion of the ureter were superimposed by the small rapid oscillations, the anesthetic affected at first the large contractions, leaving the small oscillations unimpaired, thus converting the curve into one resembling that obtainable from the renal pelvis. Furthermore the small oscillations from the renal pelvis proved to be also more resistant to anesthetics than the larger contractions from the middle part of the ureter. (See Fig. 13, upper and lower curves.) This fact is in analogy with the behavior of the large and small contractions towards fatigue. The small contractions are apparently more resistant to pernicious influences.

Finally, it must be mentioned that possibly the morphin anesthesia

is also not without detrimental influence upon the movements of the ureter, and that the actual normal peristalsis of the ureter might perhaps differ in some material points from those observed in these experiments.

#### RÉSUMÉ.

The essential points brought out in this section of my study of the ureter of dogs, by the graphic method, may be summarized as follows:

Even under morphin anesthesia the middle part of the ureter (comprising at least two-thirds) shows comparatively large contractions which recur, if the urine is permitted to flow unimpeded into the bladder, at intervals varying from six to twenty seconds.

The renal pelvis and the uppermost part of the ureter show contractions of another type, namely, small oscillations which recur much more frequently than the large contractions.

Chloroform and ether affect profoundly the large contractions of the ureter, so as even to abolish them completely, while the smaller oscillations show a greater resistance towards anesthetics.

It is a great pleasure to express my sincere gratitude to Prof. William J. Gies for the uniform kindness, encouragement, liberality, and advice I have enjoyed during my work in the laboratory of Physiological Chemistry at the College of Physicians and Surgeons, and to Dr. S. J. Meltzer, for invaluable assistance in and for rigorous and instructive criticism of all details of the work carried on at the Rockefeller Institute. I am also indebted to Prof. Stanley L. Coulter, Prof. Torald Sollmann, and Dr. A. N. Richards for suggestions and criticism during the course of my work.

# THE EFFECT OF ALCOHOL ON THE SECRETION OF BILE.<sup>1</sup>

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## INTRODUCTION.

IT is somewhat singular that studies of the action of alcohol on the digestive glands have been confined chiefly to inquiries into its influence on gastric secretion. Clinicians and physiologists have made the action of alcohol on the production of gastric juice the subject of a considerable number of rather extensive investigations, with almost concordant results. Practically all who have conducted researches on the effect of alcohol on gastric secretion agree that its administration, at least in moderate quantities, is followed by increased formation of gastric juice, both in the human subject and in fistulous dogs.

A number of investigations have been carried out on the action of alcohol on other digestive glands, but, in striking contrast to those on the stomach, they have neither been as numerous nor as extensive. Thus the action of alcohol on salivary secretion has received some attention. Excepting the work of Chittenden, Mendel, and Jackson,<sup>3</sup> and more recently that of Zitowitch,<sup>4</sup> however, no syste-

<sup>1</sup> The results of some of the experiments here described in detail have already been communicated in preliminary reports. Proceedings of the Society for Experimental Biology and Medicine, 1904, i, p. 43; Science, xx, p. 79; American medicine, vii, p. 950; Medical news, lxxxv, p. 230; Proceedings, 1906, iii, p. 34; Science, xxiii, p. 110; American medicine, p. 107; Science, 1906, xxiii, p. 335; Proceedings of the Section of Biological Chemistry of the American Chemical Society in affiliation with Section C of the American Association for the Advancement of Science, 1905; Proceedings of the Association, 1906, p. 330.

I am indebted to Professor J. G. Curtis for permission to carry out Experiments 10 and 11 in the laboratory of the Department of Physiology.

<sup>2</sup> Research Fellow of the Rockefeller Institute for Medical Research.

<sup>3</sup> CHITTENDEN, MENDEL, and JACKSON: This journal, 1898, i, p. 162.

<sup>4</sup> ZITOWITCH: Mitteilungen der militär medizinischen Akademie, September,

matic study of this particular phase of the subject has been made. The effect of alcohol on the secretory activity of the pancreas was merely touched upon by Claude Bernard,<sup>1</sup> and not until recently has it been accorded a fuller measure of attention.

Even more scanty is our information concerning the action of alcohol on the secretion of bile. The first attempt to study the subject was made by Rutherford,<sup>2</sup> who concluded, from the results of experiments on two dogs with temporary biliary fistulas, that alcohol is without any effect on the secretion of bile. Later, Dombrowski<sup>3</sup> and Barbera<sup>4</sup> made a few casual observations in this connection and came to the same conclusion. More recently Rutherford<sup>5</sup> stated that after the administration of alcohol in the form of whiskey to a patient with a biliary fistula, he also had failed to notice any increase in the flow of bile. The lack of a systematic study of the action of alcohol on the secretion of bile has made more extensive and thorough investigation of this subject particularly desirable. The present research was undertaken to meet this need.

#### METHODS.

My experiments were conducted on healthy, well-nourished dogs. At the beginning of the work the bile was obtained by means of temporary biliary fistulas. Later, a permanent complete gall bladder fistula was also made use of for the same purpose. In all the experiments in which temporary biliary fistulas were employed, the following procedure was adopted. Ether narcosis was induced without the previous use of morphin. To prevent the flow of bile from the gall bladder the cystic duct was securely ligated. A cannula attached in the usual way was then introduced into the common bile duct.

The bile was collected in graduated cylinders which were changed every fifteen minutes or, in some experiments, every ten minutes, thus affording a satisfactory means of studying the rate of secretion.

<sup>1</sup> BERNARD, CLAUDE: *Leçons sur les effets des substances toxiques*, Paris 1857, p. 433.

<sup>2</sup> RUTHERFORD: *An experimental research on the physiological action of drugs on the secretion of bile*, Edinburgh, 1880. Also *Journal of anatomy and physiology*, 1875, x, p. 215.

<sup>3</sup> DOMBROWSKI: quoted by LÖWENTON, *Dissertation*, Dorpat, 1891, p. 15.

<sup>4</sup> BARBERA: *Bulletino scienze mediche di Bologna*, Serie VII, 1894, v, p. 516.

<sup>5</sup> RUTHERFORD: *British medical journal*, 1898, ii, p. 775

In two experiments the rate of secretion was studied with the aid of the drop recorder. In these experiments, after four or five samples of bile had been obtained, alcohol was injected into the circulation from a burette connected with a cannula in the femoral vein. In another series of experiments alcohol was introduced into the stomach or into the intestines. The operation was performed usually about twenty hours after the dog had been fed. In a certain number of experiments, however, three to seven hours only were allowed to elapse between the feeding and the operation. Again, in rare instances, the period of fasting was extended thirty to forty hours.

The results of injecting alcohol directly into the circulation are presented in the following summaries:

#### EXPERIMENTAL RESULTS.

**Series A.** — *The secretion of bile after the intravenous injection of alcohol.*

*Experiment 2.* — Female dog; weight, 5.7 kilos. Fed twenty hours before the operation. Ether narcosis. Bile collected in fifteen minute periods as follows:

Period I.	0.20 c.c.	Period III.	0.25 c.c.
“ II.	0.20 “	“ IV.	0.2 “
Total (I-IV), 0.85 c.c.; average, 0.21 c.c.			

20 c.c. of 40 per cent alcohol injected into the femoral vein.

Period V.	0.10 c.c.	Period VII.	0.20 c.c.
“ VI.	0.10 “	“ VIII.	0.10 “
Total (V-VIII), 0.5 c.c.; average, 0.12 c.c.			

*Experiment 3.* — Male dog; weight, 10.5 kilos. Fed about four hours before operation. Ether narcosis. Neck of gall bladder ligated, then a cannula introduced into the common bile duct. Bile collected in fifteen minute periods as follows:

Period I.	1.8	Period III.	1.5
“ II.	1.5	“ IV.	1.4
Total (I-IV), 6.2 c.c.; average, 1.55 c.c.			

13 c.c. of 30 per cent alcohol injected into the femoral vein. Respiration ceased. No bile flow. Respiration was soon restored. 7 c.c. of 30 per cent alcohol were then injected and biliary secretion was resumed.

Period V.	1.1 c.c.	Period VI.	1.1 c.c.
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10 c.c. of 30 per cent alcohol injected slowly into the femoral vein.

Period VII.	0.9 c.c.
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10 c.c. of 30 per cent alcohol injected into the femoral vein.

Period VIII. 0.9 c.c.

Total (V-VIII), 4.0 c.c.; average, 1.0 c.c.

*Experiment 4.* — Male dog; weight, 7.5 kilos. Fed at 10.30 A.M. Ether narcosis. Neck of gall bladder ligated, then cannula introduced into common bile duct. Bile was collected in fifteen minute periods as follows:

Period I. 1.9 c.c.                      Period IV. 0.75 c.c.

“ II. 1.0 “                              “ V. 0.85 “

“ III. 0.8 “

Total (I-V), 5.03 c.c.; average, 1.06 c.c.

10 c.c. of 30 per cent alcohol injected into femoral vein. Respiration ceased; soon recovered.

Period VI. 0.75 c.c.

20 c.c. of 30 per cent alcohol injected into femoral vein. Heart stopped, soon recovered.

Period VII. 0.15 c.c.

Total (VI-VII), 0.90 c.c.; average, 0.45 c.c.

*Experiment 5.* — Male dog; weight, 11.8 kilos. Fed at 10.30 A.M. Ether narcosis. Neck of gall bladder ligated, then cannula introduced into common bile duct. Bile was collected in fifteen minute periods, as follows:

Period I. 1.75 c.c.                      Period IV. 0.2 c.c.

“ II. 0.7 “                                “ V. 0.2 “

“ III. 0.4 “

Total (I-V), 3.25 c.c.; average, 0.65 c.c.

50 c.c. of 30 per cent alcohol injected into the femoral vein.

Period VI. 0.15 c.c.                      Period VII. 1 drop

Total (VI-VII), 0.15 c.c.; average, 0.075 c.c.

*Experiment 9.* — Male dog; weight, 8.5 kilos. Fasted twenty hours. Bile was collected in fifteen minute periods.

Period I. 0.8 c.c.                        Period IV. 0.7 c.c.

“ II. 0.7 “                                “ V. 0.7 “

“ III. 0.75 “

Total (I-V), 3.65 c.c.; average, 0.73 c.c.

8 c.c. of 30 per cent alcohol injected gradually for eight minutes.

Period VI. 0.65 c.c.

8 c.c. of 30 per cent alcohol injected gradually for five minutes.

Period VII. 0.60 c.c.

8 c.c. of 30 per cent alcohol injected gradually for five minutes.

Period VIII. 0.4 c.c.      Period IX. 0.4 c.c.      Period X. 0.5 c.c.

Total (V-IX), 2.55 c.c.; average, 0.51 c.c.

*Experiment 10.* — Male dog; weight, 8.75 kilos. Fed seven hours before operation. Ether narcosis. Cystic duct ligated. Cannula introduced into the common bile duct. Rate of secretion studied by means of the drop recorder.

Period I. 16 drops in 7 min. 20 sec., or an average of 1 drop in 27 sec.

“ II. 30 “ 13 “ 40 “ “ “ 1 “ 27 “

“ III. 30 “ 14 “ 45 “ “ “ 1 “ 29 “

88 c.c. 30 per cent alcohol injected into the femoral vein.

Period IV. 28 drops in 15 min., or an average of 1 drop in 32 sec.

Fifteen minutes later 8 c.c. of 30 per cent alcohol injected into the femoral vein.

Period V. 16 drops in 9 min., 13 sec., or an average of 1 drop in 34 sec.

16 c.c. of 30 per cent alcohol injected into femoral vein.

Period VI. 16 drops in 10 min., or an average of 1 drop in 40 sec.

An analysis of the foregoing data shows that the secretion of bile was somewhat diminished after the intravenous injection of alcohol. This was especially evident in Experiment 2. In this experiment the flow of bile in each of the prealcoholic or control periods, except No. 3, was 0.2 c.c. When 20 c.c. of 40 per cent alcohol were injected, it fell in the succeeding periods to about 0.1 c.c. each. In Experiment 3 the amounts of bile obtained in the two periods immediately preceding alcohol treatment were 1.4 and 1.5 c.c. After injecting alcohol the secretion decreased within the following half-hour to 1.1 c.c. for a period of fifteen minutes. When an additional dose of alcohol was given, the quantity of bile obtained in the same length of time was only 0.9 c.c.

If the amounts of bile in Experiment 4 before and after injection of alcohol are compared, a continuous tendency in the direction of decreased flow may be noticed. This will be better appreciated if the results are considered in detail. During the first fifteen minutes 1.9 c.c. of bile were obtained, while in the next two periods only 1.0 c.c. and 0.8 c.c. respectively were collected.

The secretion of bile continued to decrease slightly in the succeeding fifteen minutes, but in the next period there was an appreciable improvement. Alcohol was thereupon injected, after which the secretion

of bile again diminished. The administration of an additional dose was followed by a still further reduction in the volume of secretion. The amount of bile obtained at the end of the next fifteen minutes was only 0.15 c.c., which was one-fifth that of the period immediately preceding.

The disproportionately rapid flow of bile in the early part of this experiment needs explanation, more especially since a similar effect was observed in the fifth experiment and in several others. In some of the animals in this investigation the ligation of the cystic duct, and the consequent exposure of the under surface of the liver which it involved, often made considerable handling at least of some parts of the liver unavoidable. It is quite possible that during these manipulations a certain amount of bile was temporarily held back in the biliary passages, and then was released later when the cannula was introduced into the common bile duct. The flow of bile which ensued during the first period of each experiment may be no index, therefore, of the rate of actual secretion during that time, since the quantity collected may represent not only the bile formed during that period, but also that which might have been accumulated previously in the biliary passages for the reason stated. Consequently, the first and sometimes also the second period samples were not regarded as controls whenever the amounts were comparatively greater than those in the rest of the four periods. Alcohol was not injected until the rate of secretion was approximately uniform for thirty or forty-five minutes, or until after it was evident that the variation was not considerable. Thus, in Experiment 5, alcohol was injected only after a uniform rate of secretion had been maintained for half an hour; otherwise no inferences could be drawn regarding the action of alcohol, for the rate of secretion shows diminution all through the experiment. In this experiment 1.75 c.c. of bile were obtained at the end of the first period, 0.7 c.c. in the next, 0.4 c.c. in the third. Secretion then became uniform, 0.2 c.c. having been obtained during each of the following two periods. Alcohol was therefore injected. The volume of secretion suffered but little change in the next fifteen minutes, but the flow soon ceased altogether.

In Experiment 9 the secretion of bile was practically uniform from the very beginning of the experiment. A small quantity of alcohol was injected, but very little change in the rate of secretion was noticed. Two additional doses were given at fifteen minute intervals. The flow of bile was appreciably retarded after the third

dose. Finally, in Experiment 10, as shown by the number of drops secreted in a given time, the injection of alcohol was likewise followed by a slight decrease in the rate of secretion.

The results of this series of experiments would therefore seem to indicate that alcohol when injected into the circulation depresses the secretory activity of the liver. The steady diminution in the flow of bile that was observed in some experiments before the administration of alcohol, however, made very desirable a comparative examination of the volumes of bile secreted in untreated dogs. In the following experiments the secretion of bile was studied for two hours or more, so that the amounts of bile collected during the second hour of the experiment could be compared with those obtained at about the same time after the injection of alcohol in the experiments already detailed.

**Control experiments (Series A).** *Experiment 12.*—Male dog; weight, 6 kilos. Fed three hours before operation. Ether narcosis. Neck of gall bladder ligated; cannula introduced into bile duct. Bile was collected in fifteen minute periods.

Period I.	2.7 c.c.	Period VI.	0.8 c.c.
“ II.	1.9 “	“ VII.	1.0 “
“ III.	1.1 “	“ VIII.	0.7 “
“ IV.	1.0 “	“ IX.	0.6 “
“ V.	0.8 “		

*Experiment 14.*—Male dog; weight, 6.15 kilos. Fed five hours before operation. Ether narcosis. Neck of gall bladder ligated and cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period I.	1.2 c.c.	Period V.	0.5 c.c.
“ II.	0.75 “	“ VI.	0.5 “
“ III.	0.5 “	“ VII.	0.4 “
“ IV.	0.4 “	“ VIII.	0.5 “

After sample VII was collected respiration ceased, but was immediately restored. Experiment 15, page 415, is also included in this series.

Leaving out of consideration the flow of bile in the first and second periods of Experiments 12, 14, and 15, since, as was pointed out on page 413, it may represent the amount secreted during these periods as well as the bile previously accumulated, it will be noticed that the rate of secretion beginning with the second hour of the experiment or

soon after was less (12), continued practically uniform (14), or even exceeded that of the control periods (15). Consequently, it seemed probable that the uniformly diminished secretion of bile in the experiments of Series A after alcohol had been injected was due to the action of alcohol. Before drawing any positive conclusions in this connection, however, it is desirable to consider the data of experiments in which the hepatic cells were stimulated to greater activity before determining the effect of injected alcohol. A summary of such experiments is appended.

**Series B.** — *Effects of alcohol after administration of a cholagogue (ox bile).*

*Experiment 15.* — Male dog; weight, 8 kilos. Fed three hours before operation. Ether narcosis. Neck of gall bladder ligated. Cannula introduced into the common bile duct. Bile was collected in periods of fifteen minutes each.

Period I.	2.2	Period V.	1.6
“ II.	2.15	“ VI.	0.6
“ III.	1.1	“ VII.	1.5
“ IV.	1.2		

Total (I–VII), 10.35 c.c.; average, 1.48 c.c.

Bile (VII) very dark. 10 c.c. of a 15 per cent aqueous solution of dried ox bile injected into femoral vein and bile collected in ten minute periods:

Period VIII.	1.0	Period X.	1.5
“ IX.	2.0		

Total (VIII–X), 4.5 c.c.; average, 1.5 c.c.

15 c.c. of 60 per cent alcohol injected into the femoral vein. Bile collected in ten minute periods.

Period XI.	1.9	Period XIII.	1.3
“ XII.	1.6		

Total (XI–XIII), 4.8 c.c.; average, 1.6 c.c.

The rise in the secretion of bile in this experiment after injection of alcohol might have been due to some ox bile retained in the proximal part of the femoral vein containing the cannula. In the following experiments with ox bile an effort was made to obviate this possibility by injecting the alcohol in two doses, the second fifteen minutes after the first.

*Experiment 7.* — Male dog; weight, 18 kilos. Fed about eighteen hours before operation. Ether narcosis. Cannula in common bile duct. Bile was collected in periods of fifteen minutes each.

Period I.	0.8	Period III.	0.2
" II.	1.1	" IV.	0.4
Total (I-IV), 2.5 c.c.; average, 0.62 c.c.			

Dilute ox bile injected into femoral vein.

Period V.	2.0	Period VI.	1.5
Total (V-VI), 3.5 c.c.; average, 1.75 c.c.			

32 c.c. of 30 per cent alcohol injected into femoral vein.

Period VII. 2.0

20 c.c. of 30 per cent alcohol injected into femoral vein.

Period VIII. 1.3

*Experiment 8.* — Male dog; weight, 43 kilos. Fed about twenty hours before operation. Ether narcosis. Neck of gall bladder ligated. Cannula in common bile duct. Bile collected in periods of fifteen minutes each. Secretion of bile scanty. Ox bile injected into the femoral vein.

Period I.	1.7	Period II.	1.2
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More ox bile injected, as before.

Period III. 1.6

10 c.c. of 30 per cent alcohol injected into the femoral vein.

Period IV. 1.3

10 c.c. of 30 per cent alcohol injected into the femoral vein.

Period V.	0.55	Period VII.	0.3
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" VI. 0.3

*Experiment 11.* — Male dog; weight, 18 kilos. Fed twenty-four hours before operation. Ether narcosis. Cannula in common bile duct. Secretion of bile estimated by means of the drop recorder. No secretion of bile obtained for some time. 15 c.c. of aqueous solution of dried ox bile (16 per cent) were then injected into the femoral vein. The rate of secretion in fifteen minutes was one drop in thirteen seconds. 40 c.c. of 30 per cent alcohol were then injected into the femoral vein. The rate of secretion of bile in the following fifteen minutes was one drop in fourteen and one-half seconds. 14 c.c. alcohol were injected as before. The flow of bile was one drop in seventeen seconds.

In Experiment 7 the injection of the first dose of alcohol was followed by an increased secretion of bile, but after the second dose of alcohol there was a marked decline. In Experiment 8 there was a moderate diminution of the flow of bile after the first dose of alcohol had been administered, but it was followed by a decided de-

cline when the dose was repeated. In Experiment 11 the diminution of the secretion of bile was slight after the first dose of alcohol, and somewhat greater after the second.

The secretion of bile after the injection of alcohol (when preceded by injection of ox bile) did not show a marked decrease, except in Experiment 8. This fact indicates that if alcohol intravenously injected exerts a depressing action on bile formation, it must indeed be very slight for the dose given, since otherwise the cholagogue effect of ox bile would have been of much shorter duration in these experiments than that exhibited. Moreover the increased secretion of bile in Experiments 15 and 7, after the first dose of alcohol, indicates, in the light of the observations just referred to, that the depressing action of alcohol on bile formation must be insignificant when it is injected into the circulation in the amounts stated, for otherwise the delayed admixture with the blood of such small amounts of bile as were contained in the cannula or the proximal part of the vein in those experiments would have been without any effect on the secretion of bile.

**Series C.** — *Does the intravenous injection of alcohol influence the excretion of the solid constituents of the bile?* To answer this question the following experiments were carried out. As before, the bile was obtained under ether narcosis by means of a cannula which was introduced into the common bile duct, the usual precaution of tying the cystic duct having been taken previously in order to prevent the flow of bile from the gall bladder. The analysis was made on samples collected during periods of fifteen minutes each. The bile secreted was received into graduated cylinders and then emptied into weighed crucibles. The cylinders were rinsed carefully with distilled water and the washings added to the contents of the crucibles. The liquids were then evaporated on the water bath and dried to constant weight in an air bath at about 100° C. The total solids thus obtained were weighed and carefully incinerated. The quantity of ash having been ascertained, the total organic matter was determined by difference. The analytic results are presented in the following summaries:

*Experiment 35.* — Dog; weight, 13.6 kilos. Fed about four hours before operation. Ether anesthesia. Cystic duct ligated. Cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period No.	Bile secreted. c.c.	Total solids.		Organic matter.		Inorganic matter.	
		Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.
I	3.5	265.3	75.8	227.3	65.0	38.0	10.8
II	2.0	146.2	73.1	122.2	61.1	24.0	12.0
III	1.7	112.5	66.1	94.3	55.4	18.2	10.7
IV	1.3	82.4	63.3	68.7	52.8	13.7	10.5
65 c.c. of 30 per cent alcohol injected into femoral vein.							
V	1.4	79.9	57.0	64.4	46.0	15.5	11.0
VI	1.2	74.6	62.1	61.5	51.0	13.1	10.9
VII	1.2	69.1	57.6	58.7	49.8	10.4	8.6
VIII	1.3	73.7	56.7	58.3	44.8	15.4	11.8

*Experiment 34.* — Male dog; weight, 18.8 kilos. Ether narcosis. Cystic duct ligated. Cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period No.	Bile secreted. c.c.	Total solids.		Organic matter.		Inorganic matter.	
		Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.
I	4.3	310.0	72.0	268.7	62.4	41.3	9.6
II	2.1	145.5	69.3	125.4	59.7	20.1	9.6
III	1.7	124.9	73.47	104.8	61.6	20.1	11.8
IV	1.7	129.2	76.0	110.0	64.7	19.2	11.3
V	1.6	131.4	82.1	113.0	70.6	18.4	11.2
80 c.c. of 30 per cent alcohol injected into femoral vein.							
VI	1.1	77.9	70.8	66.9	60.8	11.0	10.0
VII	0.5	44.6	89.2	37.9	75.8	6.7	13.4
VIII	1.0	96.1	96.1	81.8	81.8	14.3	14.3
IX	0.5	48.6	97.2				

40 c.c. of 30 per cent alcohol injected into duodenum.

X	0.9	87.6	97.3	75.8	84.2	11.8	13.1
XI	0.8	79.5	99.35	69.5	86.8	10.0	12.5

*Experiment 36.* — Female dog; weight, 18 kilos. Fed about six hours before operation. Ether narcosis. Cystic duct ligated. Cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period No.	Bile secreted. c.c.	Total solids.		Organic matter.		Inorganic matter.	
		Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.
I	1.8	116.4	64.6	102.8	57.1	13.6	7.5
II	1.3	88.4	68.0	75.4	58.0	13.0	10.0
III	1.2	76.9	64.0	64.7	53.9	12.2	10.0
IV	0.9	60.1	66.7	51.1	56.7	9.0	10.0



90 c.c. of 30 per cent alcohol injected into femoral vein.

V	0.5	34.1	68.2	28.5	57.0	5.6	11.2
VI	0.9	54.9	61.0	47.0	52.2	7.9	8.7
VII	0.9	59.7	66.3	51.8	57.5	7.9	8.7
VIII	0.5	34.2	68.4	29.6	59.2	4.6	9.2

40 c.c. of 30 per cent alcohol injected into small intestine.

IX	1.2	78.8	65.6	68.9	57.4	9.9	8.2
X	0.8	44.3	55.3	37.2	46.5	7.1	8.8

The results of these experiments agree in showing a decrease in the absolute amounts of solid matter eliminated in the bile after the injection of alcohol. But as this might be regarded as a normal variation and therefore not due to alcohol, the data of the following experiments are presented in order to show the output of biliary solids in untreated dogs.

*Experiment 26.* — Male dog; weight, 9 kilos. Fed about six hours before operation. Ether narcosis. Cystic duct ligated. Cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period No.	Bile secreted. c.c.	Total solids.		Organic matter.		Inorganic matter.	
		Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.
I	2.6	251.8	96.8	224.3	86.2	27.5	10.4
II	1.4	119.6	85.4	103.5	73.9	16.1	11.5
III	0.8	66.9	83.6	57.6	72.0	9.3	11.6
IV	0.5	49.3	98.6	42.3	84.6	7.0	14.0
V	0.5	44.9	89.8	38.8	77.6	6.1	12.2
VI	0.4	42.3	105.7	35.9	89.7	6.4	16.0

*Experiment 28.* — Female dog; weight, 9.35 kilos. Fed about six hours before operation. Ether narcosis. Cystic duct ligated. Cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period No.	Bile secreted. c.c.	Total solids.		Organic matter.		Inorganic matter.	
		Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.
I	2.3	146.8	63.8	127.1	55.2	18.9	8.2
II	1.5	83.2	55.4	69.7	46.4	13.5	9.0
III	1.0	52.8	52.8	45.2	45.2	7.6	7.6
IV	0.9	45.2	50.3	38.3	42.6	6.9	7.6
V	0.7	33.2	47.4	28.4	40.5	4.8	6.8
VI	0.6	28.0	46.6	23.2	38.6	4.8	8.0
VII	0.3	15.0	50.0	12.4	41.3	2.0	8.7

*Experiment 29.* — Male dog; weight, about 60 kilos. Ether narcosis. Cystic duct ligated; cannula introduced into common bile duct. Bile was collected in fifteen minute periods.

Period No.	Bile secreted. c.c.	Total solids.		Organic matter.		Inorganic matter.	
		Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.	Per period. mgs.	Per c.c. mgs.
I	3.0	338.7	112.9	297.7	99.2	40.8	13.6
II	2.3	271.2	117.8	240.7	104.6	30.5	13.2
III	1.6	195.9	122.4	174.4	109.0	21.5	13.4
IV	1.7	212.5	125.0	187.9	110.5	24.5	14.4
V	1.2	147.7	123.0	130.6	108.8	17.1	14.2
VI	1.0	113.5	113.5	99.9	99.9	13.6	13.6
VII	1.2	136.5	113.7	120.5	100.0	16.0	13.3
VIII	0.7	74.0	105.5	64.7	92.4	9.3	13.3

As is shown by the protocols on pages 418-419, the total elimination of the various solid constituents of the bile diminishes progressively in the course of each experiment. The volume of secretion in Experiments 26, 28, and 29 likewise shows a tendency to decline. The moderate decrease in the rate of secretion observed in the experiments with alcohol was apparently, therefore, a normal variation. It is likewise evident that the output of the solid elements of the bile was not appreciably influenced when alcohol was introduced intravenously.

**Series D.** — *The secretion of bile after the introduction of alcohol into the gastro-intestinal canal.*

On finding that the intravenous injection of alcohol affected neither the volume of, nor the content of solids in, the bile, attention was next directed to an inquiry whether the secretion of bile is influenced by introducing alcohol into the stomach or into the intestines. In one of my preliminary experiments in this connection (26, Tables I and II) the amounts of bile collected in each of three fifteen minute periods immediately preceding the injection of alcohol were 0.4 c.c. to 0.5 c.c. After 18 c.c. of 30 per cent alcohol had been introduced into the stomach the secretion of bile rose to 0.9 c.c. in the next fifteen minutes. When a second dose of alcohol was similarly administered, the results were even more marked. The effects of alcohol, when thus introduced, were still more striking in Experiment 29 (Tables I and II). The quantity of bile secreted in the fifteen minute period before alcohol was administered, was 0.7 c.c.; after the administration of alcohol it increased in fifteen minutes about 2½ times, while in

TABLE I.

The Effects on the Secretion of Bile of Alcohol injected into the Gastro-Intestinal Canal (Bile collected in Fifteen Minute Periods).

Exp. No.	Period before injection of alcohol.	Period after injection of alcohol.	Percentage of increase.
36	c.c. 0.5	c.c. 1.2	per cent 140
35	1.3	1.3	....
34	0.5	0.9	80
31 <sup>1</sup>	(1 hr.) 0.5	(1 hr.) 1.0	100
30	0.15	0.7	365
29	0.7	1.8	160
26	0.4	0.9	125
25	0.1	0.4	300
20	0.2	0.6	200
18	0.3	0.5	66
17	0.3	0.45	50

<sup>1</sup> In Experiment 31 bile was collected in one hour periods.

TABLE II.

The Composition of Bile before and after the Injection of Alcohol into the Gastro-intestinal Canal. (Bile collected in Fifteen Minute Periods.)

Exp. No.	Period before injection of alcohol.				Period after injection of alcohol.				Per cent of increase.			
	Vol.	Total solids.	Organic matter.	Ash.	Vol.	Total solids.	Organic matter.	Ash.	Vol.	Total solids.	Organic matter.	Ash.
36	c.c. 0.5	mgs. 34.2	mgs. 29.6	mgs. 4.6	c.c. 1.2	mgs. 78.8	mgs. 68.9	mgs. 9.9	p. c. 140	p. c. 130	p. c. 132	p. c. 115
31	0.5	48.6	....	....	0.9	87.6	75.8	11.8	80	80	....	....
26	0.4	42.3	35.9	6.4	0.9	85.1	74.9	10.2	125	100	108	60
29	0.7	74.0	64.7	9.3	1.8	211.1	191.4	19.7	160	185	195	112

the *next five* minutes 1 c.c. of bile was obtained. Further observations have, with few exceptions, confirmed the results of the preliminary tests.

As is shown in Table I, the rise in the secretion of bile after alcohol was introduced into the stomach or intestines was well marked in every experiment except in No. 35, in some instances amounting to 300 per cent or even more. In this connection it is of importance to note that alcohol was injected a considerable time after the operation was begun; fully two hours were allowed to elapse in some experiments before the effect of alcohol was tested. Since the rate of secretion of bile tends to diminish in the course of an experiment, as was learned in this investigation, the augmented flow, noted above, can only be attributed to alcohol. These results are therefore in harmony with the observations of the influence of alcohol on other digestive glands when that substance was introduced into various parts of the gastro-intestinal canal.

It has long been maintained by clinicians and physiologists that the flow of gastric juice is augmented after the administration of alcohol. Thus Kühne<sup>1</sup> states that alcohol is a gastric stimulant. Gluzinski,<sup>2</sup> who studied its effect on the human subject, found that in moderate quantities it increased gastric secretion. The same results on man were obtained by Wolf<sup>3</sup> and Blumenau.<sup>4</sup> Brandl,<sup>5</sup> experimenting with dogs provided with gastric fistulas, reported similar findings. Chittenden, Mendel, and Jackson<sup>6</sup> injected alcohol into the stomach and intestines of fistulous dogs, in varying doses and strengths, and obtained very large resultant quantities of gastric juice. The results of more recent investigations, in which the action of alcohol on gastric secretion (in supplementary stomachs of dogs, as described by Pawlow), have likewise shown that alcohol is a gastric stimulant. Thus Radzikowski<sup>7</sup> observed that alcohol, whether given by mouth or per rectum, induces the production of gastric juice. His results were corroborated by Frouin and Molinier,<sup>8</sup> Pekelharing,<sup>9</sup>

<sup>1</sup> KÜHNE: Lehrbuch der physiologischen Chemie, Leipzig, 1868, pp. 28, 30.

<sup>2</sup> GLUZINSKI: Deutsches Archiv für klinische Medizin, 1886, xxxix, p. 405.

<sup>3</sup> WOLF: Zeitschrift für klinische Medizin, 1889, xvi, p. 222.

<sup>4</sup> BLUMENAU: Therapeutische Monatshefte, 1890, v, p. 504.

<sup>5</sup> BRANDL: Zeitschrift für Biologie, 1892, xxix, p. 304.

<sup>6</sup> CHITTENDEN, MENDEL, and JACKSON: *Loc. cit.*

<sup>7</sup> RADZIKOWSKI: Archiv für die gesammte Physiologie, 1901, lxxxiv, p. 513.

<sup>8</sup> FROUIN and MOLINIER: Comptes rendus de la société de biologie, 1901, liii,

<sup>9</sup> PEKELHARING: Wiener klinische Wochenschrift, 1902, xv, p. 826.

and, four years later, by Zitowitch,<sup>1</sup> all of whom used the same methods.

Jackson<sup>2</sup> introduced alcohol into the intestines of dogs and likewise obtained increased secretion of gastric juice. Similar findings have been reported for the human subject. Metzger<sup>3</sup> states that enemata containing red wine, given to patients, is followed by increased gastric secretion. Spiro,<sup>4</sup> after giving per rectum 100 c.c. of alcohol dissolved in 200 c.c. of sodium chloride solution as well as various alcoholic drinks of the same strength, noticed the same effect. Meyer<sup>5</sup> found that, after administering alcohol to patients, the acidity of gastric juice was increased. Kast,<sup>6</sup> who gave alcohol by mouth or injected it per rectum in the human subject, stated that its administration produced an increased flow of gastric juice. Carnot<sup>7</sup> obtained the same results after giving his patients by mouth very small quantities of alcohol. He stated that fifteen minutes after 5 c.c. of 65 per cent alcohol had been given there was an increased secretion of gastric juice.

That the secretion of pancreatic juice is affected by alcohol in a similar way was shown by Claude Bernard,<sup>8</sup> who observed that after giving dilute alcohol pancreatic secretion was accelerated. Kuwshinski,<sup>9</sup> and later, Fleig,<sup>10</sup> have reported similar results. Likewise Gizelt<sup>11</sup> has recently shown that in dogs with permanent fistulas alcohol, when given by mouth or per rectum, is followed by increased secretory activity of the pancreas. He also made the interesting observation that when alcohol is administered, after cutting the vagi, the secretion of pancreatic juice is not affected.

That the biliary function of the liver fails to react to alcohol when injected into the circulation in the amounts employed by me, but is stimulated if alcohol is introduced into some part of the gastro-

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

<sup>2</sup> JACKSON: Proceedings of the Society for Experimental Biology and Medicine, 1904, i, p. 20.

<sup>3</sup> METZGER: Münchener medizinische Wochenschrift, 1900, xlvii, p. 1552.

<sup>4</sup> SPIRO: Münchener medizinische Wochenschrift, 1901, xlviii, p. 1872.

<sup>5</sup> MEYER: Klinisches Jahrbuch, 1905, xiii, p. 285.

<sup>6</sup> KAST: Biochemisches Centralblatt, 1906, v, p. 483.

<sup>7</sup> CARNOT: Comptes rendus de la société de biologie, 1906, lx, p. 807.

<sup>8</sup> BERNARD, CLAUDE: *Loc. cit.*

<sup>9</sup> KUWSHINSKI: Dissertation, St. Petersburg, 1888.

<sup>10</sup> FLEIG: Comptes rendus de la société de biologie, 1903, lx, p. 1277.

<sup>11</sup> GIZELT: Archiv für die gesammte Physiologie, 1906, cxi, p. 620.

intestinal canal, was further shown in experiments of my own (34 and 36).

After injecting alcohol into the femoral vein in Experiment 36, the amount of bile collected in the next fifteen minutes was only 0.5 c.c. as against 0.9 c.c. in the preceding fifteen minute period, while the introduction of alcohol into the small intestine one hour later, was followed by an increased secretion of bile amounting, in fifteen minutes, to nearly two and one-half times that obtained in the period of equal length immediately preceding such introduction. Moreover, as is shown in the protocol, the secretion of bile during the first fifteen minute period after the injection of alcohol into the small intestine was more rapid than in any of the five preceding periods of equal lengths.

In Experiment 34 the results obtained, although not so striking, are nevertheless quite marked. After the injection of alcohol into the intestine, the amount of bile obtained was 0.9 c.c. in the succeeding fifteen minutes, while only 0.5 c.c. were collected in the period immediately preceding the injection. In the same experiment, fifteen minutes after the intravenous injection of alcohol, 0.5 c.c. less of bile were collected than immediately before such treatment. The increase in the volume of secretion was attended also by an increase of the solid elements. As shown in Table II, the percentage of total solids in Experiment 36 was 130 per cent greater after the introduction of alcohol into some part of the gastro-intestinal canal. In Experiment 29 the amount rose to 185 per cent. In the other two experiments indicated in this table (II), the increase of percentage was much less, but was still considerable. The inorganic constituents were likewise increased under these conditions, but to a much smaller extent.

**Series E.** — *The secretion of bile after administration of alcohol per os to a dog with a permanent complete gall bladder fistula.*

Barbera<sup>1</sup> was the first to study the action of alcohol on the secretion of bile in a dog by means of a permanent gall bladder fistula. He performed three experiments on the same animal; each experiment was carried out on a different day. He reported that after the administration of alcohol (300 c.c., — 5 per cent, given by the mouth) the secretion of bile remained unchanged.

I carried out a number of similar observations on a dog by the same method. The operation was performed in the usual way except

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

that the cannula suggested by Pawlow<sup>1</sup> was employed. On the fourth day after the operation treatment with alcohol was begun. The bile was collected by means of graduated cylinders attached to the external projection of the cannula. The following summaries give the essential data:

*Experiment A.* — Jan. 23. Dog fed twenty hours before experiment. Bile was collected during a control period of fifteen minutes — 2 c.c. Alcohol, 50 c.c. of 40 per cent by mouth through a stomach tube. 4 c.c. of bile were collected in the next fifteen minutes.

*Experiment B.* — Jan. 24. 11 A.M. Dog fed meat, then given water at 11 A.M. 12.50 P.M. to 1.50 P.M. 4.3 c.c. of bile were collected. At 2.20 P.M. 50 c.c. of 40 per cent alcohol were given by mouth through a stomach tube. Bile was collected as follows:

Period I. 2.20 to 2.35 P.M. 4 c.c.      Period II. 2.35 to 2.50 P.M. 1 c.c.  
Period III. 2.50 to 3.20 P.M. 1.5 c.c.

The total amount of bile secreted in one hour after the administration of alcohol was 6.5 c.c., which represents an increase of 44.5 per cent over the control period. The solids were increased in about the same proportion.

*Experiment C.* — Jan. 25. Dog fasted thirty-four hours. Bile was collected as follows:

8.20 to 8.30 P.M. 0.5 c.c.      8.30 to 8.40 P.M. 0.4 c.c.  
50 c.c. of 40 per cent alcohol were given by mouth through a stomach tube.  
8.45 to 8.55 P.M. 1.8 c.c.      8.55 to 9.05 P.M. 0.9 c.c.

The rate of secretion in this experiment is increased by 200 per cent as compared with the two control periods.

*Experiment D.* — Jan. 27. Bile was collected in periods of ten minutes each.

Period I. 1.3 c.c.      Period II. 2.0 c.c.      Period III. 0.8 c.c.  
50 c.c. of 45 per cent alcohol given by mouth in the usual way and bile collected in ten minute periods.  
Period IV. 4 c.c.      Period V. 2.6 c.c.      Period VI. 1.2 c.c.

In this experiment (D) the improvement in the secretion of bile after the administration of alcohol was fully 90 per cent in half an hour, while the amount secreted during the ten minute period immediately succeeding the administration of alcohol was five times as much as in the period immediately preceding. The increase in total solids

<sup>1</sup> PAWLOW: *Ergebnisse der Physiologie*, 1902, i, p. 273.

was quite marked also, but the concentration of the bile was somewhat lower, so that the increase of solids did not keep pace with the increase in volume. In the next experiment on this dog, which was carried out seven days later (February 2), the dog was very much emaciated and showed signs of general exhaustion. At times there was no secretion of bile; flow did not begin until fifteen to twenty minutes after the commencement of the observation. In the next ten minutes 1.5 c.c. of bile were collected and only 0.6 c.c. were obtained in the following period. Fifty c.c. of 45 per cent alcohol were then given by mouth in the usual manner. The amounts of bile collected in each of the two succeeding periods of ten minutes each were 3.3 and 1.5 c.c. respectively.

#### SUMMARY AND CONCLUSIONS.

A summary of the results of the experiments reported in this investigation may now be presented.

When alcohol was injected into the circulation, a slight decrease in the secretion of bile was observed. Since a progressive diminution of the flow of bile in the course of an experiment was often noticed before the administration of alcohol, experiments were carried out on non-treated dogs to study the rate of secretion for about two hours in each case. It was found that secretion of bile in such animals may, after one or two fifteen minute periods, remain practically stationary, may be retarded, or may, on the contrary, even increase after a preliminary diminution. The amount of bile during the first or sometimes also during the second quarter of an hour from the time the experiment was begun was usually ignored for purposes of comparison, as it probably represented some bile which accumulated during the operative procedure.

A comparison of the rates of secretion in alcoholized and non-alcoholized dogs tended to show that alcohol interfered somewhat with the secretion of bile. Experiments were therefore performed to test whether alcohol when injected into the circulation would counteract the stimulating effect of ox bile on the hepatic cells of the dog. The results of this line of inquiry were negative. In another series of experiments on non-alcoholized dogs the rate of secretion of bile was studied for a period of one and one-half to two hours. A progressive retardation of the secretion of bile was noticed. Hence the conclusion is justified that the rate of the secretion of bile in



dogs is not materially changed when alcohol in the quantities used in these experiments is injected directly into the circulation. The presence of alcohol in the blood did not exert, therefore, any appreciable action on the biliary function of the hepatic cell in the dog.

The similar behavior of the salivary gland toward alcohol under like conditions was pointed out by Chittenden, Mendel, and Jackson.<sup>1</sup> Their investigations have shown that alcohol has no direct effect on salivary secretion, its action being in all probability reflex, for after the introduction of alcohol into the stomach through a gastric fistula, the formation of saliva remained unaffected. The same was shown for the gastric glands by Jackson,<sup>2</sup> who noticed that the secretion of gastric juice, which took place after alcohol was introduced into the intestine, failed to occur when that substance was similarly introduced after section of the vagi and sympathetic or after injection of atropin. The recent work of Orbeli,<sup>3</sup> on the influence of alcohol on gastric secretion, likewise indicates that alcohol does not exert any action on the gastric glands. Thus, when alcohol was given per os to dogs after the subcutaneous injection of atropin, there was no secretion of gastric juice. In this connection may also be recalled the work of Gizelt,<sup>4</sup> referred to above, who has shown that the injection of alcohol after section of the vagi fails to provoke pancreatic secretion. The evidence thus far accumulated points therefore to the conclusion that physiological doses of alcohol, in whatsoever ways introduced into the body, do not exert any direct secretory action on the digestive glands. Hence the observed stimulating effect of alcohol is in all probability nervous in its origin.

Studies carried out to test the effect of alcohol, when injected intravenously, on the solids of the bile, were likewise negative. On the other hand, when alcohol was injected into the stomach or into the intestines, there was a marked increase in the flow of bile, and the solid elements were also increased absolutely and relatively. Of the biliary solids the organic constituents were increased somewhat more than the inorganic.

A series of observations was also made on a dog with a permanent gall bladder fistula. The effects of alcohol given per os were similar to those obtained after the administration of alcohol into the gastro-

<sup>1</sup> CHITTENDEN, MENDEL, and JACKSON: *Loc. cit.*

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

<sup>3</sup> ORBELI: Archives des sciences biologique, 1930, xii, p. 102.

<sup>4</sup> GIZELT: *Loc. cit.*

intestinal canal of dogs with temporary biliary fistulas under ether narcosis.

The increased formation of bile after the introduction of alcohol into the stomach or into the intestines may be caused by secretin. The administration of alcohol per os is attended by the formation of gastric juice, which on reaching the duodenum causes the production of secretin. Secretin may excite not only the pancreas but the liver as well. The failure of Rutherford,<sup>1</sup> as well as Barbera,<sup>2</sup> to observe an increased flow of bile after administration of alcohol may be explained on this basis. It has been shown by Spiro<sup>3</sup> that alcohol fails to induce gastric secretion in achylia gastrica or in cancer of the stomach. Rutherford made his observations on a patient with a biliary fistula in a case of cancer of the head of the pancreas, and of the common bile duct. Barbera, on the other hand, performed his experiments on a dog four months and a half after the biliary fistula had been established. In both of these experiments the impaired nutrition and altered metabolism of the subject undoubtedly induced a condition similar to those which obtained in the cases of Spiro, the administration of alcohol being therefore without any effect on the secretion of gastric juice, or on the formation of secretin, and consequently also without any influence on the formation of bile.

I am indebted to Prof. William J. Gies for his interest in the subject, as well as for numerous courtesies shown to me in the course of the research.

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

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

<sup>3</sup> SPIRO: *Loc. cit.*

## THE MOTOR ACTIVITIES OF THE STOMACH AND SMALL INTESTINE AFTER SPLANCHNIC AND VAGUS SECTION.<sup>1</sup>

BY W. B. CANNON.

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

**I**N a research published two years ago attention was called to a difference in the rate of discharge of different food-stuffs from the stomach.<sup>2</sup> Since the gastric peristaltic waves normally run without cessation so long as food is in the stomach, the control of the discharge into the intestine must lie at the pyloric sphincter. Until that sphincter relaxes food cannot pass onward. The question now arises, Is the long initial closure of the pylorus when proteids occupy the stomach, and the relatively brief initial closure and the frequent relaxation when carbohydrates are present, due to reflexes from the central nervous system, or is the difference determined by local mechanisms? The question can be answered by cutting the nervous connections between the stomach and the central nervous system and studying the discharge of food through the pylorus by the method employed in the earlier investigation.

The stomach and small intestine are connected with the central nervous system by the vagi and the splanchnics.<sup>3</sup> Section of these nerves in the normal animal and subsequent study of the animal without further operation, *i. e.*, by means of the Röntgen rays, reveal the effects of severance of the nervous paths, not only on the discharge of food from the stomach, but also on the rate and character of gastric peristalsis, the rate of passage of food through the small intestine, the presence of rhythmic segmentation, and other activities.

<sup>1</sup> A preliminary report of this investigation was made at the meeting of the American Physiological Society in December, 1904, and was printed in the Proceedings, this journal, 1905, xiii, p. xxii.

<sup>2</sup> CANNON: This journal, 1904, xii, p. 387.

<sup>3</sup> STARLING: *Ergebnisse der Physiologie*, 1902, i, Biophysik, pp. 450, 454, 459.

## THE METHOD.

In the investigation here recorded one series of animals was studied with only splanchnic nerves cut, another series with only vagus nerves cut, and a third series with entire severance of vagi and splanchnics. The animals used were cats.

In every instance the animal was etherized during the operation. The hair in the region of operation was clipped short, the skin washed with soap and warm water, dried with sterile cotton, and finally wet with ether. The usual aseptic precautions were observed in preparing instruments and the operator's hands. After the operation the skin wound was covered with a collodion-cotton cocoon.

In order to reach the splanchnic nerves some of the intestines were removed from the body cavity, and during the subsequent operation were wrapped in a cloth wet with warm sterile salt solution. Usually the right major and minor splanchnics, which are more difficult to reach than the left, were cut first. A strong thread was in most cases tied very tightly about the nerve, and the nerve was then cut distal to the ligature. The ligature was not removed from the proximal part. The nerve in other cases was picked up in forceps and a piece about a centimetre long was resected.

Severance of both vagus nerves in the neck is followed in the cat by difficult inspiration due to improper functioning of the structures about the glottis. In order to remove the vagus supply to the stomach and intestines without destroying entirely the innervation of the larynx, the right vagus nerve was cut below the origin of the recurrent laryngeal branch and, usually in a later operation, the left vagus was sectioned in the neck. About one centimetre of the nerve was removed in every case.

When both sets of nerves were severed in the same animal, usually both splanchnics and the right vagus were cut first, and later the left vagus.

The cutting of the greater and lesser splanchnics on both sides was followed by almost no appreciable change in the animals. They all lived more than a month, and were finally killed because of an inflammation of the nose and conjunctiva, which spread as an epidemic among operated and normal animals alike. After bilateral vagus section the cats lived without trouble, with the exception of a temporary failure of œsophageal peristalsis, in two cases for over a month, in other cases for periods ranging from two to three weeks. They

never appeared so vigorous as the animals with only the splanchnics cut. They also suffered from inflammation of the nose and eyes, and were usually killed for that reason. After entire severance of splanchnics and vagi two of the animals were afflicted with the prevailing coryza, one was found dead twelve days after the second operation, and the remainder were killed by etherization two weeks after being operated upon. All of these animals were noticeably asthenic.

Autopsy was made in every case, and the thoroughness of the section of each nerve was completely verified.

The activities of the alimentary canal were studied in these animals at various times after the nerves were cut. As a representative carbohydrate food potato was fed, and lean beef was chosen as a representative proteid food. These foods were given in 25 c.c. amounts, with 5 gm. bismuth subnitrate added to render the mixture opaque to the X-rays. These proportions were used in the earlier investigation in which the standard rates of discharge from the stomach were determined. The temporary failure of the œsophagus to function normally after bilateral vagus section necessitated giving the food by stomach tube, in order that the standard amount should be present in the stomach at the beginning of the period of observation. To be forced through the stomach tube the food had to be slightly more fluid than it was under the standard conditions.

## RESULTS.

**The movements of the stomach.** — After bilateral splanchnic section gastric peristaltic waves were always seen, so long as food was in the stomach, passing in the normal manner and with the normal rhythm.

When both vagi were cut, the first effect was often total suppression of peristalsis. In two instances in which the second vagus was cut immediately after the animals had voluntarily eaten boiled lean beef, no gastric peristalsis was observed for four hours; and in another instance, in which this operation was done the day previous, no gastric peristalsis was seen during the first three hours after feeding. In other animals, fed the carbohydrate food, peristalsis was seen a half-hour after feeding; on one of these animals the second operation, on the left vagus, had been performed the day before. Whether, under the circumstances of these observations, the different food-stuffs have different effects on the initiation of gastric peristalsis was not

further determined. In every instance of vagus section, however, the peristaltic waves, although recurring with the normal rhythm, were at first characterized by being extraordinarily shallow. Sometimes they were hardly visible, at other times they could be seen distinctly only on the antrum. But the period during which the movements of the stomach were late in commencing and were notably weak did not long continue; as days passed, these abnormalities largely disappeared and the waves started at the usual time and had much of their normal vigor.

When all the extrinsic nerves were cut, the waves of constriction coursed over the stomach with the normal rhythm, but these waves, almost from the first, contrasted with those observed when the vagi alone were severed, in being normally deep contractions. A remarkable feature revealed at the autopsy of these animals was the strong tonus of the gastric musculature. Through most of the length of the stomach, in two instances, the diameter of the organ ranged between 1.5 and 2 cm., a smallness of size almost incredible when compared with the diameter of the stomach filled with food.

**The passage of carbohydrate and proteid food from the stomach.** — The rate of discharge of food from the stomach was judged, as in the research already mentioned, from the aggregate length of the shadows of the food masses in the small intestine at regular intervals after feeding.

1. *After splanchnic section.* — The following figures represent in centimetres the aggregate length of the food masses in the small

Hours after feeding.	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length, in centimetres, of food masses in small intestine.	23.5	32.0	32.0	35.0	20.5	18.0	14.0	12.0
	14.5	21.0	42.0	33.0	16.0	6.5	0.0	0.0
	12.0	22.5	32.5	38.0	21.5	20.0	17.0	8.0
	9.0	20.0	37.0	30.0	25.5	18.5	14.0	9.5
Averages . .	14.5	24.0	36.0	34.0	21.0	15.5	11.0	7.5

intestine at regular times after mashed potato was fed, in four cases with severed splanchnic nerves.

Comparison of the above figures with those secured in normal

cases<sup>1</sup> shows that although there is greater variation from the average in the cases with splanchnics cut, there is still preserved in every instance the characteristic rapid carbohydrate discharge. In Fig. 1 is represented graphically the average figures from the above table and the average figures which resulted after feeding potato in four normal cases. The amount of this food in the small intestine at the end of the first and second hours was less in the operated than in the normal animals, but the difference is so slight as to leave the curve from the operated animals unmistakably a carbohydrate curve. The rate of discharge of a typical carbohydrate food from the stomach is therefore not markedly changed from the normal by cutting the splanchnic nerves.

How lean beef, as a representative proteid food, is treated by the stomach after the splanchnic nerves are severed is indicated by the following figures, representing, in four cases, the total length of the food masses in the small intestine at the regular times of observation.

Hours after feeding.	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length, in centimetres, of food masses in small intestine.	0	4.5	9.0	20.5	20.5	12.0	11.0	10.0
	0	5.5	13.5	25.0	22.5	14.5	15.0	13.0
	0	3.5	15.5	21.5	27.0	25.0	21.5	17.5
	0	5.5	15.5	20.0	25.5	29.0	16.0	16.0
Averages . . .	0	5.0	13.5	22.0	24.0	20.0	16.0	14.0

Examination of the averages of these figures and the averages obtained in four normal cases<sup>2</sup> in which lean beef was fed (see Fig. 1) reveals a striking coincidence in the amounts of food in the small intestine after the same intervals in the two conditions. From these results it is clear that the discharge of a typical proteid food from the stomach persists in a quite normal manner and is characteristically different from the carbohydrate discharge after total suppression of impulses through the splanchnics.

2. *After vagus section.*—In studying the rate of discharge of

<sup>1</sup> See CANNON: *Loc. cit.*, p. 397.

<sup>2</sup> CANNON: *Loc. cit.*, p. 400.

mashed potato and lean beef from the stomach after vagus section a wide range of variation was at first observed, especially when the

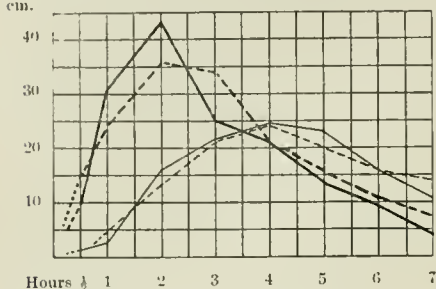


FIGURE 1.—Curves showing average aggregate length of the food masses in the small intestine at regular intervals for seven hours after feeding mashed potato in four normal cases (heavy continuous line); and in four cases with splanchnics cut (heavy dash line); and after feeding lean beef in four normal cases (light continuous line); and in four cases with splanchnics cut (light dash line).

at the regular times of observation in four cases in which potato was fed, and in two sets of four cases each in which lean beef was fed, after section of both vagus nerves. The interval since the cutting of the second vagus nerve is set down in each instance.

Examination of these figures and comparison of the averages with the averages secured in normal cases (see Fig. 2) show at once that, in the absence of impulses through the vagus and in the presence of impulses through the splanchnic nerves, the discharge of both carbohydrate and proteid is notably retarded. As the two tables for lean beef prove, however, the retardation after vagus

section is much more marked soon after the operation than it is later. In one case, not included in this table, which was observed the day

proteid food was fed. No explanation of the differences was found until the time interval between the severing of the second vagus nerve and the day of observation was regarded. Then it was evident that although the discharge of lean beef from the stomach was much retarded for the first day or two after the vagi were cut, there was later a considerable restoration of the usual activity.

The figures on the following page represent the total length of the shadows of the food masses in the small intestine

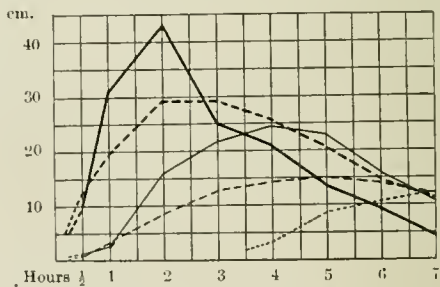


FIGURE 2.—Curves showing average aggregate length of food masses in the small intestine at regular intervals for seven hours after feeding mashed potato in four normal cases (heavy continuous line); and in four cases with vagi cut (heavy dash line); and after feeding lean beef in four normal cases (light continuous line); in four cases with second vagus cut more than nine days before (light dash line); and in four cases with second vagus cut within one day of the observation (light dotted line).

section is much more marked soon after the operation than it is later. In one case, not included in this table, which was observed the day



after the second operation, no gastric peristalsis was seen for the first five hours of observation. In none of the cases studied within

MASHED POTATO.									
Hours after feeding	$\frac{1}{2}$	1	2	3	4	5	6	7	Interval since 2d vagus cut.
Aggregate length, in centimetres, of food masses in small intestine.	12	19.5	32	30	22.0	20.0	12.0	12.0	6 days.
	15	19.0	25	26	25.0	17.5	14.0	10.0	12 "
	14	24.0	36	29	27.0	25.0	16.0	13.0	6 "
	6	15.5	24	32	27.5	23.0	16.0	10.5	9 "
Averages . .	12	19.5	29	29	25.5	21.5	14.5	11.5	
LEAN BEEF.									
Aggregate length, in centimetres, of food masses in small intestine.	0	0	0	0	0.0	6.5	8.0	11.0	same day.
	0	0	0	0	9.0	17.5	19.0	22.0	1 day.
	0	0	0	0	0.0	4.0	6.0	6.0	same day.
	0	0	0	0	5.5	6.0	9.0	9.5	1 day.
Averages . .	0	0	0	0	3.5	8.5	10.5	12.0	
Aggregate length, in centimetres, of food masses in small intestine.	0	2.5	5.5	9.0	11.5	15	14	14.0	11 days.
	0	2.5	9.5	13.0	10.0	10	8	6.0	9 "
	0	2.0	9.0	13.0	18.0	15	14	13.0	9 "
	3	5.5	9.0	16.0	17.5	19	19	15.5	16 "
Averages . .	1	3.0	8.0	12.5	14.0	15	14	12.0	

one day after the second operation did any food pass the pylorus for three hours after feeding. Clearly, when the vagi are cut, the stomach suffers at first a considerable disturbance of its normal functioning. In a few days, however, this primary defect is largely recovered from.

Even though discharge of both the carbohydrate and the proteid

from the stomach remains notably retarded after vagus section, there still persists the characteristic difference between the treatment of the two food-stuffs, — the carbohydrate passes out much more rapidly than the proteid food.

3. *After splanchnic and vagus section.* — The rate of discharge of food from the stomach when an interval has been allowed to elapse after section of all the extrinsic nerves, may be judged from the figures in the following tables. These figures, like those in previous tables, give the total length of the food masses in the small intestine, at the times designated, after potato and lean beef had been fed.

MASHED POTATO.								
Hours after feeding.	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length, in centimetres, of food masses in small intestine.	13.0	16.0	18.0	22.5	15.0	7.5	6.0	2.0
	11.0	26.0	32.0	32.0	22.5	14.0	13.0	10.5
	18.0	21.5	34.0	18.0	9.0	9.0	8.0	4.5
	12.5	15.0	17.5	22.0	14.5	6.5	6.0	4.5
Averages . .	13.5	19.5	25.5	23.5	15.0	9.0	8.0	5.5
LEAN BEEF.								
Aggregate length, in centimetres, of food masses in small intestine.	0.0	9.0	18.0	19.5	19.0	20.0	20.0	13.5
	0.0	6.0	16.0	25.0	21.0	19.0	17.5	16.0
	2.5	7.0	12.0	17.0	20.0	19.0	12.0	11.5
	0.0	4.0	8.5	17.0	14.0	14.0	13.5	10.5
Averages . .	0.5	6.5	13.5	19.5	18.5	18.0	15.5	13.0

Previously in this paper evidence has been presented that there is a considerable recovery of normal conditions after the disturbance caused by bilateral vagus section. In all the cases of the above tables there was a severance of the splanchnics and also of both vagi. Thirteen days had elapsed since the second vagus operation on three of the animals fed mashed potato, and five or six days had

passed since the second vagus operation on the animals fed lean beef. That there had been at least a partial recovery of the natural state during the intervals is shown by the following figures, averages from three cases of potato feeding one day after the second vagus section : <sup>1</sup>

Hours after feeding . . .	$\frac{1}{2}$	1	2	3	4	5	6	7
Cm. of food masses . . .	3	10	20.5	27	22	17	14.5	12

The recovery is shown also by the figures of the following table, secured by feeding shredded lean beef one day after the second vagus section :

Hours after feeding.	$\frac{1}{2}$	1	2	3	4	5	6	7
Aggregate length, in centimetres, of food masses in small intestine.	0	7.0	12.0	11	9.0	9.0	9.0	8.0
	0	6.5	8.5	9	7.5	7.5	7.5	5.5
	0	1.5	2.5	4	5.0	9.0	8.0	7.0
	0	0	0	0	7.0	10.0	14.0	15.5
Averages . . .	0	4.0	5.5	6	7.0	9.0	9.5	9.0

Examination of these tables and the comparison with the normal conditions which is made in Fig. 3 show that the passage of the carbohydrate food from the stomach occurs at about the same rate as when the vagi alone are cut, but that the proteid discharge is more nearly normal when all extrinsic nerves are divided than when the vagi alone are cut, whether the observations are made within a day of the final operation or after an interval of several days. Why, under the circumstances of these observations, proteids are more normally treated than carbohydrates is not known. The important fact, however, is that, with all the splanchnic and vagus impulses removed, a characteristic difference between the discharge of carbohydrate and the discharge of proteid food from the stomach is still maintained.

**The passage of food through the small intestine.** — As stated in an earlier research, the method used then, and used now again in this investigation, does not permit a statement of the moment when food first enters the colon; all that can be reported is the first

<sup>1</sup> In all cases the splanchnics were cut before the vagi.

observation when food mixed with bismuth subnitrate is seen in the colon. The following figures therefore show, under different circumstances, the number of cases in which, at the hours stated, the carbohydrate and proteid food was first seen in the large intestine: <sup>1</sup>

MASHED POTATO.					
Hours after feeding . . . . .	2	3	4	5	7
Normal . . . . .	1	3	..	..	..
Splanchnics cut . . . . .	1	1	2	..	..
Vagi cut . . . . .	..	1	1	1	0
Splanchnics and vagi cut . . . . .	..	1	3	..	..
LEAN BEEF.					
Hours after feeding . . . . .	4	5	6	7	
Normal . . . . .	..	..	3	1	
Splanchnics cut . . . . .	2	1	..	1	
Vagi cut { (recovery 1 day) . . . . .	..	..	..	0	
{ (recovery 9 days +) . . . . .	1	..	..	1(0)	
Splanchnics { (recovery 1 day) . . . . .	..	..	..	0	
and vagi cut { (recovery 5-6 days) . . . . .	1	..	..	1(0)	

In the cases reported in the above tables the most important variations from the normal are to be seen in the treatment of lean beef. After splanchnic section the rate of passage from pylorus to ileocolic sphincter was much accelerated. After vagus section, on the other hand, the passage was slower than normal, for in only two out of the four cases in which lean beef left the stomach at the usual time did it reach the colon before the end of seven hours after feeding. One of the two cases presented an exceptionally rapid passage through the small intestine. In two of the cases observed within a day of the second vagus operation the beef was being churned in the stomach

<sup>1</sup> A zero in the final column indicates that in some or all of the four cases nothing had appeared in the large intestine at the end of seven hours after feeding.

by peristaltic waves, and was present also in considerable amount in the small intestine, twenty-eight hours after feeding. When the splanchnics as well as the vagi had been cut, there was still a delay in the appearance of material in the colon after lean beef was fed. In one instance, however, in which the passage through the small intestine occurred in an unusually short time for lean beef (four hours), large masses of food were seen moving slowly and steadily through the coils, evidently being propelled by peristalsis. This steady movement of food in the gut I have rarely seen except in some of these cases of entire section of vagi and splanchnics and in animals that had been given strong cathartics.

The carbohydrate food passed through the small intestine under the various conditions of operation with less variation from the normal than did the proteid food. Potato reached the large intestine after not more than an hour's delay beyond the normal in all cases save two. It is noteworthy that these were cases of vagus section. Indeed the abnormally high level of the carbohydrate curve after vagus section (see Fig. 2) is undoubtedly due largely to slow progress of the food through the small intestine under these circumstances.

Rhythmic segmentation was observed in every condition of nerve section. Peristalsis was prominently exhibited in some of the animals with splanchnics and vagi cut, but only when lean beef was fed did this marked peristalsis produce unusually rapid transit from the stomach to the colon.

#### DISCUSSION OF RESULTS.

The stomach, according to most observers, is influenced by impulses through the splanchnics in an inhibitory manner alone, but

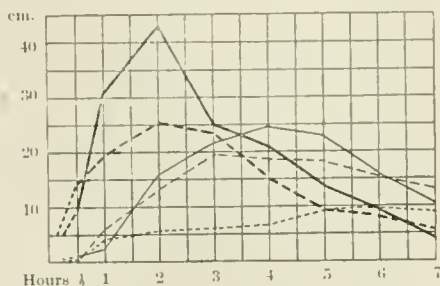


FIGURE 3.—Curves showing average aggregate length of food masses in the small intestine at regular intervals for seven hours after feeding mashed potato in four normal cases (heavy continuous line); and in four cases with splanchnics and vagi cut (heavy dash line); and after feeding lean beef in four normal cases (light continuous line); in four cases with splanchnics and vagi cut, and five or six days since second vagus operation (light dash line); and in four cases similarly operated, but with only one day since the second vagus operation (light dotted line).

Morat,<sup>1</sup> who worked mainly on dogs, noted instances of a motor effect, and May,<sup>2</sup> who used rabbits, cats, dogs, and monkeys, concluded that the splanchnic nerves have no direct influence whatever, either motor or inhibitory, on the muscular wall of the stomach. On the movements of the small intestine the very careful work of Bayliss and Starling<sup>3</sup> has disclosed only an inhibitory action of the splanchnic impulses. The quite normal mechanical treatment of the food by the œsophagus, stomach, and intestines, after splanchnic section, indicates that the splanchnics, in the presence of vagus influence, are certainly not required for the continuance of the normal motor activities of these organs.

For the vagus nerve both motor and inhibitory effects on the stomach have been described. May, working with Starling, found that after ordinary stimulation of the vagus a short initial inhibition of the movements was followed by much more vigorous peristalsis and an increased muscular tonus.<sup>4</sup> On the small intestine almost all observers ascribe to the vagi an excitatory effect. Bayliss and Starling<sup>5</sup> found invariably that stimulation of these nerves produced, after only a momentary inhibition, a marked and prolonged augmentation of the contractions of the circular muscles. Although the vagus nerves are not necessary for contractions of the walls of the alimentary canal, the presence of vagus impulses, when the splanchnics are active, is apparently required in order to maintain a vigorous activity. ✕ When the splanchnics are intact and the vagus impulses are removed, gastric peristaltic contractions are at first late in starting, and are so weak as scarcely to impress the stomach outlines; the discharge through the pylorus is slow; and the passage through the small intestine is under the normal rate. This primal depression of the movements of the gastro-intestinal canal after cutting both vagi must not, however, be regarded as persistent, for there is later a respectable recovery of efficiency by all the motor activities of the canal. ✕

Observations on the isolated stomach<sup>6</sup> and on the isolated intes-

<sup>1</sup> MORAT: Archives de physiologie, 1893, xxv, p. 142.

<sup>2</sup> MAY: Journal of physiology, 1904, xxxi, p. 271.

<sup>3</sup> BAYLISS and STARLING: Journal of physiology, 1901, xxvi, p. 138.

<sup>4</sup> MAY: *Loc. cit.* p. 264.

<sup>5</sup> BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 142.

<sup>6</sup> HOFMEISTER and SCHUTZ: Archiv für experimentelle Pathologie und Pharmakologie, 1880, xx, p. 7.

tine<sup>1</sup> have proved that these organs are capable of the performance of characteristic movements wholly independent of connections with the central nervous system. It was to be expected, therefore, that gastric and intestinal peristalsis and rhythmic segmentation would occur quite as usual after section of all splanchnic and vagus nerves in animals otherwise normal. The interesting feature of the observations on animals with all extrinsic nerves cut was the improvement in the motor activities over the condition in which only the vagi were cut. Gastric peristalsis, the discharge of proteid from the stomach, and the passage of both proteid and carbohydrate through the small intestine were all more nearly normal when there was no connection between the gastro-intestinal canal and the central nervous system than when the splanchnics alone were intact. This difference indicates that the abnormality of functioning after vagus section was due, not only to the absence of tonic motor impulses through the vagi, but also, in part, to the depressing influence of the splanchnics.

Again in these cases of total isolation of the stomach and the small intestine from the central nervous system, the immediate effect of removing vagus impulses is a failure of these organs to act as naturally as they act later. Although the motor functions of the gastro-intestinal tract can be performed by muscular contraction alone, or through local nervous mechanisms, an energetic performance of these functions appears dependent upon impulses through the vagi. The vagi seem to support the operations of these outlying mechanisms in a tonic manner. When this support is removed, the motor activities may be temporarily suspended or much less effective than normal; later, however, the local control proves itself able to manage the movements with approximate naturalness.

It is clear that the persistence of characteristically different rates of discharge of proteid and of carbohydrate food from the stomach after splanchnic section, after vagus section, and after severing both sets of nerves in the same animal, definitely proves that the control of the differential discharge is local and not mediated through the central nervous system.

#### SUMMARY.

One series of animals was studied with only splanchnic nerves cut, another series with only vagus nerves cut, and a third series with an entire severance of splanchnics and vagi.

<sup>1</sup> BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 102; 1901, xxvi, p. 125. Also MAGNUS: *Archiv für die gesammte Physiologie*, 1904, cii, p. 123.

Splanchnic section resulted in no change of the normal movements of any part of the alimentary canal. Proteid and carbohydrate food passed through the pylorus at practically the normal rates, but the transit of proteid food from pylorus to ileocolic sphincter was much accelerated. There was no acceleration when carbohydrate food was fed.

Vagus section resulted primarily in a tardiness in the starting of gastric peristalsis, a marked weakness of the peristaltic constrictions, a retarded and slow discharge through the pylorus, especially when proteids had been fed, and a slow passage through the small intestine. These primary effects were largely recovered from within a few days.

After combined splanchnic and vagus section gastric peristaltic waves, unlike those seen when the vagi alone were cut, were, almost from the first, normally deep constrictions. At autopsy of these cases the stomach was usually found strongly contracted. As in the cases of vagus section, there was after operation immediately a retardation of the discharge through the pylorus, and later a partial recovery of the normal state. After this recovery the proteid discharge was more nearly normal than when the vagi alone were cut; the carbohydrate discharge remained about the same. The transit through the small intestine was slower than normal.

Rhythmic segmentation of the contents of the small intestine was seen in every condition of nerve section.

The characteristic rapid discharge of carbohydrate food and slow discharge of proteid food from the stomach, maintained after vagus section, after splanchnic section, and after combined splanchnic and vagus section, confines the differential discharge of proteids and carbohydrates through the pylorus to a local control.



CHEMICAL STUDIES ON THE CELL AND ITS MEDIUM.  
—PART I. METHODS FOR THE STUDY OF LIQUID  
CULTURE MEDIA.

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I. INTRODUCTION.

THE Protozoa have been made the experimental objects for the solution of important physiological problems. The processes of these animals have been found so much dependent upon the conditions of their liquid environment that the subject of their culture and experimental media has become one of fundamental importance in any investigation dealing with them. It has become clear that the results obtained must be defined as certain functions of the particular conditions, physical and chemical, which prevail in the solutions that constituted the media in which they were raised. The intimate relation between a living cell and its liquid environment is still much in want of elucidation. In this first part of the present paper some methods will be described and data will be reported which relate to the study of mass cultures of Protozoa. The subsequent parts, data for which have been obtained but not yet published, will deal with the progressive chemical and physical changes in these culture media together with their simultaneous biological change, also with the relations of the contained organisms to solutions of acids and alkalies, and to pure water, as their media.

My attention was first called to a closer study of the media in which Protozoa grow by the fact that a hay infusion in which numerous species were growing abundantly failed to develop others which were introduced into it in sufficient numbers to seed it. Much later, however, in the history of the same culture the seeded species would show a period of abundant development, although the great majority of the animals that had been used for seed died soon after. Omitting details for the present, I have shown that different species of Protozoa develop in a serial order in a hay infusion made and treated as will be presently described, and further that this order is irreversible in natural cultures. The explanation of this biological phenomenon was sought in the physico-chemical conditions. Other explanations which suggested themselves will be discussed subsequently, but they do not seem to me to apply equally well to the data which were obtained.

During the progress of the present work I enjoyed the privilege of frequent conferences with Dr. A. T. Lincoln of the Department of Chemistry, and for this kindness I am under much obligation to him. I desire also to thank the authorities of the chemical department for their kindness in lending me necessary apparatus.

## II. THE PHYSICO-CHEMICAL DESCRIPTION OF CULTURE MEDIA.

In order to answer the questions whether the fixed order of succession characteristic of these cultures is due to a regular series of changes in the conditions of the media, it is necessary to devise methods for determining their physical, chemical, and biological nature and content. The biological aspect of this subject I shall defer to subsequent parts of the paper. In general, it is evident that the physico-chemical definition of a cultural or experimental medium would be of great value. It is also true that in the present state of our knowledge accurate and complete definition of the conditions of a solution such as a hay infusion which contains decomposing organic matter is impossible. Even in the case of experimental media consisting of pure aqueous solutions of salts, physical chemistry fails to give us the desired definition, especially when several salts are contained in the medium. Culture media, natural or artificial, usually consist of water, inorganic salts, dissolved gases, and organic matter which includes foods and some of the metabolic products of the organisms. Even when organic food with its liabil-

ity to decomposition is excluded from an experimental medium the complexity of the latter is still enhanced by the formation of metabolic products, both organic and inorganic. We are far from a satisfactory knowledge of organic decomposition products on the one hand, and on the other the theory of solutions fails us most in just those cases which occur with the greatest frequency, *i. e.*, in complex mixtures of salts. It is evident that only an approximate definition of the media is at present attainable. But still other limitations appear when the matter is viewed not simply from the chemist's standpoint but also from that of the biologist. The latter would like to examine these media in the actual condition in which they act upon organisms. The end results of a chemical analysis calculated back to their supposed original condition do not very well serve the purpose of the physiologist. Physical chemistry has already done much service in the description of the internal conditions of solutions so far as they are defined by direct measurement, and this much is true regardless of what the theory of the significance of these measurements may be.

At this point we may pause to consider the relation of this effort to define media by physical and chemical characters to the theory or theories of solution. All such characters as are directly observed or measured are of course independent of the changes which the theory of solution may undergo, and such determinations serve reliably for the comparison of physiological conditions in natural or experimental media, this purpose being one for which the determination of these characters is recommended. But we can go still further upon firm ground. It should be emphasized that the question whether these measurable characters or conditions of solution are physiological factors as well may also be tested by direct experiment independently of the theories of physical chemistry. Illustration of this fact will occur in the subsequent parts of this paper. Simply the determination of the interrelation between physiological activities of cells and the physical and chemical conditions of their liquid environment is one of the extensive and important problems of biology. This problem need not and should not wait for the completion of a theory of solutions. This safe and stable ground should be recognized as a permanent bond between physical chemistry and physiological biology. When physiological interpretation is made to depend for its validity upon a given theory of solutions, then of course it stands or falls with the latter. The biologist should clearly recognize the distinc-

tion between these two phases of the relation of his work to physical chemistry. Both phases are represented in the present paper. Most of the methods recommended and most of the facts presented rest upon direct observation and measurement. In some cases I have developed the results so obtained according to a theory of solutions, but I have aimed to make such development clearly recognizable by the reader.

We may now return to the consideration of some other peculiarities of this problem as it is seen from the biological standpoint. For the biologist it is impracticable to use for a single analysis the large volume of his culture liquid which the chemist is accustomed to use and which is considered necessary to insure accuracy. For the biologist it is important that the physical and chemical determinations require but small quantities of the liquid in order that he may have enough of the same medium remaining for further observation and for subsequent determinations. The physico-chemical examinations must not involve the necessity of bringing the experiments to a close. It may be possible, but in most biological laboratories it would be exceedingly inconvenient, to deal with very large quantities of the kind of media here in question. If the frequent estimations required result in the use of small quantities as the basis of ordinary chemical analysis, then the quantitative results obtained must not be regarded as correct in their absolute values, but they must nevertheless be sufficiently accurate to be valid for the purpose of comparison with other values obtained under similar conditions. Another fact resulting from the conditions under which the biologist works is that volumetric chemical determinations should be selected, whenever possible, instead of those which are gravimetric. All the above considerations lead to the conclusion that only approximate and comparative definition of media for the service of biology is at present possible. This fact by no means minimizes the great necessity and the usefulness of such a description of the conditions which prevailed in the environment of the animals when the results of a given investigation were obtained. Discrepancies between the work of different investigators upon the same species or of the same investigator at different times would thus find a probable clue to explanation. Another important advantage would be the limitation of results to a given set of conditions until their wider application had been proved. All these considerations have their valid basis in the fact, now well known to investigators familiar with the objects, that living cells are very delicately adjusted to the conditions of their liquid environment.

### III. THE MANAGEMENT AND DEVELOPMENT OF HAY CULTURES.

The scheme of determinations here presented which serves at least for an approximate description of the physical and chemical conditions of a liquid medium, was developed in connection with the study of hay infusions used for the growth of mass cultures of Protozoa. The principles which determined the applicability of the methods to this medium were then seen to apply to practically all common liquid media, although these may differ much in their quantitative aspect. The methods are perhaps best explained in connection with their application to a hay infusion, and I shall first describe the preparation and care of this medium.

The following method has been followed so often in this laboratory, both for the purpose of investigation and for the growth of classroom material, that the course of development of cultures so managed has become predictable. The final volume of the majority of cultures prepared was about 7590 c.c., or about two gallons. For this amount of liquid a small handful of timothy hay was used, and this was found to weigh about 30 gm. or about one ounce. The quantity of hay need not be accurately weighed or measured. The effect to be expected from somewhat larger or smaller quantities is simply an increase or diminution respectively in the length of time required by the culture to run through its cycle of changes to complete exhaustion, *i. e.*, to biological sterility. Other kinds of hay and other forms of organic nutriment have given considerably different results than those here described. The hay is covered with a sufficient proportion of the whole quantity of water to be used to immerse all the hay, and it is then heated to boiling, and this is continued for a few minutes. I am in the habit of performing this process in an iron kettle used for this purpose only. In the meantime the unboiled portion of the water is placed in the vessel in which the culture is to be set. Glass jars are best adapted to this purpose because they facilitate observation, but stoneware vessels may also be used. When the boiled material has partially cooled, it is transferred, including both the infusion and the solid hay, to the remaining cold water which has been placed in the culture jar. The hay has been boiled for several reasons, one of which is to drive the air out of it, soak it with water, and so make it heavy enough to sink to the bottom of the jar. This prevents it from interfering with observation of the surface and sides of the jar, which it would do for some time if it were introduced

in the dry form. Other effects of the boiling will be described subsequently. Two important features of this method of setting a culture are the boiling of only a portion of the total volume of water and the retention of the boiled hay in the medium. The absence of the solid hay will simply give a short-lived culture which would not last long enough to develop the characteristic biological cycle to be described later. I have not found the kind of water used to be a matter of much importance so far as merely raising a successful culture is concerned, but if any experiments which involve physiological conditions are to be performed upon the animals, then the question of the water used as a solvent may become of much importance. Animals of the same species but originating from different conditions have frequently shown marked physiological differences. The cultures I have studied have been made, some with tap water that contained a considerable proportion of inorganic salts in solution, some with the water of a flowing stream or of a stagnant pond, and some with distilled water. Rain water might also be used. Barring natural water containing poisonous mineral salts, it is of less importance to select a given kind of water than it is to be able to determine the character of the medium prepared with it. After a given water has had boiled hay added to it, its original character is no longer of much significance. A culture medium prepared as above described is now ready to be seeded. If proper precautions were taken, these cultures could be seeded with one or a few species of Protozoa, but the presence of bacteria is also necessary to produce the normal course of development or of decomposition in the culture. In these studies and in the growing of class material the object was to produce a mass culture of as many different organisms, plant or animal, as were at any time adapted to the varying conditions. Seed for a new culture was taken from any or all old cultures in the laboratory, and consisted of some of the liquid, portions of zooglœa, solid and decaying hay, and a representative collection of the organisms to be found in the growth area which develops on the walls of the jar near the surface of the liquid. A new culture may also be seeded with aquatic material of all sorts collected in the field. Decaying leaves, slimy sticks, and small portions of the water which has been agitated so as to disturb the sediment at its bottom furnish good material. Frequently the seed, wherever obtained, does not, upon examination, seem to be rich in organic forms, but unobserved and unexpected forms may nevertheless appear in the culture later in abundance. Nor is it by any means cer-

tain that forms which are introduced in abundance will flourish, and in some cases they will nearly all die off at first, only to appear in large numbers later. For the explanation of these peculiarities a ready indication may be obtained from a comparison of the physico-chemical conditions as shown by quantitative methods, of the medium from which the seed material originates, and of the hay infusion into which it is introduced. Old cultures that have nearly or entirely run through the characteristic cycle may be revived and caused to repeat the cycle in apparently every particular by re-feeding the culture with solid hay and its infusion prepared exactly as for a new culture. For this purpose re-seeding is of course omitted. After preparing the media and seeding them, the culture jars are to be kept covered, preferably with a pane of glass, to prevent undue evaporation which would give rise to a concentration of the dissolved salts not due to the metabolism of the medium or of its contained organisms. The cultures are to be labelled with a date number, so that their age is easily determined when they are under observation. They should be kept in a warm place at or above room temperature, and preferably with always the same side towards the light. Upon the illuminated side a green layer of algæ will gradually appear. If gas bubbles raise the hay to the top, it may be pressed down with a glass rod, otherwise the culture liquid should never be agitated except for definite experimental reasons. Through some cultures I have led constant or intermittent currents of air which somewhat modify the course of development.

I shall next describe some of the processes which occur in the culture liquid and their measurement. On the day when prepared the liquid has a straw color. When the culture is a day old, this color is still lighter, and if the temperature has been warm enough, some gas bubbles appear which may become sufficiently abundant to lift the hay to the surface. This gas is principally  $\text{CO}_2$ . Other gases distinguished from this by their odors may be evolved in greater abundance later. Old cultures have a darker or brown color, and the same culture liquid passes through all gradations from light straw to dark brown colors. Treatment of a portion of this liquid in a test tube alternately with acid and with alkali shows that the hay contains a coloring matter, sensitive enough to act as a rough indicator for H and OH, and hence mere inspection shows to the experienced eye the condition of a culture, and, moreover, its relative age. The light and yellowish shades of color are due to acid, the darker and brownish shade to alkali.

Another condition easily ascertained by direct observation is that the liquid, though colored, is clear when first prepared, but after a day or longer it has become turbid. Microscopical examination of the clear liquid shows relatively few bacteria, but the turbid liquid is found to contain millions of them. I have never determined the specific kinds of bacteria, but I have observed sufficiently to see that practically the same forms are characteristic of the same cycles of these cultures.

The data thus far obtained indicate that the bacteria introduced with the seed material are the primary agents in the metabolism of the culture. To their fermentative action upon the carbohydrate extracted from the hay we may ascribe the abundant production of  $\text{CO}_2$ , and the very acid condition of the medium during the first few days of its history. Of course other acids, *e. g.*, lactic, are no doubt also produced, and other matter than carbohydrate is also fermented, but probably in much smaller amount, and more so at a subsequent period. Appropriate methods of determination, described below, will give us some information on this point. The bacteria themselves may be regarded as constituting the primary source of food for the Protozoa, *e. g.*, *Paramæcium*, which soon begin to multiply with great rapidity. Observation will show that some of the Protozoa, *e. g.*, *Stentor cœruleus*, prey upon the animals of the same group. Frequent microscopical examination of the food content of various Protozoa has shown that this mode of feeding is not of much quantitative significance, and that bacteria and algæ are the main food supply of the various animals found in the cultures. Observation shows that the number dying as prey is relatively small compared to the number of new organisms arising by a rapid rate of multiplication when the organism preyed upon multiplies under favorable conditions. The succession of organisms in the culture cannot be satisfactorily explained by the unaided supposition that the animals prey upon each other. Upon the theory that oxidizable food matter is osmotically absorbed from the surrounding solution by the Protozoan cell, I am content to leave the burden of proof for that proposition. The first biological period in the history of the culture may then be regarded as that of the bacteria, and other cycles characterized by predominant species of Protozoa follow in fixed and regular order. Without determining this order at present we may now turn to the determination of the conditions under which these animals will develop.



#### IV. QUALITATIVE EXAMINATION OF CULTURE MEDIA.

If a few cubic centimetres of the straw-colored liquid be placed in a white porcelain evaporating dish and the indicator phenolphthalein in solution added, the liquid fails to develop the characteristic red color which would appear in the presence of a certain excess of OH. Hence, according to the theory of indicators, the liquid contains more H ions than are produced when phenolphthalein dissociates, thus permitting the red color of its anions to become visible. In other words, the concentration of H ions is too great to permit the dissociation of phenolphthalein. If now the same liquid be boiled even a very short time, the phenolphthalein becomes red. Evidently the original acidity was due to a volatile acid, mostly carbonic. If the reaction of another portion of the culture liquid be tested with methyl orange, it will fail to develop the characteristic pink color which would appear in the presence of a certain excess of H. According to theory the liquid contains fewer H ions than are produced when methyl orange dissociates, and so permits its yellow anions to become visible. In other words, the concentration of H ions is too small to interfere with the dissociation of methyl orange. The behavior of the liquid with these two indicators points to the presence of acid salts, which in this case will be found to be mostly bicarbonates.

Evaporation of 10 c.c. of the filtered culture liquid in a crucible leaves an abundant yellowish brown residue, which chars upon the application of a red heat, finally leaving a colorless mass of inorganic salts. The charring is due, of course, to the presence of organic matter held in solution and not to organisms suspended in the medium, provided the filtration which preceded evaporation was properly performed. All organisms larger than the bacteria should be separated from those portions of the culture liquid which are to be used for most of the determinations to be described. Hard pressed filter paper (Schleicher and Schuell's No. 575) serves well enough for approximate results. Under some circumstances the paper may easily clog. A better and more rapid method consists in the use of a filter pump to partially exhaust the air from underneath an asbestos filter made of any desired thickness and compactness from fine washed asbestos.

At this stage an ordinary qualitative chemical analysis is indicated if the operator is dealing with a kind of culture liquid which he has never examined in this way. After he has once well oriented himself regarding a particular kind of medium which he may be preparing

repeatedly, the qualitative tests may be omitted when fresh quantities are prepared by the same rule. The experimenter with organic culture liquids must not deceive himself with the proposition that because such a medium is repeatedly prepared by the same empirical method the quantitative results produced are also uniform. It is just this point that requires objective proof before reliance is placed upon it in a continued series of experiments. Particularly is this true when the ensuing biological phenomena are supposed to be due to the physical and chemical conditions of the medium. When in this case the experimenter presents neither qualitative nor quantitative investigation into the character of the medium with which he is producing the results described, his interpretation of these results may very justly be regarded as wanting in sufficient and necessary evidence. These observations are worth making in order that the experimental zoölogist be not less critical with his means of investigation than the chemist and the physicist. If the zoölogist is to deal efficiently with problems in the physiology of animals, he will have to add to scalpel, microtome, and microscope, highly valuable as they are, a working knowledge of at least some chemical and physical determinations. The fact that chemistry and physics cannot yet give a complete account of an organic culture liquid does not diminish the force of the preceding observations. The logical analysis of biological phenomena requires all the data now obtainable. All the above observations apply with equal or greater force to the preparation and use of experimental (not cultural) media consisting of solutions of pure inorganic salts. A general principle which applies to the study of the interaction between organisms and liquid media, whether the latter be organic or inorganic, is that the experimenter must determine objectively the successive changes which any medium necessarily undergoes when a living organism inhabits it. This constitutes a laboratory study in ecology made upon a kind of environment, the liquid, which, from the standpoint of the cellular structure of all organisms, is of almost universal occurrence.

From this digression we may return to the qualitative analysis. As a guide Prescott and Johnson, :05, may be used with much advantage. After the preliminary tests already described the operator may proceed with a systematic scheme of analysis. For a description of the necessary operations reference must be made to the above-named authority. I have never found the quantity of organic matter in hay infusions made as previously described, to be sufficient to interfere

with the ordinary tests, except in some cases, by reason of the pronounced color of the liquid. In the examination of any medium where it becomes desirable or necessary to remove organic matter, this is best done by the methods recommended by Hoppe-Seyler, :03, pp. 391-394. The liquid is treated with some excess of sodium carbonate, evaporated, and then charred and ignited at as low a temperature as possible. This process reduces losses from volatilization to a minimum. The residue may then be used for the ordinary course of analysis. Na and  $\text{CO}_3$  must of course be sought in another portion of the original liquid. In some cases it may be permissible for the operator to assume the absence of the metals of the first and second analytical groups, as classified by Prescott and Johnson, because they are poisons not likely to occur in the materials which he carefully selects, but for certainty demonstration is indispensable. Lead in waters, fixing agents from laboratory utensils, etc., must be guarded against. Usually the examination for metals of the third group, *i. e.*, Fe, Cr, Al, will be the first part of the analysis that will show the positive presence of certain metals. As the presence of phosphoric acid would complicate the analysis of the third and of succeeding groups, and as this acid may frequently occur in organic liquids, its presence or absence must be established before proceeding. The residue from 10 c.c. of hay infusion obtained as previously described was dissolved in water, acidified with nitric acid, and tested for phosphoric acid with ammonium molybdate. No precipitate and no color change whatever followed the use of this reagent, thus demonstrating the absence of phosphoric acid. As a control test, a trace of  $\text{K}_2\text{HPO}_4$  was added to 10 c.c. of the original culture liquid, and exactly the same procedure for phosphoric acid was performed, and a positive result obtained, as evidenced by the yellow color due to a small quantity of ammonium phosphomolybdate. For certain purposes it might become advisable to conduct the qualitative examination upon the residue from the concentration of a large amount of liquid. Using only small quantities of the hay infusion, the following radicals were proved to be present: Fe, Al, Ca, Mg, K, Na,  $\text{NH}_4$ ,  $\text{CO}_3$ , Cl,  $\text{SO}_4$ . Examination of the medium at a different stage of its development might show additional substances present, *e. g.*,  $\text{H}_2\text{S}$ . These would consist of transition products standing between organic matter and its final mineralized products. I am indebted to the State Water Survey of Illinois for the following mineral analysis of the University Water Supply, with which the above-described hay infusion was made:

Hypothetical combinations	Parts per million	Hypothetical combinations	Parts per million
KNO <sub>3</sub> . . . . .	0.4	MgCO <sub>3</sub> . . . . .	121.2
KCl . . . . .	4.8	CaCO <sub>3</sub> . . . . .	175.2
NaCl . . . . .	2.0	FeCO <sub>3</sub> . . . . .	2.1
Na <sub>2</sub> SO <sub>4</sub> . . . . .	3.6	Al <sub>2</sub> O <sub>3</sub> . . . . .	2.5
Na <sub>2</sub> CO <sub>3</sub> . . . . .	62.1	SiO <sub>2</sub> . . . . .	18.9
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> . . . . .	6.1		398.9

It appears that the hay added no qualitatively new constituent in appreciable amount except its organic matter, but of course the quantitative relations are probably much altered, and on this point the determinations to be described later furnish satisfactory data. The progressive alteration of this organic matter begun by the bacteria furnishes the explanation for the fact that this medium is not one environment, but is a series of these, each characterized by a fauna delicately adjusted to its special conditions. Our immediate problem is to find some means of distinguishing these stages from each other.

#### V. SELECTION OF QUANTITATIVE DETERMINATIONS.

At the beginning the most evident change is the production of CO<sub>2</sub>. There is not enough base present to hold all the CO<sub>2</sub> produced in chemical combination, nor is there sufficient liquid to hold it all in solution under the existing atmospheric partial pressure. If we desired to measure all the CO<sub>2</sub> produced, this would be entirely feasible by the method which was devised by Barratt, :05, pp. 66 ff.

In the present case we desire a measure of only that quantity of H<sub>2</sub>CO<sub>3</sub>, or of any other free acid, which may arise from organic processes with which the organisms in the medium must live in contact. This can be accomplished by titrating under certain conditions a given volume of the culture liquid with a standard 0.01 m NaOH solution, using phenolphthalein as an indicator. I shall call this determination simply the *phenolphthalein acidity*. Its significance is both chemical, with reference to the carbon metabolism of the culture medium, and physiological, owing to the great activity of acidic H upon living organisms. On account of the chemical relations of these determinations the technique of making them will be described together later, and some results given for illustration.

The gradual breaking down of dead organic matter and the disin-

tegration of at least some animal or plant bodies may be expected to affect the quantity of inorganic salts present in the medium at different times. In any case a culture liquid is, for physiological reasons, a salt solution of some kind, and this is also true of many experimental media. Hence the quantitative inorganic salt content (and gaseous content also) is of much significance as an indication of the condition and changes of the medium, chemically considered, but the physiological importance of this content is still greater. The qualitative differences between the action of salt radicals (or ions) is well known, including such variety as poisons, apparently indifferent substances, and stimulants of specific character. In addition to their specific action all the salts are important on account of their possible osmotic action. The interchange between the cell and its medium is one of most far reaching consequences in the life of the cell, and the salts play a prominent part in this process. At this point it is necessary to distinguish between what for want of a better name I shall have to call a physical osmosis, on the one hand, and a physiological osmosis on the other. It is here, again, that the theories of solution must be taken into consideration. If the kinetic theory of gases be regarded as applicable to solutions, then we may calculate from certain measurements an osmotic pressure, which is regarded as existing in the solution independently of the properties of any membrane. Perhaps this quantity is more accurately described as the maximum osmotic pressure which the solution could exert upon a strictly semi-permeable membrane. It is to be noted that this theory of osmotic pressure is rejected by some investigators, and for the biologist the quantity above described can be of use only as one of the physical characters for the comparison or identification of solutions. Two different points of view on this question are well represented by Lothar Meyer, '90, pp. 23-27, and a reply by J. H. van't Hoff, '90, pp. 174-176. Further discussion of osmosis, with special reference to biological phenomena, is made by J. Traube, '04, pp. 704-715. Physiologically, osmotic pressures without reference to the particular membranes involved are of no significance, and the existence of semi-permeable membranes is highly improbable. Hence the permeability of the natural membrane is the osmotic factor of greatest significance for physiological processes. But this may vary according to the specific kind of cell under consideration, also with different physiological conditions of the same cell, and possibly according to the kind of salt surrounding the cell wall. But of the permeability of animal

cells we have only the most limited knowledge. How seriously this limitation affects our physiological conceptions may be understood from a consideration of the significance of the vital interchange between cell and medium. It is not the purpose of this part of the present paper to describe investigations on this subject. I shall, however, in one case at least, describe the method of obtaining the physical osmotic pressure above referred to.

The preceding considerations show the importance of obtaining the inorganic salt content as one of the descriptive quantities of a culture or experimental medium. I shall describe some methods adapted to this purpose. In the hay medium here used the qualitative examination has shown the presence of acid salts, mostly or wholly bicarbonates, and in many media this same condition will present itself. This class of salts can be easily estimated by titrating a given volume of culture liquid with 0.01 M HCl, using methyl orange as indicator, and I shall call this determination the *methyl orange alkalinity*. In the quantitative examination of the hay infusion here used the total quantity of nearly all the salts present will have been estimated in the determination of bicarbonate. Nearly all the base present is combined in this form, there being comparatively little chloride, sulphate, etc. Hence, here the quantity of bicarbonate is especially significant as an indicator of the osmotic conditions. The phenolphthalein acidity and the methyl orange alkalinity, two simple determinations, are sufficient to show great differences between various proposed organic culture media, and they also show some well-marked changes in the history of a given organic medium. If the medium contains quantities of other salts, *e. g.*, chlorides or sulphates, etc., estimable by ordinary methods when applied to small quantities of culture liquid, these must also be determined by the various special volumetric methods applicable to each constituent sought. For chlorides, even in very small concentration, the method of Volhard, which gives accurate results and in which the liquid is made strongly acid, is to be most recommended. In the selection of methods for the titration of other acid and basic radicals the operator is strongly limited by the fact that these methods must be sensitive and accurate enough to estimate quantities in such dilution that 0.01 normal standard solutions are appropriately used. The operator does not command large quantities of liquid which could be concentrated by evaporation as in mineral analyses of waters. This limitation does not prevent the titration of substances for which very sensitive

methods exist, *e. g.*, chlorides and iron, and of those substances which occur in relative abundance in the liquid, *e. g.*, calcium. I have not tried any of the newer methods for the volumetric estimation of alkali metals. Such determinations would give desirable factors in the history of a culture liquid, especially hay infusions. A further difficulty in the selection of methods is that they must be practicable in the presence of some organic matter. Incineration of the evaporated residue of a portion of the liquid would defeat one of the purposes of these determinations, which is to find how much of the organic constituent has become mineralized from time to time. If for any reason it becomes important to know accurately the relative quantities of inorganic salts, some gravimetric methods may have to be used, even if infrequently. I have made only those estimations which seemed to me to be of most significance for the correlation of the chemical and physiological events in the culture. My selection is admittedly incomplete.

From the above point of view some indication of the total amount of organic matter is desirable and only an indication is possible. Probably the best we can do is to adopt the permanganate method for *oxygen consumed* to be executed under certain uniform conditions later to be described. In spite of the well-known limitations in the significance of the results obtained by this method they are nevertheless of considerable value for comparative purposes, and it is from this standpoint only that we shall interpret them.

It is evident that the amount of dissolved oxygen in a given volume of the liquid is a chemical and physiological factor which it would be desirable to obtain. Some experiments which I have made in this direction were not carried to completion.

I have added to the preceding the measurement of the *electrical conductivity* by the method of Kohlrausch. According to the theory of electrolytic dissociation this quantity is an index of the ionic concentration. The conductivity is one of the important physical characters of a solution. As this determination assists us in the correlation of physiological facts with physical characteristics, it is for that reason desirable, even though its physical meaning may be in dispute. The use is evident of this or any other methods that will assist in the explanation of the fact that the same concentrations of different acids as determined by ordinary chemical analysis act with very different intensities, both physically and physiologically. Other examples could be added. It is true that this measurement

taken independently of other determinations upon the same liquid has only a limited and ill-defined significance, and still more so when taken upon a complex mixture of salts such as most culture media. But in the latter case and in natural media, *e. g.*, waters, the inorganic salt content, to which the conductivity is mainly due, may be almost completely classified into the alkali, alkali earth, and earthy metal groups of bases, and the acidic radicals combined with these are also limited to a certain small list. The conductivity of these salts taken separately is known as well as the conductivity relations of members of the same group and of the different groups to each other, so that averages can be used for approximate results. Furthermore, one is frequently dealing with repetitions of qualitatively and roughly similar media, *e. g.*, hay infusions. In all these cases the electrical conductivity serves well for the identification of similarity in conditions and for the physical comparison of the solutions. The method is so sensitive that small differences are expressed in easily measurable magnitudes. Kohlrausch, '98, pp. 131-132, has emphasized the use of the method for the approximate estimation of the concentration of salts in solutions whose general character is known. In the present scheme of investigation more precise knowledge of the constituents is contemplated, and this measurement is to be accompanied by others which may be brought into physical or physiological relation with it.

The methods thus far described give information regarding the:

1. Acidity;
2. Salt content;
3. Carbonaceous content;
4. Electrical conductivity.

The organic nature of the culture liquid suggests the question whether the nitrogenous metabolism of the culture might not also be an important chemical and physiological factor. Organic culture media resemble a mild sewage, and it is evident that the chemical data sought are much the same as the analyst of waters and of sewage seeks, the methods used being slightly modified by the ultimate physiological aim. A consideration of the conditions prevailing in these media led me to make numerous estimations of nitrogen in the form of free and saline *ammonia* by the Nessler process, and of *organic nitrogen* by a modified Kjeldahl process due to Rideal. The serial nitrogen values obtained in the history of individual cultures showed that, owing to several *compensating* processes in the nitrogenous metabolism of these cultures, these values are not useful in demarcating developmental stages unless other inconvenient and time-consuming estimations are made at the same time. Nitro-



gen in the form of nitrites and nitrates would not be expected to occur, as will be explained later, in any quantity greater than traces during the time that these cultures are under examination, and they were never found when tests for them were made. Nitrates even disappeared shortly after they had been purposely added, these salts serving as a source of oxygen which became available upon their decomposition.

Guided by the principle that the estimations which are to be made by the biologist should yield important information, either individually or combined with others, and that they should be capable of sufficiently rapid execution to permit serial determinations, I venture to propose the following series: 1. Phenolphthalein acidity. 2. Methyl orange alkalinity. 3. Total salt content and special methods for individual constituents. 4. Electrical conductivity. 5. Oxygen consumed. 6. Dissolved oxygen (not developed). 7. If desired, organic nitrogen. I have purposely omitted the determination of the depression of the freezing point from this list, regarding that method as more useful in certain special cases than for the frequent application which was contemplated in making the above selection. It may be said again that, on the one hand, this list is not comprehensive enough to yield sufficient results for a full description of a medium, and, on the other, it may be abbreviated and still give results which define some important conditions of physiological activity.

The use of these quantitative methods in the case of an unknown culture liquid is advantageously preceded by a qualitative analysis. The biological examination that is to run parallel with these determinations will, of course, vary according to the special aim of the investigator, and the method of examination which was applied to hay cultures will be described later. The practical application of each method will be outlined or references will be given to descriptions of the processes. These methods will be illustrated by practical applications actually made, and the significance of results obtained or obtainable from culture liquids will be explained briefly. For guidance in method and for assistance in the interpretation of results, general reference is here made to certain works which are nearly indispensable. For a clear, concise, and concrete treatment of the elements of volumetric analysis reference may be made to the excellent short treatise of O. Kübling, :04. To this should be added Sutton, :04, "Volumetric Analysis"; Hoppe-Seyler, :03; Rideal, :01; Kohlrausch und Holborn, '98. Specific references to these and to other authorities will be made in the following pages.

## VI. APPLICATION AND RESULTS OF QUANTITATIVE METHODS.

For the practical application of the preceding methods it has seemed to me advantageous to have a system of interrelated standards so devised that they all or nearly all depend upon a single original standard solution which can be most carefully prepared, or, if necessary, obtained by the biologist from a chemical laboratory. For a variety of reasons dependent upon the chemical point of view I have selected HCl for this standard. This HCl is used to obtain the methyl orange alkalinity directly. The standard HCl is also used to standardize the NaOH solution required to determine the phenolphthalein acidity in the manner to be described. The standard HCl is also used for the standardization of a sodium thiosulphate solution by means of which, again, a standard potassium permanganate is prepared, the thiosulphate and the permanganate to be used in the determination of oxygen consumed. Furthermore, from the standard hydrochloric, after neutralization with the NaOH, a  $\text{AgNO}_3$  solution is standardized by Mohr's method. In this case the absence of Cl from the NaOH must be first proved by the  $\text{AgNO}_3$  test. The standard so prepared is used for the direct determination of chlorides, or better, by means of it, a standardized KCNS solution is made for use in the Volhard method for chlorides. In addition to the preceding a pure saturated aqueous solution of  $\text{CaSO}_4$  is required for the purpose of determining the resistance capacity of the electrolytic cell or electrodes which the operator uses in conductivity measurements. The operator is advised to take the conductivity of his standard 0.01 m HCl solution and compare the value found with that given in the tables of Kohlrausch and Holborn, '98, p. 160. This furnishes him a desirable control on both his chemical and electrical measurements. To any one to whom a system of standards which are interdependent seems objectionable, the alternative of preparing any or nearly all of the above standards by processes depending upon independent weighings is of course open. The principle of making interdependent volumetric standards has been recognized by good authorities (Sutton, :04, pp. 43-44 and references there).

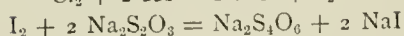
For the preparation of a standard HCl, F. Sutton, :04, pp. 47, 42-43, and for more detail, O. Kühling, :04, pp. 46-52, may be consulted. I have followed the practice of finding, from the specific gravity of a pure strong HCl and from a table, the number of cubic centimetres of this acid required to make one or several litres of approximately

0.01 m HCl. Having made this dilution, I have accurately standardized it by means of a weighed quantity of pure, dry  $\text{Na}_2\text{CO}_3$ , using methyl orange as indicator. It is not worth while to attempt to dilute these solutions to exactly the desired 0.01 m, but its actual value must be accurately determined and this quantity used in the calculation of results obtained by means of this standard. The value of the HCl may also be accurately checked by precipitation as  $\text{AgCl}$ . The absorption method may also be used for the preparation of a standard HCl.

The preparation and keeping of a standard NaOH solution are described in F. Sutton, :04, pp. 48, 20-21, and O. Kühling, :04, pp. 53-59. An approximately 0.01 m solution of accurately known value is desired. Its exact value as determined by means of the standard HCl should be ascertained both for methyl orange and for phenolphthalein used as indicators, and these standardizations should be repeated at intervals. The standard NaOH should be connected by tubing with the burette, and should be protected from the  $\text{CO}_2$  of the air by means of soda lime. It is not necessary that it be free from  $\text{CO}_3$ , but the two values above mentioned must always be known.

A standard 0.01 m  $\text{AgNO}_3$  and 0.01 m KCNS are made as has been previously indicated, and in addition reference may be made to F. Sutton, :04, pp. 144-146, 401, and to O. Kühling, :04, pp. 141-149.

For the preparation of standard thiosulphate and permanganate solution, see F. Sutton, :04, pp. 472, 477, 124, 135; also O. Kühling, :04, pp. 84-94, 121-123. I have standardized the thiosulphate by means of the standard HCl by a method based upon a suggestion in F. Sutton, :04, pp. 138-139, concisely stated as follows: "Recently precipitated oxides, or the natural oxides when reduced to fine powder, are readily dissolved and decomposed by very weak acid in the presence of potassium iodide (Pickering)." A bottle is selected whose ground glass stopper fits or is ground to fit gas tight. In this are placed 20 to 50 c.c. of the standard 0.01 m HCl. Then there is added a quantity of pure pulverized  $\text{MnO}_2$ , an unnecessary excess being avoided in order to prevent too dark a color of the liquid when it is shaken. Then a sufficient quantity of KI in solution is quickly added, the bottle tightly stoppered and set in hot water (about  $75^\circ\text{C}$ .) for a half-hour. The bottle is cooled and the liberated iodine is titrated, using starch indicator with a thiosulphate solution which has been prepared by a rough weighing. The following equations represent the reactions involved:



From these relations the value of the thiosulphate is calculated, and by means of this the permanganate, also prepared by a rough weighing, is standardized by the usual method, using KI and  $\text{H}_2\text{SO}_4$ . Concentrations of about 0.01 m  $\text{Na}_2\text{S}_2\text{O}_3$  and about 0.002 m (= 0.01 n) of  $\text{KMnO}_4$  are desirable for this work.

The saturated aqueous  $\text{CaSO}_4$  solution used for determining the resistance capacity of the electrolytic cell was made by the precipitation of  $\text{CaCl}_2$  with  $\text{H}_2\text{SO}_4$  and washing the precipitate by decantation. The water finally used must have a conductivity of  $5 \times 10^{-6}$  reciprocal ohms, or less. For some purposes, particularly those involving conductivity measurements, particular attention must be given to the quality of the water as determined by this test, and in some volumetric processes it becomes important to use pure water freed from  $\text{CO}_2$  by boiling. Water of sufficiently low conductivity is easily produced by a single redistillation of common distilled water treated with  $\text{Ba} \ 2 \ \text{OH}$ , the first and last portions of the distillate being rejected.

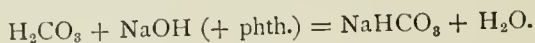
The free and saline ammonia was determined by the usual colorimetric process, using the Nessler reagent. Distillation was not done except for comparison. The colored hay infusion was treated with a slight excess of  $\text{NaOH}$  in the cold, and rapidly filtered into a 50 c.c. Nessler tube, which had been previously nearly filled to the mark with cold ammonia-free water. By this means a colorless liquid was obtained ready for Nesslerization. The total organic nitrogen (including ammoniacal nitrogen) was converted by the Kjeldahl process into ammonium sulphate. A quantity of 5 to 20 c.c. of the culture liquid was boiled with strong  $\text{H}_2\text{SO}_4$ , and crystallized  $\text{Na}_2\text{SO}_4$ , cooled, washed into a 100 c.c. stoppered measuring flask with cold ammonia-free water, neutralized with strong  $\text{NaOH}$ , and a measured portion of the whole when cold was Nesslerized. Processes involving distillation were used for comparison. F. Sutton, :04, and Rideal, :01, pp. 30, 38-40, may be consulted for the preparation of the standards required for the above methods, and also for the usual methods followed in making the same determinations.

With some few exceptions the use of the described standard solutions for the estimation of the quantities specified in connection with

the description of their preparation needs no discussion beyond that which has already been given, or is readily found in the list of references previously cited. A few practical points are worth mentioning. Owing to the small quantities (5 to 10 c.c.) upon which titrations are made, it is important to find, if possible, what is the least quantity of the standard reagent required to make the end point clearly perceptible in the volume of liquid under titration at the time when the end point is reached. A titration performed upon this volume of distilled water gives an approximate correction which is to be subtracted from the actual volume of standard solution used in the case of culture liquid. The use of more methyl orange than necessary makes the result worthless. With practice the eye becomes very sharp in detecting the *orange-brown* transition color which the indicator shows between pink and yellow. White porcelain evaporating dishes of small size are suitable vessels for titrations. When flasks are used a sheet of white paper under them serves well for a background. Increased certainty regarding the end point may be obtained by the use of a second dish of the same liquid for comparison, especially when the latter is somewhat colored. Finally, the operator must not fail to determine his limits of error on the different estimations by obtaining a series of results under the same conditions, and noting his deviations from the average. If the absolute value of the estimations comes in question, or if accurate comparisons between different results are to be made, then the calibration of measuring apparatus by the analyst himself must be made.

In estimating the free acidity, consistent and correct results cannot be obtained if the small quantity (5 c.c.) to be titrated and the standard solution also are exposed to the air during the process. This difficulty is easily overcome by performing the whole process under neutral xylol or kerosene; these serve admirably as temporary protectives. My experience is that these agents are not permanent protectives for the stock solution of NaOH. In a narrow white porcelain dish (crucible) kerosene is placed to the depth of one half to one centimetre. With a volumetric pipette 5 c.c. of culture liquid is taken from the culture jar and placed under the kerosene. Some solution of phenolphthalein is added. The necessary volume of standard NaOH for neutralization, as shown by the rose color of the indicator, is introduced under the surface of the kerosene, the liquid being stirred under the latter with a glass rod. The results are conveniently stated in millionths, *i. e.*, in  $10^{-6}$  units, of a gram mole-

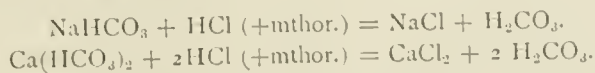
cule of the reagent used per cubic centimetre of culture liquid. Thus a certain culture, No. 6-3-8 1, on the second day after setting showed the usual light color and abundant evolution of gas. Upon looking through it towards the light, it was seen to be turbid, which condition was due to the enormous number of bacteria found by microscopical examination to be suspended in the liquid. Five c.c. of this liquid required for neutralization 1.2 c.c. of 0.01 M NaOH =  $1.2 \times 10^{-5}/5 = 2.4 \times 10^{-6}$  gram molecules of NaOH per cubic centimetre of culture liquid. It is this value, 2.4, which is used in tabular or serial statements to express the acidity of the culture, and it is also of convenient magnitude for use in plotting the curve of acidity. Five c.c. of another culture, No. 6-3-8 2, set at the same time and by the same method, required 1.0 c.c. of 0.01 M NaOH =  $1.0 \times 10^{-5}/5 = 2 \times 10^{-6}$  gmol. of NaOH per c.c. Upon the assumption, which would approximately represent the facts, that all the free acid was  $H_2CO_3$ , the following equation represents the reaction when phenolphthalein is used as indicator:



From this relation it is evident that in the first culture above estimated the *gram molecular* concentration of  $H_2CO_3$  was  $2.4 \times 10^{-6}$  gmol. per c.c. The *gram equivalent* concentration per c.c. is  $2 \times 2.4 \times 10^{-6} = 4.8 \times 10^{-6}$ , and the gram equivalence per 1000 c.c., *i. e.*, the *normality* of the acid here present, is  $10^3 \times 4.8 \times 10^{-6} = 4.8 \times 10^{-3}$ ; in other words, the culture liquid is a 0.0048 normal solution of acid. A small part of the acid present may be monovalent, *e. g.*, lactic from fermentation, and this would somewhat reduce the above magnitude. But from the physiological standpoint it is necessary to consider that chemical estimations show the total amount of acidic hydrogen that can be produced by the process of titration operating upon the total amount of material present. This may be, and in the majority of cases is, a very different quantity than the amount of *free* acidic hydrogen, *i. e.*, of free  $H^+$  ions which exists in the liquid before the process of titration begins and with which the organisms of the culture live in contact. It is at this point that physical chemistry can add to the data which to a greater or less extent define the physiological environment. Of the various methods which may be used to determine the ionic hydrogen, I shall select the conductivity method for application in the present case. It must be remembered that here we are not dealing with a pure solution of  $H_2CO_3$ , but that

this acid is accompanied by a relatively large quantity (presently to be determined) of bicarbonates, *i. e.*, of salts having a common anion. Neglecting the influence of these upon the dissociation of the  $\text{H}_2\text{CO}_3$  until the discussion of the conductivity measurements, it is required to find the maximum of ionic  $\overset{+}{\text{H}}$  which would exist in the culture if it were a pure solution of the  $\text{H}_2\text{CO}_3$  previously estimated. To do this the dilution formula  $\frac{a^2}{(1-a)v} = k$  is applied, since  $\text{H}_2\text{CO}_3$  is a weakly dissociating acid. Walker and Cormack, :00, p. 11, have determined the value of  $k = 304 \times 10^{-9}$ . The gram molecular dilution in litres,  $v$ , is equal to the reciprocal of the concentration per litre  $= 1/2.4 \times 10^{-3} = 417$ . Let  $\frac{a^2}{1-a} = vk = e$ , then  $e = 417 \times 304 \times 10^{-9} = 126.7 \times 10^{-6}$ . From which  $a = 0.0113$ , *i. e.*, about 1.1 per cent of the total amount of the acidic H, obtained by titration, actually exists as free ionic  $\overset{+}{\text{H}}$ . This gives the *ionic concentration* of  $\overset{+}{\text{H}} = 2.4 \times 10^{-6} \times 0.0113 = 27 \times 10^{-9}$  gmion per c.c. It is this magnitude which would become significant if we were trying to compare different cultures with reference to their physiologically effective acidities. The titrated values alone would be misleading for the purpose of such comparison. These observations are by no means intended to involve the proposition that the undissociated portion of the molecule is physiologically inactive. The problem of apportioning the effect between the ionized and the undissociated portions of molecules awaits experimental solution.

Five c.c. of the first culture whose acidity was above described required for the neutralization of the methyl orange alkalinity 3.4 c.c. of 0.01 m HCl =  $3.4 \times 10^{-5}$  g =  $6.8 \times 10^{-6}$  gmol. per c.c. of HCl. The numerical value, 6.8, is used for purposes of comparison and for plotting the curve of alkalinity. It is directly comparable with the 2.4 obtained for the acidity of the same culture at the same time. From the qualitative analysis it may be inferred that the following are *types* of the reactions that occur, one for alkaline, the other for alkaline earth bicarbonates:



The qualitative examination has shown that the methyl orange alkalinity is equal to practically the total salt content, and this quantity

becomes especially significant as an indispensable factor for the calculation of the physical osmotic pressure. But for this purpose it is necessary to know at least how much of the salts consists of alkali metal compounds and how much of alkali earth compounds. This is due to the fact that the value,  $n$ , which expresses the number of the dissociated products of a single molecule is involved in van't Hoff's osmotic factor,  $i, = 1 + (n - 1) \alpha$ , and that  $n = 2$  for the alkali group, and  $n = 3$  for the bicarbonates of the alkali earth group. The question of the dissociation of the bicarbonate molecules will be taken up again presently. Of course a greater approach to accuracy in the calculation of osmotic pressure would be possible if the quantities of Ca, Mg, etc., were separately determined. These estimations are hardly worth making for the little to be gained thereby, unless in a given case a specific physiological action of these cations were also in question. For the purpose of estimating the alkali group directly and the alkali earth group by difference, the following method was pursued: 5 c.c. of the same culture liquid whose methyl orange alkalinity had been determined upon another portion, was evaporated to dryness. The temperature was kept short of charring the organic matter and the heating was continued for some time. The residue was treated with small quantities of boiling distilled water and the solution of alkali carbonate so obtained was filtered hot. The quantity of water used, including one washing of the filter, should not much exceed the original volume of culture liquid. After cooling, the methyl orange alkalinity is again found with the same reagents and under the same conditions as in the first titration. Since both these titrations show the total Na or K content present, regardless of whether the carbonates are normal or acid, the second titration shows directly how many gram molecules of alkali bicarbonate were present in the original 5 c.c. of culture liquid, and the difference between the first and second may be taken to represent the amount of alkali earth bicarbonate. This method would not be applicable if other alkali earth salts than carbonate and bicarbonates were present. In such case the method of *Hehner*, described in *Sutton*, :04, pp. 70-71, could be used to ascertain the amount of alkali earth bases.

The determinations previously given apply to the first culture above described at a very early period in its history. I shall now take data from the same culture after it was several weeks old. It had by that time shown the characteristic succession of animals later to be described, had become again clear with but comparatively few non-



encysted bacteria to be seen, and had changed its color to a dark brown indicative of a low free acidity. Its acidity without correction measured about 0.2 c.c. of 0.01 m NaOH for 5 c.c. of culture liquid, and hence was negligibly small. The methyl orange alkalinity and the calculations which follow were taken for this culture at this stage of its development, because the data will serve both for illustrating a general problem of frequent recurrence and also as part of the necessary data in an experiment upon *Paramæcia* taken from this culture. In a subsequent part of this paper dealing with the relation of Protozoa to distilled water, the results of the present discussion will be again referred to and applied.

Five c.c. of this older culture liquid required 3.95 c.c. (corrected) of 0.0102 m HCl for neutralization, using methyl orange as indicator. After evaporation for the separation of alkali earth bicarbonates according to the process previously described, the methyl orange alkalinity required 1.4 c.c. (corrected) of 0.0102 m HCl for neutralization. Let us calculate the gram molecular concentration per cubic centimetre, assuming the type reactions above written (p. 465). For alkali bicarbonates the concentration =  $1.4 \times 102 \times 10^{-7} / 5 = 2.86 \times 10^{-6}$  gmol. per c.c. For alkali earth bicarbonates the concentration =  $\frac{(3.95 \times 102 \times 10^{-7}) - (1.4 \times 102 \times 10^{-7})}{5 \times 2} = 2.6 \times 10^{-6}$  gmol. per c.c. It is to be observed in the type equation for the alkali earths that the use of 2 gmol. of HCl shows the presence of only 1 gmol. of  $\text{Ca}(\text{HCO}_3)_2$ .

Having obtained these data, we are ready to consider the question of the osmotic properties of the liquid. As has been previously explained, this question is, from the physiological standpoint, a complicated one, which involves the quantity of each special constituent, the complex physical system constituted by the mixture of these in solution, and more especially the permeability of the cell walls under the simultaneous action of all these constituents. The physiological osmotic relations are, in the present state of our knowledge, unapproachable by any method of calculation. Only direct and extended experimentation will yield these highly desirable data. But if on the physiological side we assume a strictly semi-permeable membrane, and on the physical the kinetic theory of gases as applied to solutions, thus involving the theory of electrolytic dissociation, then we can approximately calculate, from the data above obtained, the quantity which I have called the physical osmotic pressure. In this cal-

culatation I shall assume that the alkali earth salts are sufficiently alike in physical osmotic properties, that this whole group of salts can, for purposes of calculation, be treated as if it consisted entirely of  $\text{Ca}(\text{HCO}_3)_2$ . Similarly the alkali earth group will be treated as if it consisted entirely of  $\text{NaHCO}_3$ .

Let the pressure of a gram molecule per litre of a substance in undissociated condition =  $P_0$ , and in condition of dissociation =  $P$ . Comparison of different substances belonging to the two classes shows that  $P$  for electrolytes, such as we are dealing with, is greater than  $P_0$ , and the ratio  $\frac{P}{P_0}$  has been called =  $i$ , and the application of the theory of electrolytic dissociation to these cases has developed the formula  $i = 1 + (n - 1) a$  in which  $n$  = the number of the dissociated products of a molecule, and  $a$  = the fraction of the total number of molecules which have dissociated. In the culture medium above titrated we have for the total salt content  $P_0 = (2.86 + 2.6) \times 10^{-3} = 5.46 \times 10^{-3}$  gmol. per litre. Since one gram molecule in 22.4 litres exerts a pressure of one atmosphere  $P_0 = 22.4 \times 5.46 \times 10^{-3} = 0.122$  atm. In order to find the desired quantity  $P$ , we must have  $i$ , and for this the value of  $a$  must be known. Let us assume for the moment that the dissociation of both salts is practically complete, *i. e.*, that  $a = 1$ , and that for  $\text{NaHCO}_3$ ,  $n = 2$ , for  $\text{Ca}(\text{HCO}_3)_2$ ,  $n = 3$ . Then for  $\text{NaHCO}_3$ ,  $i = 2$ , and for  $\text{Ca}(\text{HCO}_3)_2$ ,  $i = 3$ . Hence for  $\text{NaHCO}_3$ ,  $P = 2 \times 2.86 \times 10^{-3} \times 22.4 = .128$  atm. For  $\text{Ca}(\text{HCO}_3)_2$ ,  $P = 3 \times 2.6 \times 10^{-3} \times 22.4 = 0.175$  atm. The total  $P = 0.128 + 0.175 = 0.303$  atm., which is the physical osmotic pressure as previously defined and under the above assumptions. As will be shown directly, these pressures are greater than the theoretically correct values.

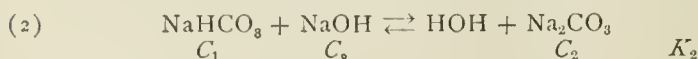
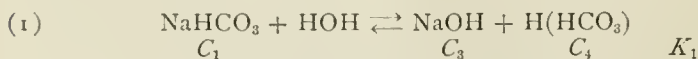
Evidently  $a$  is the most significant quantity involved in the determination of the osmotic pressure, and I shall describe the method by which  $a$  was obtained from data of electrical conductivity. We may make a digression here to discuss one other method, that for the determination of the depression of the freezing-point, which might also be used for the same purpose when the molecular concentration as here found by titration is known. By this method we would get a depression of  $1.85^\circ$  for 1 gmol. in 1 litre, or  $\frac{1.85^\circ}{22.4}$  for 1 gmol. in 22.4 litres, and this would be the depression corresponding to a concentration which would give an osmotic pressure,  $P = 1$  atm. Since an ion and a molecule give the same depression, the 0.303 atmospheres

which we have above found to be the maximum physical osmotic pressure, would give a depression =  $0.303 \times 1.85^\circ / 22.4 = 0.025^\circ \text{C}$ . This small range on the temperature scale represents the total concentration, which on the conductivity scale is represented by  $642 +$  of  $10^{-6}$  units (reciprocal ohms). The sensibility and accuracy of the latter method greatly exceed that of the cryoscopic method, at any rate under the conditions of the ordinary use of both methods. The depression of the freezing-point has the advantage of giving the value of  $a$  more directly than calculation from conductivity data.

In calculating the value of  $a$  I shall regard the medium as consisting of the known molecular concentrations above found of the weak electrolytes  $\text{NaHCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$ . Other electrolytes are not present in appreciable concentration, and the amount of free H acidity is very near the phenolphthalein neutral point as previously observed. We have then practically a system composed of  $\text{NaHCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$  in solution in  $\text{H}_2\text{O}$  and in a condition of equilibrium. We desire to obtain the  $a$  value for the salts when they are constituents of the system. This we shall attempt to obtain by the application of the law of mass action. In the process we shall need at least for comparison the  $a$  values of the respective pure aqueous solutions of the salts. These values are to be found directly given, or they can be interpolated in the tables or calculated from the data of Walker and Cormack, :00, and from Kohlrausch und Holborn, '98. By titration with methyl orange we have found the gram atomic concentration of  $\text{Na} = 2.86 \times 10^{-6}$  per c.c. = 1 gram atom in 350 litres, *i. e.*, the dilution  $v = 350$ . By interpolation in the table of conductivities of  $\text{NaHCO}_3$  given by Walker and Cormack, :00, p. 10, and by using 79.5 as given by these authors as the conductivity of  $\text{NaHCO}_3$  at infinite dilution, we obtain  $\frac{\mu_v}{\mu_\infty} = \frac{74.67}{79.5} = 94.0$  per cent =  $a$ . For  $\text{Ca}(\text{HCO}_3)_2$  we have found the concentration of  $\text{Ca} = 2.6 \times 10^{-6}$  per c.c. = 1 gram atom in 385 litres, *i. e.*, the dilution  $v = 385$ . For  $\text{Ca}(\text{HCO}_3)_2$  no table of directly given conductivities was available. The conductivity  $\mu$  (Siemens units) of  $\text{Ca}(\text{HCO}_3)_2$  at  $v = 385$  and also at infinite dilution was derived from partial values, *i. e.*, from the ionic conductivities of the respective ions at these respective dilutions. Walker and Cormack's value, 38, was used for the ionic conductivity of  $\text{HCO}_3$  at infinite dilution. To obtain  $\text{HCO}_3$  at  $v = 385$ , the molecular conductivity  $\mu$  for  $v = 385$  was interpolated in Walker and Cormack's table for the conductivity of  $\text{NaHCO}_3$ , and

from this was subtracted the ionic conductivity of Na at  $v=385$ . The latter value was taken by interpolation from Kohlrausch und Holborn, '98, p. 200, the value of  $\Delta$  being reduced to  $\mu$ . Thus was found  $\text{NaHCO}_3 - \text{Na} = 74.95 - 38.6 = 36.35 = \mu$  for  $\text{HCO}_3$  at  $v=385$ . In an entirely analogous manner the ionic conductivities of Ca at  $v=385$  and at infinite dilution were obtained. For this purpose the tables of Kohlrausch und Holborn, '98, p. 161, for  $\text{CaCl}_2$ , and p. 200 for Cl were used, although the value of Ca could as well have been taken from the table of ionic conductivities on p. 200. The values of  $\Delta$  were reduced to  $\mu$ . Thus was found  $\mu$  at  $v=385$  for  $\text{CaCl}_2 - \text{Cl}_2 = 199.8 - 118.4 = 81.4 = \mu$  for Ca at  $v=385$ . Then  $\mu$  at  $v=385$  for Ca +  $(\text{HCO}_3)_2 = 81.4 + 72.7 = 154.1 = \mu$  for  $\text{Ca}(\text{HCO}_3)_2$  at  $v=385$ . The ionic conductivity of Ca at infinite dilution was taken from Kohlrausch und Holborn, '98, p. 200,  $\Delta$  being reduced to  $\mu$ , giving  $\text{Ca} = 99.71$ . Thus  $\mu$  for  $\text{Ca}(\text{HCO}_3)_2$  at infinite dilution =  $\text{Ca} + (\text{HCO}_3)_2 = 99.71 + 76 = 175.7$ . We now have  $\frac{\mu_v}{\mu_\infty} = \frac{154.1}{175.7} = 87.7$  per cent =  $a$ .

We may now consider the conditions which prevail in this system. It is well known that when substances like  $\text{NaHCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$  are dissolved in  $\text{H}_2\text{O}$  a reaction known as hydrolysis occurs between these salts and the water. The result is that only a portion of the salt originally placed in the water remains as such, the remainder being distributed under the form of other compounds. In the consideration of the present system it is therefore necessary first to study the question of the hydrolytic decomposition of the two salts. The methyl orange titrations previously made show the total gram atomic content of Na and Ca with which one could have made the actual solution that constitutes the culture liquid here considered. According to McCoy :04, pp. 4 and 5, the hydrolysis of  $\text{NaHCO}_3$  is represented by the *two* following reactions:



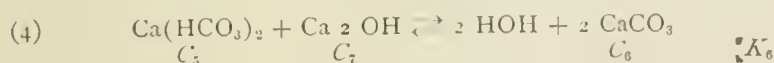
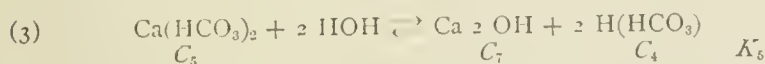
Let the equilibrium constant of (1) be represented by  $K_1$ , of (2) by  $K_2$ , and of the system composed of (1) and (2) by  $K_3$ . By applying the law of mass action McCoy developed the following relations:

$$\frac{C_1^2}{C_2 C_4} = \frac{K_2}{K_1} = K_3$$

He found the value of  $K = 5320$ . He found that "in a decinormal solution made from solid bicarbonate and water in such a way that no carbon dioxide is allowed to escape" the hydrolysis amounts to 2.68 per cent (l. c. pp. 12-13).

Walker and Cormack, :00, p. 9, also investigated the amount of hydrolysis of  $\text{NaHCO}_3$  in pure solution, by reducing the hydrolysis by the addition of a solution of carbonic acid, as recommended by Bredig, and found "that the extent of hydrolysis of sodium hydrogen carbonate would not exceed a fraction of a per cent, even at the greatest dilution we investigated." The  $\alpha$  value above obtained for a pure solution of  $\text{NaHCO}_3$  may therefore be taken as practically correct for the dissociation of  $\text{NaHCO}_3$ , even for the supposition that no hydrolysis occurs.

We may now consider the hydrolysis of  $\text{Ca}(\text{HCO}_3)_2$ . If the reactions are of the same nature as those McCoy has described for  $\text{NaHCO}_3$ , we would have the following relations:



From the law of mass action we would obtain the following relation for a pure solution of  $\text{Ca}(\text{HCO}_3)_2$  in  $\text{H}_2\text{O}$ :

$$\frac{C_5^2}{C_6^2 C_4^2} = \frac{K_6}{K_5} = K_9$$

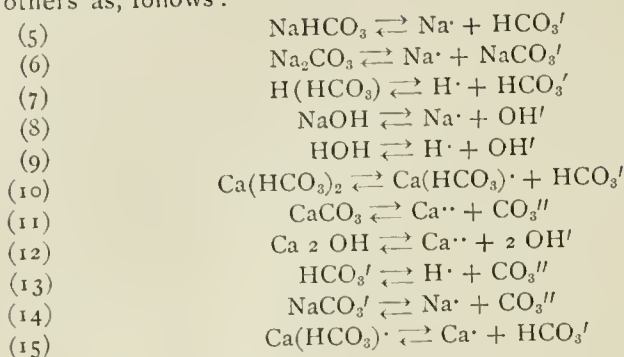
The entire system of relations containing the simultaneous conditions of equilibrium represented by reactions (1), (2), (3), and (4) can be deduced from the law of mass action, and is expressed as follows:

$$\frac{C_2^2 \times C_6^2}{C_1^4 \times C_3^2} = \frac{K_9}{K_8^2} = K_{10}$$

For want of the experimental data for  $\text{Ca}(\text{HCO}_3)_2$  as represented in (3) and (4) we cannot solve the equation for  $K_{10}$ .

For our purpose, which is in part to determine the osmotic pressure from the amount of molecules and ions and in part to gain a conception of the complexity of the biological factors represented by the molecules and ions, it will be necessary to consider also the ionization of the products of hydrolysis. Besides the four main equilibria

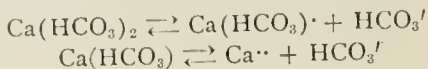
represented in reactions (1) to (4) there will exist at least eleven others as, follows :



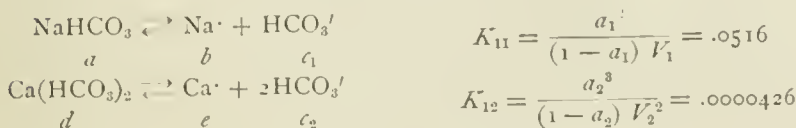
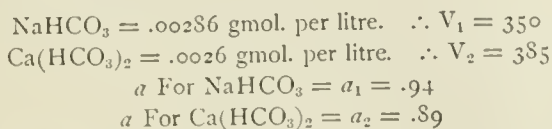
The constants required by the law of mass action are known for most of these, but I am not aware of values for the constants of (10) and (15) and of (6) and (14). If we may neglect hydrolysis, the system of relations becomes at once simpler and can be more easily solved for the amount of each of its kinds of particles. From what has already been said, the amount of hydrolysis is probably small. The ionization of  $\text{NaHCO}_3$  without appreciable hydrolysis may be considered comparable to that of  $\text{NaNO}_3$ . The latter solution when at the same concentration as the  $\text{NaHCO}_3$ , *i. e.*,  $v = 350$ , has an  $\alpha$  value of 95.2 per cent, as determined from a table for  $\text{NaNO}_3$  constructed from data in Kohlrausch und Holborn, '98, p. 200. As above described, Walker and Cormack found 94.0 per cent for a solution of  $\text{NaHCO}_3$ . Likewise the ionization of  $\text{Ca}(\text{HCO}_3)_2$  without hydrolysis may be considered comparable to that of  $\text{Ca}(\text{NO}_3)_2$  at the same concentration, which is  $v = 385$ . From Kohlrausch und Holborn's tables we obtain 89.1 per cent =  $\alpha$ . For an actual solution of  $\text{Ca}(\text{HCO}_3)_2$  we have above found, by a method of interpolation,  $\alpha = 87.7$  per cent.

We shall treat the system without reference to hydrolysis and shall adopt  $\alpha$  for  $\text{NaHCO}_3 = 94.0$  per cent, and  $\alpha$  for  $\text{Ca}(\text{HCO}_3)_2 = 89.0$  per cent.

Without hydrolysis we would have a system composed of the two weak electrolytes  $\text{NaHCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$  dissolved in water. A portion of each salt will be ionized until equilibrium is established and the two equilibria so produced exist simultaneously. It is probable that  $\text{Ca}(\text{HCO}_3)_2$  ionizes in two stages as follows :



The calculation of the ionic concentrations involved here, according to the law of mass action, would require certain constants. It is probable that at the dilution in question, *i. e.*,  $\tau = 385$ , the formation of the  $\text{Ca}(\text{HCO}_3)_2$  ion may be disregarded for approximate results. Not having the necessary constants, we shall treat the system as if one reaction represented the ionization of  $\text{Ca}(\text{HCO}_3)_2$ . The system may then be represented as follows:



For  $K_{12}$  I have used the formula as given by Mellor, :04, p. 193 (cf. Ostwald, '99, p. 408). Since the undecomposed  $\text{NaHCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$ , *i. e.*,  $a$  and  $d$  must stand in equilibrium with all the  $\text{HCO}_3'$  ions present in the system, *i. e.*,  $c_1 + c_2$ , regardless of which molecule produced them, we may write  $c = c_1 + c_2$ . Furthermore we shall adopt one litre as the volume of the system. Hence the letters  $a, b, c, d, e$ , represent the actual concentrations of their respective substances in gram ions or gram molecules, *i. e.*, in mols. Direct application of the law of mass action gives two equations from the two equilibria, the necessary equality between all the kations and all the anions gives a third, and the known concentrations of each salt give two more (cf. Ostwald, '99, pp. 415-416). These equations are as follows:

$$\begin{array}{lll} (1) \quad bc = K_{11}a & (2) \quad e^2 = K_{12}d & (3) \quad c = b + e \\ (4) \quad a + b = \frac{1}{350} & (5) \quad d + e = \frac{1}{385} & \end{array}$$

When a distinction between  $c_1$  and  $c_2$  was observed in the statement of the equations, difficulties arose and the attempt was abandoned. Solution of the above system of equations gives the following values:  $c = .00285$ ,  $b = .00271$ ,  $a = .00015$ ,  $e = .00216$ ,  $d = .00044$ . These values make  $a$  for  $\text{NaHCO}_3$  under the conditions of the system = 98.6 per cent, and for  $\text{Ca}(\text{HCO}_3)_2 = 83.1$  per cent. According to the

above values the total concentration of mols in the system is  $a + b + c + d + e = .00831$ , and the concentration of undissociated mols required to produce the system  $= .00286 + .0026 = .00546$ . Hence the  $i$  value required to find the physical osmotic pressure is  $831/546 = 1.52 = P/P_0$ , previously defined (p. 468). This value has no claim to accuracy, but it is probably a fair approximation.

In this connection we may consider the results obtained from the measurement of another one of the physical properties of the same culture liquid which has just been under discussion. The electrical conductivity of this medium was determined by the Kohlrausch method on the same date when the data for the preceding calculation were obtained. We are therefore able to compare calculated and observed conductivities. Having above found for both constituents,  $m$  ( $= a$ ) under the conditions of the system, and knowing  $\mu_\infty$  for both, we have all the data necessary for calculating the theoretical conductivity. The values we have used are expressed in Siemens units, and we desire to convert them to reciprocal ohms to correspond with the denomination of the results obtained by actual measurement. We apply the formulas  $\mu_0 = m \mu_\infty$ ,  $\Delta = \mu_0 1.063$ , and  $n = \Delta y$ . For comparison of units see Kohlrausch und Holborn, '98, p. 4. We thus obtain for the  $\text{NaHCO}_3$ ,  $n = 238 \times 10^{-6}$ , and for the  $\text{Ca}(\text{HCO}_3)_2$ ,  $n = 404 \times 10^{-6}$ . The sum of both gives total  $n = 642 \times 10^{-6}$ . The above calculations are all based on  $T = 18^\circ\text{C}$ ., and the actual measurement of the conductivity of the culture liquid at this temperature gave  $n = 643 \times 10^{-6}$ .

The total salt content, *i. e.*, the total ash, is a quantity which gives so little information regarding the culture liquid itself, and its determination is subject to so much inaccuracy (volatilization, cf. Hoppe-Seyler, :03, pp. 391-394, especially pp. 409-410) that the result for our purpose is scarcely worth the necessary effort. It is a quantity which presumably would not vary much in the same culture, but might sometimes be useful for the comparison of different cultures. One objection to these estimations, from our point of view, is that they include the mineral matter of both the organic and inorganic constituents of the original liquids, and there is no method of separating these. In any case, for rapidity and uniformity of results the processes of mineralization in the wet way, by A. Neumann, :00, :02, :05, should be followed, as described in Hoppe-Seyler, :03, pp. 393-394. I have found it practicable to perform the operation upon 5-10 c.c. of culture liquid contained in a 50 c.c. to 100 c.c. porcelain crucible, which had



been weighed, and which during the process was kept nearly closed by the inverted cover of the crucible. The liquid was treated by slow additions of small quantities (drops) of a mixture of equal volumes of concentrated  $H_2SO_4$  and  $HNO_3$ , and was heated cautiously. When the mineralization is complete the clear and light-colored liquid is evaporated, and the residue is dried and weighed in the crucible. In this manner was obtained, from 5 c.c. of the culture liquid with which we have been dealing, a residue = 0.0083 gm.

I regard the total salt content as a quantity of much less importance than the several amounts of different salts or radicals which can be obtained by the use of special methods for each constituent. The preliminary qualitative examination serves as a guide to show which of these need be sought quantitatively. There will be most frequent use for the estimation of Cl, and this I have done by the Volhard method, using the standard solutions prepared as previously described. The use of a perforated porcelain funnel upon which a layer of fine asbestos was prepared, and in connection with a filter pump attached to the water supply, greatly expedited the removal of  $AgCl$  as required in this method. The amount of Cl in 5 c.c. of the above culture liquid was found to be equivalent to 0.2 c.c. of a 0.01 n  $AgNO_3$  solution. Hence the  $Cl = 0.2 \times 10^{-5}/5 = 0.4 \times 10^{-6}$  gmat. per c.c.

We pass next to the determination of the oxygen consumed. The meaning and limitations of this determination are well stated by Rideal, :01, pp. 31-34. The absolute values obtained have no uniform significance, but when the process is performed under the same conditions and upon materials that are qualitatively comparable, *e. g.*, hay infusions, the results may be regarded as giving at least some indication, but by no means an accurate measure, of the amount of easily oxidizable organic matter. I have performed the process essentially as described by Rideal, :01, pp. 32-33, except that I permitted the action of the permanganate to continue fifteen minutes at room temperature. Thus 5 c.c. of the same culture liquid as has been used in the other previously described measurements showed, under these conditions, a loss of 1.6 c.c. of a 0.012 n  $Na_2S_2O_3$  solution. In conformity with our general scheme we desire to express this in  $10^{-6}$  gm. of oxygen per cubic centimetre of culture liquid. Equations for the above process show that  $2 KMnO_4 = 5 O = 10 l = 10 Na_2S_2O_3$ , *i. e.*, 1 gmol. of  $Na_2S_2O_3$  used represents 8 gm. O. Hence 1.6 c.c. of 0.012 n  $Na_2S_2O_3$  represents  $1.6 \times 12 \times 10^{-6} \times 8/5 = 30.7 \times 10^{-6}$  gm. O consumed per c.c. of culture liquid.

The method used to obtain the free and saline ammonia, and the total organic nitrogen (including the ammoniacal nitrogen) have been sufficiently described in connection with the description of the preparation of standard solutions. 5 c.c. of the same culture liquid with which we have been dealing, when operated upon in the manner referred to, was found to have a content of total organic nitrogen =  $12 \times 10^{-6}$  gm. N/c.c., and of ammoniacal nitrogen =  $1.8 \times 10^{-6}$  gm. N/c.c.

The entire collection of data which have been obtained from this culture No. 6-3-8/I, by both direct observation and by calculations based thereon, is recorded in the following table. The determinations marked \* represent the quantity of reagent used, not that of the substance estimated, the complete identity of which was in such cases not certainly determined. The significance attached to the numerical results appears in the preceding discussions.

CULTURE No. 6-3-8.		
Age.	2 days.	14 days.
*Phth. Ac. . . . .	$2.4 \times 10^{-6}$ gmol./c.c.	
H <sub>2</sub> CO <sub>3</sub> . . . . .	$2.4 \times 10^{-6}$ gmol./c.c.	
Ionic H <sup>+</sup> . . . . .	$27.0 \times 10^{-9}$ gmion/c.c.	
*Mthor. Alk. . . . .	$6.8 \times 10^{-6}$ gmol./c.c.	
*Phth. Ac. . . . .		Trace.
*Mthor. Alk. : Obs. . . . .		$8.06 \times 10^{-6}$ gmol./c.c.
“ “ Calc. fr. Bicarb. . . . .		$8.06 \times 10^{-6}$ gmol./c.c.
Alk. Bicarb. . . . .		$2.86 \times 10^{-6}$ gmol./c.c.
a for Alk. Bicarb. . . . .		98.6%.
Alk. Earth Bicarb. . . . .		$2.6 \times 10^{-6}$ gmol./c.c.
a for Alk. Earth Bicarb. . . . .		83.1%.
Total P/P <sub>0</sub> . . . . .		0.186 atm.
Electr. Cond. : Obs. . . . .		$643. \times 10^{-6}$ I/ohm.
“ “ Calc. fr. Bicarb. . . . .		$642. \times 10^{-6}$ I/ohm.
Sulphated Ash per 5 c.c. . . . .		0.0083 gm.
Cl . . . . .		$0.4 \times 10^{-6}$ gmat./c.c.
O consumed . . . . .		$30.7 \times 10^{-6}$ gm./c.c.
Total Organic N . . . . .		$12.0 \times 10^{-6}$ gm./c.c.
Ammon. N. . . . .		$1.8 \times 10^{-6}$ gm./c.c.

In the next following part of this paper will be described the results obtained in the study of cultures under continuous observation, and of which the biological record was simultaneously obtained.

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# THE RELATION OF THE NORMAL HEART RHYTHM TO THE ARTIFICIAL RHYTHM PRODUCED BY SODIUM CHLORIDE.

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## I.

THE experiments here recorded were undertaken primarily with the view of determining what part, if any, the intrinsic nervous tissue in the heart takes in the production of rhythm in parts of the heart not normally automatic when immersed in an isotonic sodium chloride solution. The fact that curarized skeletal muscle twitches more or less rhythmically in a pure sodium chloride solution suggests that the rhythm produced by this same chemical in non-automatic parts of the heart is idio-muscular. But it is also possible that the local nervous plexus in the heart strips is involved in the artificial rhythm, and this may account for the fact that the rhythm of isolated heart strips in sodium chloride approaches in regularity more closely to the normal heart rhythm than does the sodium chloride rhythm of skeletal muscle. This question cannot be attacked directly in the vertebrate heart, because the nervous and the muscular tissues cannot be separated for the necessary experiments, let alone the fact that the function of the intrinsic nervous tissue in the vertebrate heart is still, at least according to some physiologists, an unsettled question.

None of these difficulties confronts us in the *Limulus* heart. The function of ganglion and the nervous plexus in this heart is no longer a matter of controversy. The two tissues can be isolated so that the action of a chemical on either tissue may be accurately studied apart from that on the other. And finally, a pure sodium chloride solution (isotonic) produces, to all appearance, the same type of transient rhythm in the non-rhythmical part of the *Limulus* heart as in the classical strips from the apex of the vertebrate heart.

For these experiments the *Limulus* heart is prepared as shown in

diagram *B* (Fig. 1). The median dorsal nerve cord or ganglion is extirpated for the whole length of the heart. The heart is transected in the middle of the second and the third segments, and the intervening part removed, thus leaving the anterior and posterior ends of the heart connected by the lateral nerves or rather nerve plexus only. Both ends of the heart can now be suspended in separate chambers for graphic registration, and either end immersed in sodium chloride while the other end is bathed in plasma or sea water. It has been shown in a previous paper in this journal that most of the fibres of the lateral nerve plexus in the middle region of the heart are motor fibres to the muscle of the anterior segments. Hence, if the posterior end of the preparation (*B*, Fig. 1) is placed in sodium chloride, the anterior end remaining in plasma, the anterior end would begin to beat in synchrony with the artificial rhythm of the posterior end, provided the solution produced a rhythm in the lateral nerve plexus directly or in case the idio-muscular rhythm was in some way communicated to the nerve plexus.

Some experiments were also made with a preparation similar to that shown in diagram *C* (Fig. 1), the lateral nerve plexus on both sides being isolated from the middle of the second to the fourth or fifth segment and placed in the sodium chloride solution. If the sodium chloride produces rhythmic activity in the nerve plexus, the anterior end of the heart would begin to contract rhythmically in the plasma or sea water bath.

I desired, in the second place, to study the changes in the response of the heart muscle to the normal stimulus produced by the sodium

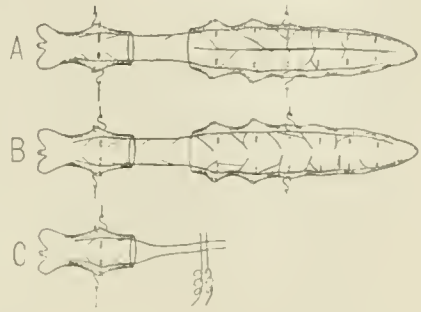


FIGURE 1. — Diagrams to illustrate the preparation of the *Limulus* heart for studying the relation of the sodium chloride rhythm to the normal rhythm. Dorsal view of the heart. Diagram *A*, ganglion extirpated in the first three segments, and part of the second and third segments removed, leaving the two ends of the heart connected by the lateral nerves. Anterior end is immersed in the sodium chloride, while the posterior end is kept in plasma or sea water. Diagram *B*, heart prepared as in *A*, with the exception that the ganglion is extirpated throughout its whole length. Preparation used for studying the relation of the nerve plexus to the sodium chloride rhythm. Diagram *C*, preparation of the first two heart segments and the lateral nerves for studying the changes in the response of the muscle to stimulation of the nerves during the sodium chloride rhythm.

chloride in developing the rhythm in the ganglion free part of the heart. So far as I know, this has not yet been done, despite the numerous researches on artificial rhythm in the vertebrate heart, but the question is now being investigated on the vertebrate heart in this laboratory by Professor Lingle. The *Limulus* heart lends itself admirably to an accurate study of this question. The heart preparations made use of for these experiments are illustrated in diagrams *A* and *C* (Fig. 1). The preparation *A* is arranged for simultaneous tracings from the two ends of the heart, and the plasma or sea water in the chamber containing the anterior end replaced by  $\frac{6}{10}n$  sodium chloride. In such a preparation the anterior end beats in synchrony with the posterior end, while the sodium chloride rhythm is being developed in the former. In the tracing from the posterior end we have a check on the rhythm of the ganglion so that the changes in the amplitude or rate of the contractions of the anterior end can be traced directly to the action of the sodium chloride.

The preparation in diagram *C* was used as follows. The muscle was immersed in the isotonic sodium chloride solution, and the isolated lateral nerves placed in a watch glass filled with plasma. From time to time the nerves were lifted out of the solution and stimulated by induction shocks, and the variation in the muscular response during the different phases of the sodium chloride rhythm recorded.

## II.

*When the dorso-median nerve cord or ganglion has been removed from the heart, the intrinsic nerve plexus takes no part in the rhythm produced by immersing the heart in isotonic sodium chloride.* This is shown by the following experiments. 1. By placing the lateral nerves of preparation *C* (Fig. 1) in the sodium chloride, and leaving the muscle part in plasma or sea water, no rhythm is produced in the anterior end. 2. When the posterior end of preparation *B* (Fig. 1) is placed in sodium chloride and the anterior end in plasma or sea water, the rhythm developed in the posterior end never affects the anterior end, despite the fact that the lateral nerves connecting the two ends of the heart remain intact and capable of functioning. It is therefore evident that the rhythm produced by sodium chloride in the ganglia free heart is idio-muscular, and that this idio-muscular activity does not affect the superficial nerve plexus after the ganglion has been removed. Furthermore, the sodium chloride is not able to

produce a rhythm in the dorsal nerve plexus in the absence of the ganglion.

In a previous paper in this journal I have shown that an isotonic solution of sodium chloride may inaugurate a transitory rhythm in a quiescent heart ganglion of *Limulus*. The latent period of the ganglionic rhythm in sodium chloride is much shorter than that of the heart muscle. In fact in some preparations the ganglionic activity in sodium chloride has run its course before the idio-muscular rhythm appears, so that there is a definite pause between the neurogenic and the myogenic rhythm. In other preparations the ganglionic and the idio-muscular rhythm may overlap. The ganglion continues in activity for a while after the idio-muscular contractions have begun, but the latter invariably persist much longer than the former.

### III.

As shown by the response of the heart muscle to electrical stimulation of the lateral nerves, *there is a gradual increase in excitability of the heart muscle immersed in an isotonic sodium chloride solution up to the time when the idio-muscular contractions appear.* The heart muscle not only responds to the same stimulus with contractions of increasing amplitude, but shortly before the idio-muscular rhythm begins, the heart muscle may respond with two or three contractions to the same stimulation of the lateral nerves that called forth only one contraction at the beginning. In several experiments the electrical stimulation of the lateral nerves after a bath of the muscle in sodium chloride for 35 to 45 minutes actually started the idio-muscular rhythm. The heart muscle continues to respond to the stimulation of the lateral nerves for some time (20 to 40 minutes) after the inauguration of the rhythm, but towards the end of the rhythm the stimulation of the lateral nerves has no effect, although the heart muscle is affected by direct stimulation.

The changes in the heart muscle leading up to the sodium chloride rhythm are therefore in the direction of increased sensitiveness to the normal stimulus, but the heart muscle can also contract rhythmically in sodium chloride when in such condition that it can no longer respond to the normal stimulus. The same will probably be found to be true for skeletal muscle in the vertebrates. For the vertebrate heart it must be left undecided till we have succeeded in separating the two tissues for experimental purposes.

## IV.

*The heart muscle immersed in an isotonic sodium chloride solution continues to respond to the normal impulse or stimulus to the rhythm reaching it from the ganglion through the lateral nerves for some time after the idio-muscular contractions have developed, but*

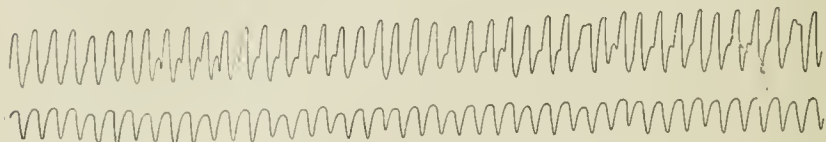


FIGURE 2. — One-half the original size. Simultaneous record from the two ends of the Limulus heart, prepared as shown in diagram *A*, Fig. 1. Upper tracing from anterior end, forty minutes after immersion of the anterior end in  $\frac{6}{10}$  *n* NaCl. Showing development of the sodium chloride rhythm while the muscle still responds to the nervous impulses from the ganglion through the lateral nerves.

*towards the end of the sodium chloride rhythm the heart muscle no longer responds to the normal stimulus just as it fails to respond to the artificial stimulation of the lateral nerves. The preparation used in these experiments is that represented in diagram *A* (Fig. 1), the anterior end being immersed in the sodium chloride solution. Under these conditions the normal rhythm is superimposed on the artificial rhythm for a while. The appearance of the tracings of this composite rhythm is very variable, depending on the regularity, or rather irregularity of the idio-muscular contractions. In rare instances the idio-muscular contractions were strong and fairly regular from the beginning, in which case the rate of the rhythm of the anterior and the posterior end of the heart usually presented the ratio of two or three to one. A typical record of this type is reproduced in Fig. 2. The upper record is from the anterior end. The neurogenic or stronger contractions are superimposed on the idio-muscular or weaker contraction. The rate of the latter rhythm is nearly the same as that of the former at the stage of the experiment represented on the tracings, but such a regularity is not long maintained, as the idio-muscular contractions become more rapid and irregular before the final cessation of the rhythm. In Fig. 3 is reproduced the most common form of this compound rhythm. By close inspection of the upper record it will be seen that the sodium chloride contractions start as a rapid series of more or less rhythmical twitches or beats, on which the normal or neurogenic rhythm is superimposed. The former increases*



in strength and then tends to obscure the normal rhythm, but the latter can be plainly made out by artificially accelerating the ganglionic rhythm as well as by abolishing the former. This is rapidly ac-

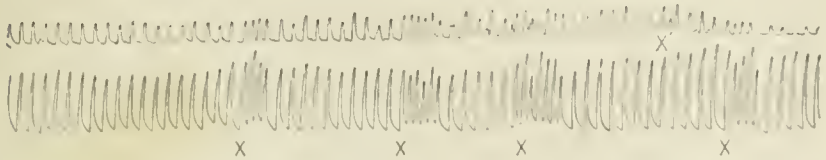


FIGURE 3. — About one-third the original size. Simultaneous tracings from the two ends of the Limulus heart, prepared as in diagram *A*, Fig. 1. Upper tracing from anterior end, immersed in  $\frac{1}{10} N$  NaCl. Showing the normal rhythm superimposed on the idio-muscular sodium chloride rhythm. *X* = acceleration of the normal rhythm by mechanical stimulation of the ganglion on the posterior end. *X'* = the  $\frac{1}{10} N$  NaCl surrounding the anterior end replaced by plasma, showing the quick cessation of the sodium chloride contractions as well as depression of the neurogenic contractions.

complished by replacing the sodium chloride by plasma or sea water, as shown on the right-hand side of Fig. 3 at *X'*.

When after development of the idio-muscular rhythm, the sodium chloride is replaced by plasma or sea water, not only are the idio-muscu-

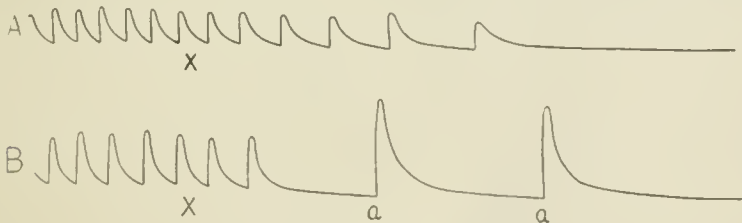


FIGURE 4. — Tracings from the anterior end of the Limulus heart prepared as in diagram *A*, Fig. 1, after forty minutes immersion in  $\frac{1}{10} N$  NaCl. The contractions in *B* are the normal or neurogenic rhythm, in *A* the sodium chloride rhythm. *X* = the sodium chloride surrounding the anterior end replaced by plasma. *a* = stimulation of the lateral nerves by a weak interrupted current showing depression of the normal rhythm by plasma after a previous bath in an isotonic sodium chloride solution, despite the fact that the ganglion on the posterior end of the heart is active, and the lateral nerve able to conduct.

lar contractions quickly abolished, but the heart muscle may for a time cease to respond to the normal stimulus from the ganglion. The anterior end of the preparation (diagram *A*, Fig. 1) thus comes to a complete standstill for a longer or shorter period until the normal rhythm is resumed. The striking thing about this cessation of the response to the normal stimulus is this, that on electrical stimulation of the lateral nerves the heart muscle can still be made to contract although it does

not respond to the nervous impulses from the ganglion. Escape of the current directly to the muscle was guarded against. The fact that the muscle contracts on stimulation of the lateral nerves shows that the nerves and nerve endings remain functional. The heart muscle contracts also on direct stimulation. The failure of the

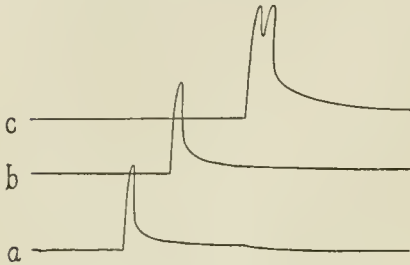


FIGURE 5. — Tracings from the anterior end of the *Limulus* heart, prepared as in diagram C, Fig. 1. The muscle part immersed in an isotonic solution of sodium chloride. *a, b, c*, stimulation of the lateral nerves by the same strength and duration of the interrupted current at fifteen-minute intervals. Showing increased response of the heart muscle to stimulation of the lateral nerves with the length of time of immersion in the sodium chloride solution.

normal stimulus to produce contractions is therefore difficult to explain, except on the assumption that the electrical stimulation of the nerves gives rise to nervous impulses of so much greater intensity than those coming from the ganglion that in this state of reduced excitability the muscle responds to the former but not to the latter.

## V.

The *Limulus* heart muscle immersed in an isotonic sodium chloride solution exhibits the following changes in tonus. When

the heart muscle is completely isolated from the ganglion, there is a gradual lengthening of the muscle in the sodium chloride up to the time when the idio-muscular contractions appear. It is not clear to me whether this lengthening is an actual tonus relaxation or merely a passive response to the pull of the light recording lever. As the contraction begins there is a gradual increase of the tonus till the end of the rhythm. During the latter part of the rhythm tonus waves usually appear on the records simultaneously with the fundamental contractions, and the former usually persist for some time after the latter have ceased. With the cessation of the more rapid or fundamental rhythm, the heart muscle begins to lengthen again. The tonus rhythm may persist for a while during this relaxation process. When the preparation is transferred from sodium chloride to plasma or sea water, the tonus is rapidly diminished as the idio-muscular rhythm disappears.

VI.

It has been shown in a previous paper in this journal that in the Limulus heart the conduction takes place in the nerve plexus and not from muscle cell to muscle cell. The proof of this is conclusive. We have now, furthermore, seen that the sodium chloride rhythm is idio-muscular and that the nerve plexus (minus the ganglion) takes

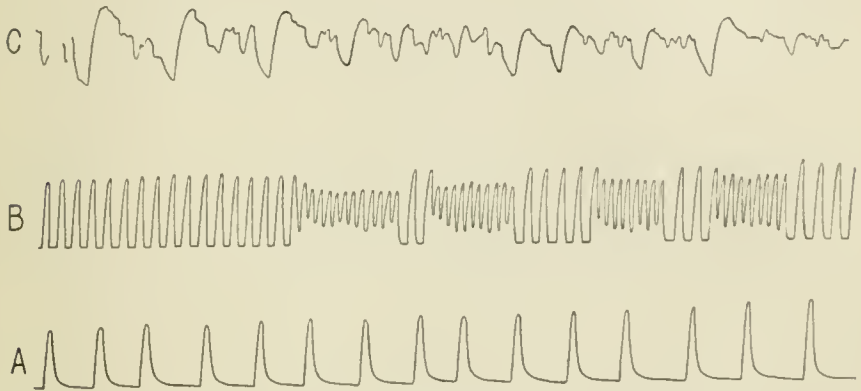


FIGURE 6.—Tracings from ganglion free segments of the Limulus heart immersed in  $\frac{1}{10}$  n NaCl, showing some types of the idio-muscular sodium chloride rhythm.

no part in it. In view of these facts it is significant that the *sodium chloride rhythm may closely approach the regularity of the normal rhythm*, because such a regularity involves practically the simultaneous contraction of all the muscle cells in the preparation. How is such a co-ordination effected in the Limulus heart in the absence of conduction from muscle cell to muscle cell when the tissue that normally serves to correlate the activity is not involved?

It is in a very few preparations, however, that such a regular sodium chloride rhythm is obtained. In the majority of the preparations the rhythmical twitchings are quite as irregular as the corresponding contractions of skeletal muscle, and in the preparations exhibiting the greatest regularity of the contractions it is invariably superseded by inco-ordination and irregularity before cessation of the activity. The sodium chloride rhythm may begin with a perfectly regular and gradually augmented series of beats which finally pass into a state of inco-ordination. It may begin by irregular and inco-ordinated twitchings and change for a brief period to a regular co-ordinated rhythm involving simultaneous contraction of all parts of

the preparations to again be superseded by inco-ordination. Or, thirdly, the sodium chloride rhythm of a preparation may be irregular throughout its whole course. Typical tracings of these different forms of the sodium chloride rhythm are represented in Fig. 6.

The irregular rhythm requires no special explanation, as that type of activity is what is to be expected under the circumstances, but the presence of an to all appearance perfectly regular rhythm under these conditions is more difficult to account for. There appear to be only two alternatives. Either the sodium chloride alters the muscular tissue so that a wave of contraction can be conducted from muscle cell to muscle cell, a process which does not occur normally in this heart; or the co-ordination is effected by means of an intercellular nerve plexus so related to the dorsal or superficial plexus connecting the ganglion with the heart walls that the impulses do not pass from the former to the latter. These suggestions are, of course, only working hypotheses. My observations so far rather tend to discredit the second alternative, but it must at the same time be admitted that we have no direct evidence of the truth of the first, either for the *Limulus* or the vertebrate heart. We do not yet know whether the contraction wave is normally conducted from muscle cell to muscle cell in the vertebrate heart, but according to Guenther<sup>1</sup> the sodium chloride rhythm in strips from the tortoise ventricle is at first confined to the part of the strip immersed in the solution. The same author states that this is also true for curarized skeletal muscle. In other words, the sodium chloride rhythm of skeletal muscle is of such a nature that one end of the muscle cell may be in rhythmic activity while the other end is at rest, despite the fact that the whole fibre retains its normal conductivity and contractility. The interpretation of the fact for the tortoise ventricle may be the same as suggested above for the *Limulus* heart. The main difficulty in testing the hypothesis in the vertebrate heart is the unsettled question of the normal mechanism of conduction.

<sup>1</sup> GUENTHER: This journal, 1905, xiv, p. 73.

## OBSERVATIONS ON HUMAN CHYLE.

By TORALD SOLLMANN.

[From the Pharmacological Laboratory of Western Reserve University, and the Surgical Service of Lakeside Hospital, Cleveland, Ohio.]

### INTRODUCTORY.

WOUNDS of the thoracic duct or its branches are not infrequent in operations on the neck.<sup>1</sup> The permission of Dr. D. P. Allen to utilize a case of this kind at Lakeside Hospital gave occasion to the following observations. I am indebted to Dr. J. A. Hofmann for the collection of the fluids.

*Clinical abstract.* — A branch of the thoracic duct was cut during the excision of some tubercular cervical lymph glands, on April 19, 1906. The lymph flowed from the wound in a continuous stream. The flow continued free for some days. On April 27 (eight days after the operation) it is estimated as about 300 c.c. in the twenty-four hours. The amount is distinctly less four days later. On May 4 (fifteen days) it is noted as "very small." The discharge has ceased entirely by May 14 (twenty-five days). The patient is discharged on May 21, with the wound completely healed.

The circumstances of the case rendered it impossible to make the physiologic and pharmacologic experiments as extensive as I would have wished; but the following may not be without some interest:

**Rate of flow.** — On the first day of the regular observations (April 27, eight days after the operation) the chyle flowed freely through a catheter placed into the wound, and could be collected very thoroughly and accurately. Breakfast (consisting of four ounces of cooked cereal, coffee, one egg, and one piece of toast) was given at 7 A.M. One gram of potassium iodid was administered at 8.52 A.M. The chyle was collected continuously from 8.45 A.M. until noon, into test-tubes which were changed every five minutes until 10 A.M., then less frequently. The contents of the test-tubes could not be measured

<sup>1</sup> ALLEN, D. P., and BRIGGS, C. E.: American medicine. Sept. 14-21, 1901.

directly because of clotting; but a close approximation was secured by comparison with a control tube of similar size. The quantities in Table I are therefore not quite accurate.

TABLE I.  
FLOW OF CHYLE (APRIL 27).

Time.	Quantity in c.c.	C.c. per five minutes.	Time.	Quantity in c.c.	C.c. per five minutes.
8.45-8.50	14.0	14.0	9.40-9.45	9.4	9.4
8.55	8.7	8.7	9.50	9.0	9.0
9.00	8.8	8.8	9.55	7.2	7.2
9.05	10.0	10.0	10.00	11.6	11.6
9.10	10.4	10.4	10.10	9.4	4.7
9.15	9.4	9.4	10.20	0.6	0.3
9.20	9.4	9.4	10.30	0.4	0.2
9.25	10.2	10.2	10.45	0.3	0.1
9.30	11.1	11.1	11.15	1.0	0.15
9.35	11.0	1.10	12.00	2.5	0.25
9.40	8.0	8.0			

For the seventy minutes between 8.50 and 10, the flow remained remarkably constant, from 7.2 to 11.6 c.c. per five minutes, averaging 9.6 c.c. It then fell quite suddenly to 4.7 c.c., and then remained for two hours between 0.3 and 0.1 c.c. per five minutes. The diminution could not be ascribed to clogging, nor could it be connected with the administration of the iodid; it was probably due to the completion of digestion (three hours after eating). A light meal poor in fat (egg, toast, custard, and soup), taken at 11.45, had no effect within fifteen minutes.

**Appearance and clotting.**—The fluids had a more or less creamy appearance, except when collected in the early morning, *i. e.* after fasting, when they became almost or quite clear. All the samples showed some clotting when brought to the laboratory. The size of the clot varied greatly, corresponding to the degree of cloudiness of the fluid, *i. e.* to the quantity of fat enmeshed by the clot. The coagulation occurred promptly. In those samples which were of

creamy appearance, the entire fluid set into an opaque, fairly consistent jelly, sufficiently firm to remain in the tube when this was reversed and shaken rather roughly. This clot gradually contracted into a thin cord of fibrin, by squeezing out the fat and serum. The clear samples did not form a jelly, the clot in this case being delicate and transparent and easily overlooked.

**Chemical composition.**— I am indebted to Dr. E. D. Brown for the determination of the data of Table II.

TABLE II.  
CHEMICAL COMPOSITION OF THE CHYLE (IN 1000 GM.).

Sample.	A.	B'.	B''.	C.	D.
Water . . . . .	....	928.90	927.47	....	....
Total solids . . . . .	....	71.10	72.53	....	....
Inorganic solids (ash) . . . . .	....	6.04	....	....	....
Chlorids as NaCl . . . . .	....	....	6.80	....	....
Organic solids . . . . .	....	65.06	....	....	....
Proteids (N × 6.25) . . . . .	....	....	....	18.52	....
Fatty substances:					
Chloroform extraction . . . . .	0.73	....	....	....	....
Ether extraction . . . . .	....	....	....	....	19.30

Sample A : Morning of April 26, four hours after last meal (19.23 gm. used for analysis).

Sample B : Evening of April 25, four hours after first, and one-half hour after second meal (milk and eggs), 15.394 gm.

Sample C : Afternoon of April 25, three hours after meal, 21.118 gm.

Sample D : Mixed collection between April 26 and 27, 168.485 gm.

The figures for total solids, ash and NaCl are fairly representative for human chyle.

The proteids appear variable: direct estimation in C showed 18.52 per thousand, while B must have contained nearly 65 per thousand. Similar variations are shown by the data of other investigators: thus, Paton found 13.7 per thousand, Rees 70.8 per thousand.

The fat content of the human chyle varies generally between 20 and 150 per thousand. In this case it is unusually low: the chloroform extract of sample A represented only 0.73 per thousand; the ether extract in D, however, approaches closely to the normal lower

limit, namely, 19.30 per thousand. This ether extract differed from ordinary fat in that 96 per cent of it was soluble in 95 per cent alcohol. The alcohol soluble fat (weighing 2.8823 gm.) contained free fatty acid corresponding to 0.1165 gm. KOH per gm. of fat; this would correspond to 0.587 gm. of free acid, expressed as oleic acid per gm. of fat. This free acidity is probably due to decomposition of the sample before the examination was started. The Koetsdorfer saponification number (combined fatty acid) corresponded to 0.1362 gm. KOH per gram of fat. The unsaponifiable residue amounted to only 2.5 per cent of the fat. This did not contain any phosphorus, hence no lecithin.

**Appearance of drugs in urine and chyle.**—It was planned to utilize this case mainly for observing the relative rapidity of the appearance and disappearance of drugs in the chyle and urine. The limited number of results, however, are only valuable for orientation.

**Potassium iodid; Method**—The iodid in the urine was determined by adding to 5 c.c. of the urine 1 c.c. of 1:4 sulphuric acid, a 1 per cent solution of sodium nitrite drop by drop, and 5 c.c. of carbon disulphid, and comparing the depth of color. The same method was applied to the first twenty samples of chyle with negative results. The failure was doubtless due to the combination of the liberated iodine with the fat of the chyle. It was attempted to save these spoiled samples by adding an excess of sodium hydroxid, evaporating and incinerating, and testing the ash as above. The results were again negative, as also in a control mixture of iodid and olive oil, treated in the same manner. Subsequent samples were tested with satisfactory results by evaporating directly with sodium hydroxid, charring the residue strongly, extracting with water, neutralizing, and proceeding as for the urines.

The iodid experiments were begun on April 27, 1 gram of potassium iodid being administered by mouth at 8.52 A.M., two hours after a light breakfast. The chyle was collected continuously through a catheter, the receiving tube being changed at frequent intervals; the bladder was emptied spontaneously every ten minutes for an hour, then less frequently. The urine flow declined very slowly from 6.7 c.c. per ten minutes at 9 A.M. to 4.25 c.c. at noon.

The flow of the chyle is reported in Table 1. The iodid did not cause any noticeable change.

The excretion by the urine began between eight and twenty minutes after the administration; the iodid concentration was almost



maximal in thirty-eight minutes, but increased slightly to one and one-half hours. In one and three-fourth hours it had again fallen slightly, being about the same as in one hour. This decline continued to two and one-fourth hours, when it resembled that of one-half hour; at this level it remained to the seventh hour. In twenty-four and twenty-six hours it was very much less. None was detected after four days.

**Excretion by the chyle.**—The first appearance of the iodid could not be determined on account of the faulty method. The earliest specimen examined correctly was taken three hours after the administration of the drug. This contained a fair quantity of iodid, as also the subsequent samples up to twelve hours. In the specimens of twenty-four and twenty-five hours there were only minute traces. None could be discovered in twenty-seven hours or later.

It would appear therefore that *the iodid, after a single administration, disappears at about the same time from the urine and chyle.*

**Sodium salicylate.**—0.3 gram of the salicylate was administered by mouth on April 30. The flow of chyle had become quite small by this time so that it could not be collected by catheter. The dressings were changed two and one-half and twenty-three hours after giving the drug, and exhausted with boiling water. They *failed to give a plain salicylate reaction* with ferric chlorid, either directly, or after acidulation and extraction with ether. The urine was not collected.

**Santonin.**—0.13 gram was given by mouth on May 1. The fluids were not tested for santonin itself, but for the peculiar product which gives a red color with alkalies. The *urine* gave no reaction in an hour; a slight red color in two hours, a deeper color in three hours. The tint remained nearly uniform in the hourly samples to the end of the seventh hour, when the collection was discontinued. *The chyle did not give the reaction*, the gauze being changed and examined every hour for seven hours.



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