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THE AMERICAN JOURNAL
OF
PHYSIOLOGY.

EDITED FOR

The American Physiological Society

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THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME XIX.

BOSTON, U. S. A.
GINN AND COMPANY

1907

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University Press
JOHN WILSON AND SON, CAMBRIDGE, U. S. A.

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THE
American Journal of Physiology.

VOL. XIX.

JUNE 1, 1907.

NO. I.

A CLINICAL METHOD FOR DETERMINING THE
ALKALINITY OF THE BLOOD.¹

BY HERMANN M. ADLER.

THE desirability of a clinical method for determining the alkalinity of blood—that is to say, the OH and H ionization—has long been recognized. The recent determinations of this ionization by Friedenthal,² Höber,³ Fränkel,⁴ Farkas,⁵ and Tangl⁶ and his pupils, have shown that the alkalinity is very slight and subject to very little variation. Accordingly, a clinical method has seemed difficult to seek. The recent study of indicators by Salm,⁷ however, characterizing with the greatest accuracy a whole series of indicators, has suggested the possibility of devising a method at once certain, accurate, and easy of application. The present paper is concerned with experiments to this end.

Two general methods of measuring quantitatively the reaction of a fluid may be pursued in any case: 1. titration to an end point;

¹ This work was done in the Laboratory of Biological Chemistry and in the Laboratory of Clinical Pathology of the Harvard Medical School. The clinical work was done in the Pathological Laboratory of the Boston City Hospital.

² FRIEDENTHAL: *Zeitschrift für allgemeine Physiologie*, 1902, i, p. 56; 1904, iv, p. 44.

³ HÖBER: *Archiv für die gesammte Physiologie*, 1900, lxxxii, p. 522; 1903, xcix, p. 572; HOFMEISTER's *Beiträge*, 1903, iii, p. 525.

⁴ FRÄNKEL: *Ibid.*, 1903, xcvi, p. 601.

⁵ FARKAS: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 551.

⁶ BENEDICT, H.: *Archiv für die gesammte Physiologie*, 1906, cxv, pp. 106-117.

SZILI, ALEX: *Archiv für die gesammte Physiologie*, 1906, cxv, p. 72: *Ibid.*, p. 82.

⁷ SALM, EDUARD: *Zeitschrift für physikalische Chemie*, 1906, lvii, p. 471.

2. determination of the H and OH ionization. The former method yields information concerning the amount of base and the amount of acid present in a solution, but no direct evidence concerning the nature of the equilibrium. According to such a method HCl and NaH_2PO_4 in equivalent amounts are equally acid. The latter furnishes no direct information regarding the absolute amounts of base and acid in solution, but determines the intensity of alkalinity, — the condition of equilibrium in the solution. For clinical purposes, any procedure that discloses the condition of equilibrium or variation in alkalinity is to be desired. For the variations, rather than the absolute quantities, are important to the clinician.

All the methods so far described depend upon titration. There are two reasons for this: first, because investigations of the OH and H ionization of blood have hitherto been possible only with the aid of the concentration cell, involving difficult procedure and expert technique; secondly, because the indicators which have been in use do not change color within the range of ionization found in blood. Recently, however, Salm has carefully studied the exact point of H and OH ionization at which a number of indicators turn, and accordingly it has been possible to pick out from this list a certain number of these that give sharp color reactions at about the H-ion concentration of the blood, — in particular two, rosolic acid and neutral red.

Rosolic acid is orange yellow in the presence of acid, and red in the presence of alkali. There is an intermediate pink color at neutrality. According to Salm, it is orange yellow at 1.10^{-6} n. H, pink at 1.10^{-7} n. H, and red at 1.10^{-8} n. H. The changes are well marked, and intermediate shades were easily distinguished. The variations of H ionization of the blood lie between 0.1×10^{-7} and 3×10^{-7} or less, a variation that falls within the bounds of neutrality as ordinarily conceived. It will be seen, however, that this variation lies at about the middle of the color change of rosolic acid from yellow to red or, to be more exact, between the pink and the red; and it was to be expected that, if sensitive enough, this variation would be noticeable in a difference in shade of the indicator.

In the present investigation rosolic acid was used. The first examinations were made on rabbit's blood. 1 c.c. of fresh rabbit's blood was caught in a centrifuge tube in which 10 drops of a 1 per cent solution of NaF had been placed, and 10 c.c. normal salt solution added.¹ This was placed in the centrifuge for a few minutes, and the superna-

¹ Both the NaF and NaCl solutions were precisely neutral to rosolic acid.

tant liquid then removed with a pipette to a clean test tube. Two drops of a 0.1 per cent alcoholic rosolic acid solution were added, and the pink color produced was compared with a standard. The test was repeated with laked blood from which the proteids had been removed by coagulation by heat. The same degree of color was obtained in this case as in the preceding experiment; that is, the reaction was neutral, or slightly more than 1×10^{-7} . The objections to the clinical application of this method are that a comparatively large quantity of blood is required, that a standard to compare it with must be at hand, and principally that the color of the blood itself interferes with the color reaction. It was therefore determined to exclude, for the time being, the formed elements, and to use the serum alone. The test-tube method also seemed cumbersome for clinical work, so ash-free filter paper was prepared with the alcoholic solution of rosolic acid. The paper was soaked in the solution and then hung up in the air to dry. While still moist the paper was yellow, but on drying, which occurred rapidly, it became a delicate salmon pink. The serum was obtained in the usual way from the finger or ear of the patient, by capillary tubes which, after the blood had clotted, were placed in the centrifuge. After the serum had separated, the tubes were opened with a file and a small drop of the serum removed by a capillary pipette and placed on the filter paper. The normal serum produced a strongly marked red color, which contrasted well with the unchanged parts of the paper.

The serum in a case of diabetic coma did not produce this change in color; the same observation was made in the case of a patient suffering with acute meningitis, and at the time of examination in a comatose condition. The urine of this patient contained acetone, but no sugar. The serum of a case of acute jaundice acted in the same manner on the test paper. All these cases had a marked decrease in alkalinity. Slighter variations in the same direction were observed in the serum of a case of lymphatic leucæmia, of two cases of scarlet fever, and in post-mortem blood. An increase in alkalinity above the normal has so far not been determined.

Henderson and Black¹ have shown that in a system containing both free and combined carbonic acid, in which there is not great variation in the tension of carbonic acid, the H and OH ionization correspond to those of blood serum, and that enormous variation in NaHCO_3 results from slight variation (corresponding to that found in blood) in

¹ HENDERSON and BLACK: This journal, 1907, xviii, p. 250.

H and OH ionization. Therefore it follows that variations in reaction to rosolic acid indicate not only H and OH ionization but also NaHCO_3 content of blood, perhaps the most important factor in acidosis.

This investigation shows that the slight variation in H and OH ionization heretofore detected only by the aid of the concentration cell can quite certainly be observed with the aid of properly chosen indicators, thus making possible the clinical investigation of the subject. It remains to determine which the best indicator or indicators may be, and then to proceed to a quantitative clinical study.

THE ACTION OF MAGNESIUM SULPHATE UPON THE HEART AND THE ANTAGONISTIC ACTION OF SOME OTHER DRUGS.

BY S. A. MATTHEWS AND D. E. JACKSON.

[From the Laboratory of Biochemistry and Pharmacology, University of Chicago.]

WHILE the intensely poisonous effects of magnesium salts when injected into the circulation in mammals have long been recognized, Meltzer and Auer¹ have added a new interest to magnesium by calling attention to its depressing action upon the central nervous system and to its power of blocking nervous impulses in both directions, the sensory before the motor, when applied to nerve trunks or to the spinal cord. These investigators were able to induce a state of anæsthesia by subcutaneous injection of magnesium salts, and by applying it to the spinal cord by way of the subdural space were able to block all sensory impulses. In fact, from their observations it would seem that magnesium possessed all the requisites of a true general anæsthetic, and of a local anæsthetic equal to cocaine.

But there are certain parts of the nervous system so affected as to interfere with the practical application of magnesium as an anæsthetic. For instance, the respiratory centre and probably certain nerve elements in the heart are depressed to such a degree as to jeopardize the life of the animal before general anæsthesia is complete. It was with the hope of finding some means by which these effects might be lessened that this series of experiments was undertaken.

In our work we have dealt with amphibians, reptiles, birds, and mammals. A few preliminary experiments were performed upon frogs for the purpose of determining the condition of the spinal cord during the general anæsthesia induced by magnesium sulphate. In these experiments the frogs (both pithed and intact) were given injections of 1 to 2 c.c. of a $\frac{2}{1}$ solution of magnesium sulphate into the anterior lymph sac. In ten to thirty minutes a condition of com-

¹ MELTZER and AUER: This journal, 1905, xiv, p. 366, 1906; xv, p. 387, xvi, p. 233, xvii, p. 313.

plete general anæsthesia was produced. It was then found that strong stimulation immediately over the cord, or at the medulla, produced violent motor results. Stimulation of the sciatic nerve also produced motor results.¹ This would show that motor impulses passed readily along the nerve trunks after the establishment of complete general anæsthesia.

OBSERVATIONS ON THE HEART.

A number of preliminary experiments were performed for the purpose of determining the action of magnesium sulphate upon the heart

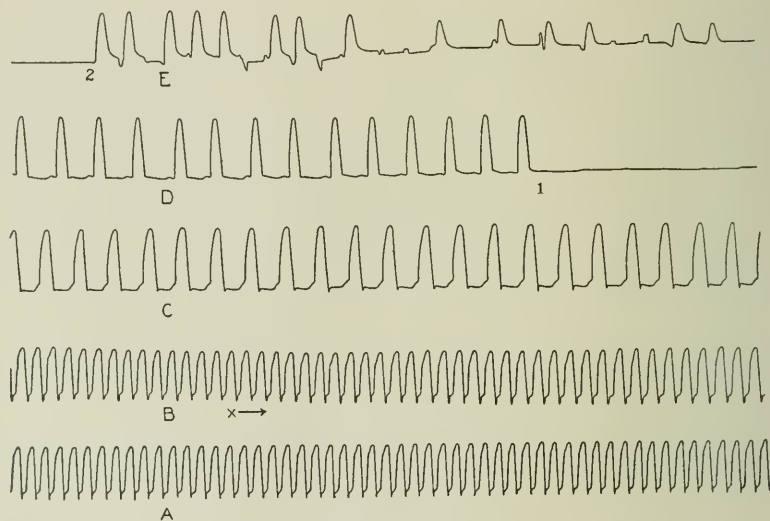


FIGURE 1.—About two thirds the original size. Frog. Heart tracing by the suspension method. Read from left to right and from bottom to top. The first line, *A*, is normal. Beginning at *X* in line *B*, about twenty-five drops of a $\frac{2}{1}$ solution of magnesium sulphate were slowly dropped on the heart. Lines *B*, *C*, *D*, and *E* show the continued effect of the magnesium. At 1 in line *D* the heart was brought to a standstill. At 2 in line *E* mechanical stimulation of the heart tissue by means of pinching with a pair of forceps was begun. (It is worthy of mention that the effects of osmotic pressure are necessarily produced in the heart tissue by this method of application. Aside from this, however, it is quite evident that the magnesium sulphate has a specific action of its own.)

in frogs. The animals were pithed, and the thorax was opened so as to expose the heart. A normal tracing was taken by the suspension method. Then magnesium sulphate $\frac{2}{1}$ solution, was dropped on

¹ M. B. WIKI: Comptes rendus, July, 1906, lx.

the heart. At first there was some mechanical excitation of the heart, soon followed by slowing and a loss of tone (Fig. 1). While the auricles beat normally, there was a blocking of the systolic impulse at the auriculo-ventricular junction, so that the contraction passed slowly from the auricles to the ventricle. Magnesium sulphate solution was continuously, or at most at very short intervals, dropped on the heart, so that the cardiac tissue might be considered to be bathed in the magnesium sulphate solution. As the application continued, the rhythm became progressively slower and the blocking more marked. The amplitude of the ventricular beats was not much affected, but that of the auricular beats, however, may have been slightly lessened.

The slowing of the rhythm continued to a certain point, when the ventricular beats suddenly ceased, while the auricular contractions often continued for a few seconds longer. Then generally all movement of the heart stopped permanently; sometimes a few feeble beats would again occur spontaneously after an interval of some minutes.

It was noticed that as the rhythm became much slowed there was a tendency toward a grouping of the beats. As the poisoning proceeded, the number of beats in a group became less, three, two, and finally one. This tendency toward grouping was not so great, however, as is sometimes observed in other drugs, *e. g.*, cocaine, digitalis, aconitine, etc. It is to be noted that the contractions, at least the ventricular, were of almost full size until the last. After the spontaneous rhythm had ceased, mechanical stimulation of the heart tissue by means of pinching with a pair of forceps resulted in inducing almost full-sized contractions at each stimulation. The muscle was still irritable, and when the initiative stimulus was applied it showed itself to be able to contract with almost normal force. But the heart itself seemed to be no longer capable of spontaneous movement. In other words, the heart tissue seemed to behave in a manner similar to ordinary skeletal muscle. It would contract when stimulated, but if undisturbed it remained perfectly quiet. It is to be remarked that in these experiments the solutions were merely dropped on the heart, and not injected directly into the circulation. Solutions of ten, fifteen, and twenty-five per cent showed exactly the same action, with the one exception, that the stronger solution acted much more rapidly. In order to avoid any possible vagus action atropin was dropped on a number of hearts. It did not affect the charac-

ter of the results in general, and hence indicated that no stimulation of the inhibitory apparatus was occurring.

Turtles and birds.—A few experiments were performed upon turtles and birds (roosters and geese) for the purpose of determining the effect of magnesium sulphate upon the reptilian and avian hearts. The results of these experiments show that the action of magnesium sulphate upon reptiles and birds is practically identical with that upon frogs.

Dogs.—In the work upon dogs particular attention was paid to the action of magnesium sulphate upon certain parts of the cerebrospinal and sympathetic nervous mechanisms, upon the movements of the intestines, upon the salivary glands, and upon the circulatory system. The animals were anæsthetized with ether. In the majority of cases a Cushny¹ myocardiograph was used to record the actions of the heart. The artificial respiration used served not only to keep the animals alive while in the normal condition, but was also a means of avoiding the immediate respiratory paralysis, which is the usual cause of death.² An induction coil was arranged for single shocks, and the electrodes were attached to the hooks of the myocardiograph. In this manner it was possible to send single shocks through the heart at any time desired. Usually the vagus nerve was dissected out and arranged for electrical stimulation. In most cases the abdominal cavity was opened and the intestines exposed.

When these preparations were completed, the drum was started and a normal tracing obtained. Usually, then, a slow injection of 2 c.c. of a $\frac{2}{1}$ solution of magnesium sulphate was given the animal through the femoral vein. The result of this injection was a distinct slowing of the heart rhythm and a considerable decrease in the amplitude of the beats (Fig. 2). The effect usually lasted about three minutes. Then a second injection was given exactly as the first had been. This produced a more marked effect than the first injection. The slowing was much greater and sometimes would stop the heart. The amplitude was also greatly reduced. If this injection was made rapidly, and in a very short time after the first injection, the heart was almost invariably stopped. Magnesium chloride, in corresponding strength, had similar results. Of course, if artificial respiration had not been used, the animal would have died much earlier from respiratory failure. This description applies

¹ See STEWART'S Manual of physiology, 5th edition, November, 1905, p. 167.

² MELTZER and AUER: This journal, 1906, xvi, p. 233.

to the majority of mammals upon which we experimented. But to this general description at least two marked exceptions must be made. In these two dogs it appeared that the magnesium was very much less poisonous than in all the other cases. Very large injections in these two, however, had practically the same results as were observed in the other dogs.

When the heart had completely stopped and a sufficient interval had elapsed to indicate that spontaneous beats would not return, electrical stimulation was begun. Single shocks were sent through the heart at about the rate of the normal rhythm. The result was a contraction of that organ at each shock. The artificial stimulation was kept up until the heart again regained its normal beat. This usually

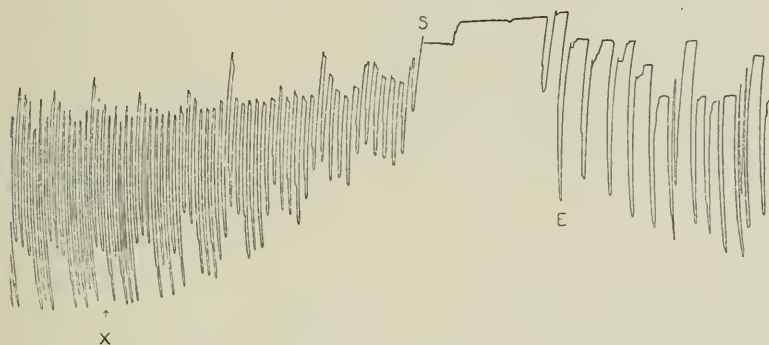


FIGURE 2. — About two thirds the original size. Dog. Myocardiograph tracing. Read from left to right. At *X*, 2 c.c. of a $\frac{2}{1}$ solution of magnesium sulphate was injected intravenously. At *S* the heart ceased to beat. At *E* electrical stimulation of the heart tissue was begun. The downward stroke represents the systole.

required about two minutes, but varied considerably. An occasional wait was made during this artificial stimulation process to see if the heart had sufficiently recovered to beat of its own accord. It was also during this period of cardiac weakness that injections of other drugs were made in the attempt to offset the poisonous effects of the magnesium sulphate on the heart. The most important of these drugs were adrenalin, squills, and a mixture of barium chloride with calcium chloride.

Adrenalin. — Injections (1 c. c. of one ten-thousandth solution) were made into the femoral vein during the cardiac depression following the injection of magnesium sulphate. The results were by no means marked. A very slight increase in the amplitude, and possibly some increase in the rate of heart beat, were occasionally observed. It seemed as if the adrenalin might have increased the irritability of

the heart muscle to a slight degree. That is, after the heart had been brought to a standstill by the magnesium sulphate, adrenalin rendered the heart muscle more responsive to electrical stimuli, but did not impart to it any increased power to originate a spontaneous rhythm.

There are three possibilities for the action of magnesium upon the heart, namely, the cardiac muscle may be depressed, or the nervous mechanism (at least that part of it involved in the origination of the cardiac impulse) may be partially or wholly paralyzed, or there may be a combined action upon both muscle and nerve. The general results of our experiments have led us to believe that the last is the true condition. If we may be permitted to assume that under normal conditions the heart beat has its initiative in some nervous mechanism,¹ then it would seem that under the action of magnesium these nervous elements were depressed first, or at least that during the earlier stage of cardiac inactivity the nervous mechanism was more nearly paralyzed than was the muscular tissue. It appeared that the heart muscle was almost changed, for the time being, into the condition of an ordinary skeletal muscle. (To be sure, we did not study in detail possible changes in the refractory period, the "all-or-none" response, the resistance to tetanus, etc.) The heart would contract if stimulated, but if undisturbed it remained perfectly quiet. And further, aside from the "staircase" effect, each contraction was of considerable amplitude. This would indicate that the muscle had not so far suffered any very great depression, but that merely the initiative stimulus to which it ordinarily responded had been withdrawn.

If the electrical (or sometimes mechanical) stimulation were continued for a little time, the normal rhythm would return. Of course, if a sufficient quantity of the salt had been injected to completely paralyze the heart muscle, it became impossible to restore the normal beat. In those cases where the heart was restored it is to be presumed that the poison had passed out of the circulation to a degree sufficient for the return of the normal function.²

Squills.— It was expected that the action of this drug on the heart and vessels might in some measure counteract the depression caused by the magnesium sulphate. In order to avoid any vagus action from the squills a small injection of atropin was usually given. It was found, however, that the slowing still occurred. But in the recovery

¹ CARLSON: This journal, 1906, xvii, p. 5.

² LUCAS and MELTZER: Proceedings of the Society for Experimental Biology and Medicine, 1906, iv, No. 1, p. 10.

there appeared to be some gain. Undoubtedly the stimulant action of the squills upon the heart muscle had a beneficial effect. However, the antagonistic action was entirely too feeble to be of any practical importance. Like adrenalin, it simply increased the irritability of the heart muscle to mechanical or electrical stimulation without increasing its ability to originate spontaneous beats.

Barium chloride and calcium chloride.— This solution consisted of barium chloride and calcium chloride in the proportions of 2.44 gm. ($\frac{m}{1000}$) of barium chloride and 2.19 gm. ($\frac{m}{1000}$) of calcium chloride, both dissolved in a litre of distilled water. Its action consists largely in a

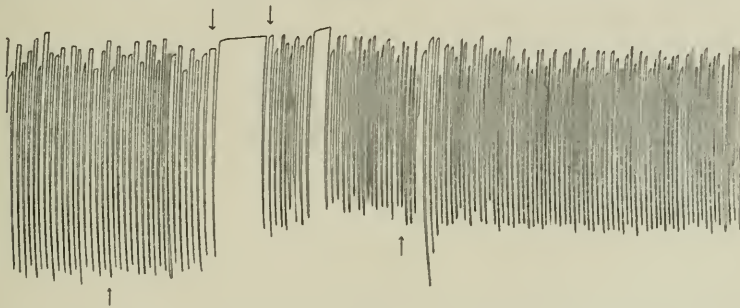


FIGURE 3. — Dog. Myocardiograph tracing. Read from left to right. The animal had previously received several injections of magnesium sulphate. At the first arrow the dog received 2 c.c. of a $\frac{2m}{1}$ solution of magnesium sulphate. This stopped the heart, and after a short interval electrical stimulation was begun (second arrow) and continued to the end of the record. Shortly after the electrical stimulation was started the animal was given an injection of eserine (third arrow). It was then found that stimulation of the sciatic nerve produced perfectly normal motor responses¹ in the hind limb. At this time the physostigmine action upon the intestines was very marked.

stimulation of unstripped muscle, especially cardiac and intestinal. It was found that this mixture had a very marked effect in overcoming the magnesium depression of the heart. The slowing caused by magnesium was much reduced, the return of the normal rhythm was considerably accelerated, and the amplitude of the restored beats was distinctly greater. Often the effects of a second injection of magnesium sulphate were almost unnoticeable after one or two injections of the barium and calcium solution. While the barium and calcium had a greater effect than either the adrenalin or squills, it still had little or no effect on the heart when once brought to a complete standstill by magnesium sulphate. That is, it increased the muscular

¹ M. B. WIKI: Comptes rendus, 1906, lx.

irritability to a greater degree, but seemed to have little effect on the power of the heart to originate spontaneous beats.

INTESTINAL MOVEMENTS.

No especial departure from the normal peristaltic movements was observed upon the intestines after magnesium sulphate injections. It was found that barium still had its characteristic effects upon the intestinal muscle.¹ The contractions may, however, have been somewhat less tetanic than is usual after barium. It was also found that magnesium sulphate did not prevent the usual action of pilocarpine and physostigmine¹ upon the intestinal contractions (Fig. 3). Pilocarpine also had its ordinary vagus effect upon the heart. Physostigmine still produced the muscular tremors¹ of the skeletal muscles in general, even after repeated large injections of magnesium sulphate, sufficient to completely stop the heart so that it had to be kept beating by means of electrical stimulation.

SPLANCHNICS.

It was found by direct stimulation of the splanchnic nerves that their endings were still intact. The rise in blood pressure, however, was not quite so great as was normally found.

EYE.

Stimulation of the cervical sympathetic still caused dilatation of the pupil.

SALIVARY GLANDS.

It was found, both by stimulation of the chorda tympani nerve and by the injection of pilocarpine, that these glands were still capable of secreting abundantly even after repeated large injections of magnesium sulphate.

SUMMARY.

In the frog during complete anæsthesia from magnesium sulphate, impulses pass readily throughout the entire length of the cord, and produce their normal effects upon the motor endings.

¹ MELTZER and AUER: This journal, 1906, xvii, p. 313.

The action of magnesium sulphate upon the heart is practically the same throughout the mammalian, avian, reptilian, and amphibian classes.

This action consists of a very marked depression, characterized by an immediate decrease in the amplitude of the heart beat and of simultaneous progressive slowing, which soon leads to a complete standstill, from which the heart may be recovered by artificial stimulation.

Adrenalin and squills cannot be used to offset this depression.

Barium chloride and calcium chloride solution is much more effective in attempts to restore the heart when magnesium standstill has been induced; but even with the use of this solution magnesium is far too poisonous to be safely injected intravenously. Neither of these solutions seems to have much influence toward increasing the power of the heart to spontaneously originate its own contractions.

Magnesium sulphate injected intravenously has but little, if any, effect upon the muscles, nerves, or peristaltic movements of the intestines.

The nerve endings of the vagus, sympathetic, motor,¹ and secretory (chorda tympani) nerves are unaffected by magnesium.

We desire to express our thanks to Prof. A. P. Mathews and Prof. A. J. Carlson for valuable suggestions in the preparation of this paper.

¹ M. B. WIKI, Comptes rendus, Société de biologie, July, 1906.

THE PHYSIOLOGICAL STUDY OF A CASE OF MIGRAINE.

BY SHEPHERD IVORY FRANZ.

[From the Laboratories of the McLean Hospital, Waverley, Mass.]

THE reason for attempting to describe another case of migraine is to record the results of some physiological and psychological tests which were made on a patient, with a view to determining what conditions were present or concomitant with the headaches. Numerous writers have considered migraine, and there are several hypotheses to account for it. These hypotheses may be briefly characterized as (1) metabolism defect, (2) sensory epilepsy, (3) fatigue, and (4) vasomotor.

Those who hold that migraine is due to some disturbance of the metabolic processes of the body have observed cases in which there have been undoubtedly changes in the nutrition and functions of the body associated with the migraine. The nausea and vomiting which sometimes, and rather often, accompany migraine have been taken to indicate that the disturbance was of gastric or intestinal origin.

The periodicity in the course of the attacks, and the fact that the headaches may be ushered in by sensory symptoms similar to the aura of epilepsy — some have photophobia and the fortification figures — have led some to conclude that the migraine is a sensory disturbance corresponding to the motor disturbances which are found in true epilepsy.

On the fatigue side I would group all those hypotheses which account for the headaches on the ground of ocular disturbance and of disturbance of the other parts of the head or face, as, for example, the nose. On the vasomotor side the hypothesis has been advanced that the aches are due to disturbances of the circulation, and more especially to the increase in blood pressure in the brain and about the head. The general vasomotor hypothesis has been modified by some in that the suggestion is made that the vasomotor change is local, a spasm of the arterioles in the neighborhood of the head and neck.

The present case was referred to the scientific department of the hospital, by Dr. W. A. Lane of Milton, Mass., for investigation into her physiological and psychological conditions. Attempts were made to determine any metabolism disorders which might be present, and to carefully note so far as possible other sensory disturbances and the concomitant changes in the blood circulation.

The patient came to the hospital on April 23, 1906. She is married and aged forty-two. So far as is known, there is no history of similar attacks among her ancestors. The only thing of possible interest in the family history is that her father was rather delicate as a boy, but grew up strong and well, and is so now at the age of seventy-two. He has had "gout" and eczema. Her mother was strong and well, but died at twenty-five of puerperal fever ten days after the birth of the patient. No member of the family, so far as known, was ever subject to neuralgia, headaches, epilepsy, or other neuroses. The patient's family physician reports, however, that there is, or has been, insanity on the father's side, the character of which is not known.

The patient was somewhat delicate as a child, although not sickly. During early childhood, until about ten, she had occasional nightmare, which was not associated with headache or nausea. No other night terrors. From ten until about eighteen she had attacks of nausea and vomiting, with severe pain in the stomach lasting several hours, coming on every two or three weeks. She did not have headaches with these, and they came on without obvious cause. Menses began at fourteen, and were painful until about twenty-two. The periods at that time lasted a week, and she would sometimes be a little faint, but never actually fainted. Since twenty-two the menses have been pretty regular, coming every twenty-three to twenty-six days, lasting three or four days without pain. When about sixteen she became run down, left school, and a year later came to America, at which time she was fairly strong, but not robust. Shortly after coming to this country she had typhoid fever, but did not have an unusual amount of headache or any nausea. She made a good convalescence and has been well ever since, except for the few acute illnesses to be mentioned. She had nasal catarrh and sore throat some years ago, and about seven years ago she had her nose cauterized, since which time she has been free from these troubles. She has never had any fright or emotional shock which she can remember. The headaches are reported to have begun soon after the death of some

of the patient's brothers, one of whom was drowned. This, however, the patient asserts, was not any greater emotional shock than would be expected from the death of several members of the family within a short period of time.

For the last thirteen years she has been subject to attacks of unilateral headache (occasionally bilateral), sometimes on one side, sometimes the other, accompanied by anorexia, but not nausea. There seemed no special cause for their appearance, and the patient reported that they came at somewhat irregular and uncertain intervals, usually about the period of menstruation, and once or twice between. They varied in severity and duration, the most severe lasting from twenty-four to sixty hours. The headache sometimes began on one side, continued throughout the day, and recurred on the other side for a second twenty-four hours. The attack usually set in with a vague feeling of general discomfort and lack of appetite, hyperæsthesia to sound, and some photophobia. These symptoms increased in severity, and after some two or three hours the head began to ache. The eyes felt large and swollen, and there was pain on turning the eyes, especially on the side on which the head ached. There was occasional dizziness or faintness on suddenly turning or rising, but not otherwise. The face was flushed and felt hot, and she felt some beating of the pulse in the head, especially on the aching side — "It felt as though the veins were full." During such an attack she did not sleep very well, though always some, and was apt to dream disagreeable dreams, in which she did a lot of work without accomplishing anything. There was no real nightmare, and no one dream was recurrent. There was no numbness, no flashes of light, no double vision, no anæsthesias. Occasionally she felt a little weak after an attack, especially if it were a long one. The periodicity was so irregular, she reports, that it could not be counted on, and she had to give up making engagements of any kind in advance. The patient reported that the attacks were apt to come on if she got very tired or had any unusual excitement, but not invariably so. On the other hand, they had come on during a prolonged period of rest.

She has tried climate, dieting in various ways, sanitarium treatment, and has had her eyes repeatedly examined and worn prescribed glasses, all without material benefit. The general health is good, and she would consider herself very well if she were not so often incapacitated by the headaches. She reports that she has been subject to attacks of "rheumatism" several times a year, in which she has

had pains in shoulders, hips, or knees, with some limitation of motion, but no fever and no swelling of the joints. These attacks have lasted two or three weeks. She has never had acute inflammatory rheumatism. The headaches occur during these attacks as usual.

About four years ago she was a little run down, then got tonsillitis, then the glands of the neck enlarged, and following this she had an attack of facial erysipelas and otitis media, all within a period of about two weeks. She had an unusually severe migraine, with constant noises in the ears. It took her a month to recover her strength.

She was married about four years ago and has one child, born two years ago. She had only two days of nausea in the beginning of pregnancy, and reports (see page 31) only two or three very mild attacks of headache throughout the nine months. During the period of pregnancy a fibroid tumor was found to be present in the abdominal cavity. The patient was operated upon and this removed, without interruption of the pregnancy. A similar operation for a similar growth took place previously at the time of an earlier pregnancy, namely, in 1902, and this first operation produced a miscarriage. It is of interest, therefore, that the removal of these fibroids, which have been supposed by gynæcologists to be the cause of migraine, had no effect on the number or on the severity of the attacks. She was delivered of a strong, healthy baby, and within a few days began to have her headaches again. Catamenia began two months after childbirth. She nursed the baby for four months.

She lives moderately, is out of doors a good deal, eats a simple diet, avoiding sugar from choice, sleeps well except when she has the headaches, and is inclined to be constipated. She has no worries, and has no mental symptoms. The last attack of headache was two weeks before entrance to the hospital, coincident with menstruation. She wears glasses prescribed for her "eye strain."

Physical examination showed a woman of medium height, grayish hair, florid complexion. Pupils rather large, equal, and react to light and accommodation. Glasses for correction of asymmetrical myopic astigmatism. No arcus senilis. No facial asymmetry, but the right eye seems slightly turned outwards. The heart area is normal in size. No murmurs. Second sound is short and accented over the base. Pulse is regular, of good volume, and rather high tension. The radial arteries are not thickened or tortuous. The temporal arteries are not tortuous or thickened, and the pulse is of equal tension on both sides.

The patient remained at the hospital from April 23 to May 6, went away for a week and returned on May 14, remaining until the 26th. During these periods she was under constant observation, and we had an opportunity of observing her during four of her attacks of migraine. The first of these attacks came on April 26 and 27, the second from May 4 to 5, the third on May 17, and the fourth on May 23 and 24.

April 26, at eight A. M., the patient felt uncomfortable, and believed that an attack was coming on; from the vague sensations which were present she thought the headache would be on the right side, as it afterwards proved to be. At ten the headache was somewhat worse, and remained until about noon the following day. When seen on the evening of April 26, the patient reported that the ache was on the right side. No difference in the appearance of the sides of the head was noticed. The pupils were large but equal. The conjunctival veins were engorged equally on both sides, and the face had the same color all over. The surface temperature taken on the temples showed on the right 95.9° , on the left 96.4° . The arterial tension was high in both radials and carotids, but, so far as could be estimated, equal on both sides. The area of the ache was all over the right side of the head, extending on the forehead to the left, about half an inch beyond the middle line. At the back it extended about two inches below the occiput, and of the face it included the right eye, the right side of the nose, and most of the maxillary area, but did not include the ear. When the patient awoke at four A. M., April 27, no aching was felt, but soon the ache reappeared, though not in so severe a form. At nine, when the patient was seen, the ache did not extend to quite the middle line on the forehead. On the face the area was the same as previously described, and at the occiput the area had decreased about an inch. The ache gradually disappeared toward evening.

The patient was put on starch diet for the laboratory experiments, and other physiological tests were begun.

During the next six days the patient was observed to have an unusually hard pulse. On May 1 and 2 the arterial pressure seemed to be lower than it had been previously. On the 3d the pressure went up, but was equal on both sides. No difference in color on the sides of the face.

About noon on May 4 a second headache began, but the patient continued to read and to occupy herself until about three o'clock,

when the pain became too severe. At five-thirty she retired, and when seen at this time it was found that the arterial tension was somewhat higher than in the morning, but equal on both sides. The left side of the face was a trifle more flushed than the right, and the left eye was watery and the eyelid drooped somewhat lower than the right. The tongue was coated. The extent of ache mapped out at this time corresponded to that noted a week previously, but was on the left side. The ache existed over the left side of the head, but was particularly severe in the frontal and occipital regions. The ache extended on the forehead to the right, about an inch beyond the middle line. At eight-thirty P. M. the patient was found to be a little more comfortable, but about half an hour later the ache became more severe, and continued so for part of the night. The patient reported that during part of the night she could not breathe well through the nose, and that occasionally in other attacks this has also occurred. The headache continued throughout the following day, but became less severe toward the afternoon. Menstruation began that morning. The left eye when opened soon filled with tears, the face was equally colored on both sides. The arterial tension was high, but apparently not so high as on the previous day. For a short time, about eleven P. M., the ache became more severe, but at this time it was localized chiefly in the left temple, and no ache was felt in parietal or occipital regions. Laughing and other movements of the head during the attack caused an increase of the pain. There was no double vision, and no nausea or vomiting.

At no time during the course of the attack were there found any areas of tenderness on head, chest, back, forearms, and legs. The following morning the arterial tension was not so high as it has been for the past three days. The patient's weight had been quite constant.

The patient returned to the hospital on May 14. She reported that she had a headache on the right side on May 8, which began at noon and continued for about twenty-four hours.

Before starting for the hospital (May 14) the patient had an uncomfortable feeling, as if a headache was coming on, but during her journey to the hospital she improved, and the feeling did not develop into an actual hemicrania. A series of experiments was begun on May 15. Two days later a right-sided ache began, which lasted all day and part of the night. When observed at three P. M., it was noticed that the right eyelid hung lower than the left, and that the

right eye did not seem so bright. Pupils large, but equal. There was a high tension pulse in both radials and the carotids, and it seemed to be higher on the right than on the left, particularly in the radials. The headache did not extend quite to the middle line of the head and forehead. In the frontal and occipital regions the ache was most severe, and the patient could not be sure of any ache in the parietal region. There were no areas of tenderness on forehead, head, or face.

On May 19 the patient left off her glasses, went to the theatre in the afternoon (saw biograph pictures, etc.), and read the remainder of the day — to try to produce a headache for experimental purposes. This was the first time the patient had gone without glasses (with exception of a few hours because of accident) for twelve years. May 20, 21, and 22, the patient continued to use (and to abuse) her eyes with hard work, but without any feeling of an ache. On the third day (May 21) her eyes seemed heavy and tired, and there was a tendency to shut them involuntarily to exclude the light, but on the following day this slight photophobia had disappeared. Only after four days of very hard "eye strain" did a headache commence (May 23). On that morning the patient reported having premonitory symptoms, but she worked in the laboratory part of the day until the ache became very severe (on the left side). The arterial tension was found to be high, but equal on both sides; the left eye was watery, and the left nostril was choked, and secreting mucus. A hot bath (95°) was ordered, with cold compress for the head. The bath was given for an hour, but owing to misunderstanding the cold compress was not placed on the head. In the bath at first the patient felt more comfortable, although the headache did not disappear, but after half an hour the improvement abated. Immediately before being taken from the bath patient was observed and found to have low tension pulse in radials, high in carotid — equal on both sides. A cold compress was put on the head at six P.M. and continued until eight, but did not ameliorate the condition. For some time after the end of the bath the ache was worse, but became a trifle better when food was taken and gas expelled from the stomach. When seen at eight P.M. the arterial tension in carotids and radials was high; the left carotid pulse was considerably harder than the right. On waking the following morning it appeared as if the ache had disappeared; it reappeared soon but not in so severe a form, and then gradually disappeared. The left eyelid still drooped to some extent and the left eye

was filled with tears. Nothing unusual was noted for the following two days, and on May 26th patient was discharged.

One of the objects of the experiments being to determine any possible change in the metabolism, the patient was put on the diet which has been extensively used in the hospital by Dr. O. Folin, and the results from this diet are given below. In addition to these experiments the author had the following series made each day, and at times the series was made both night and morning.

(a) Temperature. The temperature of the patient was taken both night and morning in the axilla. In addition to this the temperature was taken on the surface of the body at the temples at each time the other series were being made.

(b) Pulse and respiration were counted several times a day, and the weight of the patient was regularly kept.

(c) The blood pressure was estimated by means of a simple apparatus, which will be later described, taken on the head and hand.

(d) The threshold for the sensations of touch and pain was taken on the face, hands and arms, back, chest and abdomen, and on legs.

The temperature, taken in the axilla night and morning, at each time two determinations being made, did not show any differences between the times when the patient had a headache and the times when she was in a normal condition. The surface temperature taken at the temples showed some slight differences, which we may summarize as follows: During the periods in which she had headache there were 13 determinations made, the average temperature on the right side being 96.3° F. and on the left side 97.1° . At other times the temperature on the right side averaged 96.6° and on the left side 98° (17 determinations). If we analyze still further the temperature on the skin at the time of the headaches, we find 13 determinations on the side of the ache, disregarding right and left, showed 96.97° F. On the other side the same number of determinations average 96.4° . Whether this difference of over half a degree is characteristic of the condition which is present is not certain, although the number of determinations would indicate that such is possible. The details of these experiments are given in the table which follows.

No differences that were marked were noticed in the rate of pulse and respiration for the headache and non-headache periods.

The blood-pressure experiments gave more definite data, and the differences in this case were more marked than even those of the surface temperature on the head. The blood pressure was determined

by means of a spring dynamometer, which was pressed against a microscopic slide cover glass.¹ This gave some idea of the capillary blood pressure, and it was so convenient and easy to work, and so easy of adjustment, that I felt unwilling to undertake the more extensive series that would be necessary with one of the standard blood-pressure apparatuses used ordinarily for the arm and leg. The cover glass was placed upon the skin and pressure exerted by the spring apparatus, from which could be read directly the pressure in grams necessary to blanch the skin under the cover glass. The area of the circular cover glass was 122.6 sq. mm. The pressure which was exerted usually to produce the blanching of the skin under normal circumstances varied from 100 to 275 gm. On the side of the ache at the time of the ache 33 determinations on the head gave an average pressure of 247.8, and on the other side 228.4. On the head at the time of the ache both sides averaged for 66 determinations 238.1, and at other times for 114 determinations 236.8. On the hands the capillary blood pressure was tested by using the same pressure instrument and pressing upon the nails of the index and ring fingers. Two determinations were made each time on each hand. The average area of the finger nails was found to be 117.5 sq. mm. This is slightly less than the area of the cover glass used on the head, but it was a much more convenient method to use. On the side corresponding with and at the time of the ache, the pressure necessary to produce blanching under the nail was found to be 165.2 (20 determinations) and on the other side at the same time 160.7. At the time of the ache on both sides the average pressure was 162.9 and at other times 124.2. The differences between the pressure found on the side of the ache and that on the other side at the time of the ache and between the pressure on both sides at the time of the ache and that at other times, is sufficiently great to enable us to say that the pressure during the attacks of migraine in this case is greater than in her normal condition, and possibly this may be one of the etiological factors in the condition. A tabular account of these results is given in the table that follows, which includes also the results of the sensory tests.

The touch threshold was determined by means of an instrument similar to that used by Bloch, illustrated in the accompanying figure. To a piece of wood was attached a spring steel wire (*A*) which was

¹ An instrument designed for this particular purpose is made by VERDIN, of Paris, and is called an *achromatometer*.

bent at a right angle (*B*); the long part of this wire (*A*) measured six inches. The area of cross section of the wire was about 0.4 sq. mm. A scale (*E*) attached to the instrument enabled the experimenter to determine the pressure made by the wire in its bending. The instrument measured pressures as high as $1\frac{1}{4}$ gm. The right-angle piece of the wire was pressed against the patient's skin, care being taken to keep it vertical all the time, and when the subject reported that the pressure was perceived, the reading was taken from the scale and recorded. The following points on the skin were selected for the determination of the touch thresh-

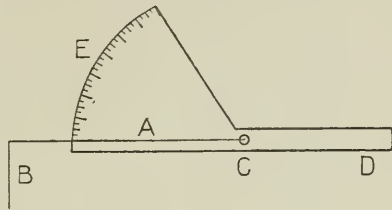


FIGURE 1. — The figure is about one fifth the size of the instrument.

old: on the head on either side about an inch from the middle line and about an inch above the orbit, and on either side about an inch above the centre of the zygomatic arch, in front of the ear, at the angle of the jaw, on the point of the chin a half inch from the median line, at the angle of the mouth and on the nose about a quarter of an inch from the tip; on the hands and arms on the volar surface at the tips of the thumb, index and ring fingers, on the thenar and hyperthenar eminences, at the wrist, at the bend of the elbow, and half-way between wrist and elbow; on the dorsum of the forearm and hand, a half-inch below the olecranon, at a point midway between the elbow and wrist, at the wrist, and above and about the middle of the fourth metacarpal; on the abdomen and chest, on either side above the breasts about two inches from middle line, at the insertion of the zypoid cartilage, and on the left and right hypochondriac regions; on the back immediately below the shoulder blades, in the lumbar region about an inch and a half on either side of the spinous processes, and points half-way between these points; on the legs below the patella, points half-way between the ankle and the knee on both the inner and outer aspects, and on the middle of the arch of the foot.

The determination of the pain thresholds was made with an instrument similar to the well-known Cattell algometer. The area of stimulation was approximately 2.5 sq. mm. The measurements of pain threshold were always made after those on touch and some of the points corresponded. Only one determination was made each day. The following are the points selected for the experiments on pain:

TABLE I.

Results of surface temperatures, capillary blood pressure, pain and touch thresholds. Where two figures are given opposite a date the upper one refers to the average for the right side, the lower for the left side. The days on which headaches were present are starred.

Averages.	Temperature.		Capillary blood pressure.		Pain threshold.			Touch threshold.			
	Temple.	Head.	Hand.	Head.	Head.	Hand and arm.	Chest and back.	Head.	Hand and arm.	Leg.	Chest, abdomen, and back.
No. of exp.	1	3	2	2	2	5	7	7	12	8	11
April											
26 A. M. *	95.9	175	213	213	365	365	312	1.00
	96.4	142	138	138	343	343	312	1.00
27 *	96.8	217	119	119	335	335	273	1.14	5.62	11.75	6.91
	96.0	238	175	175	360	360	273	1.00	3.38	6.63	6.09
28	171	212	212	477	477	336	1.00	5.69	12.13	13.00
	125	182	182	427	427	336	1.00	2.46	14.38	8.27
	96.0	137	206	206	470	470	320	1.00	9.54	10.63	6.18
29	96.8	176	188	188	433	433	320	1.00	4.54	11.75	10.73
	96.7	187	150	150	530	530	348	1.43	6.31	10.13	14.64
30	97.8	200	193	193	390	390	348	1.00	4.31	11.50	10.36
May											
1	95.8	179	194	194	553	553	479	1.00	4.69
	97.4	187	200	200	450	450	479	1.00	4.08
	96.4	217	200	200	545	545	473	1.00	3.31
2	97.6	212	219	219	493	493	473	1.00	2.84
	95.9	204	94	94	605	605	491	1.00	4.46
3	97.1	179	119	119	553	553	491	1.14	4.38
	96.8	229	163	163	607	607	484	1.14	8.00
4 A. M.	97.9	225	131	131	583	583	484	1.00	4.85
	95.8	237	163	163	523	523	1.14	4.16
P. M. *	97.3	267	119	119	425	425	1.29	4.84

on the head on either side about one inch from the middle line and about an inch above the orbit, and on either side about an inch above the centre of the zygomatic arch, on the hands and arms on the volar surface about two inches from the bend of the elbow, on the thenar and hyperthenar eminences, and at the end of the index and ring fingers; on the chest on either side above the breasts about two inches from the middle line, and at the insertion of the zyphoid cartilage; on the back immediately below the shoulder blades and in the lumbar region about an inch and a half on either side of the spinous processes.

An examination of the results given in Table I, page 24, shows that there was no regular difference between the thresholds of touch on the days on which headaches were present and those on which no headaches were felt. On the head and face at the time of the headaches there was no change in the sensory appreciation for these light pressures. Any difference that would be present at the times of headaches should be more noticeable, I judge, on the head and face than on other parts of the body, and the results of the numerous tests are negative.

It is noticed that the pain thresholds differ considerably at the times of headache from those immediately before and those immediately after. The rise in the threshold for pain is a characteristic one, which is found in all normal people, the rise being more as more experiments are performed, and it is noticeable that the pressure needed to cause pain at the beginning of the second visit to the hospital was higher than it had been at the end of her first period. On the days and times when headaches were present there is a drop in the amount of pressure needed to cause the ache. This result is not what would be expected, for in normal people when we have at the time of the determination of pain threshold other concomitant sensations, we find there is a decided increase in the amount of pressure needed to be appreciated as pain, and the results here are, therefore, the more striking in that the patient did appreciate as pain much less pressure than in her otherwise normal condition. At each time the headache occurred there was a noticeable drop in the curve for the appreciation of pain. These results indicate that at the time headaches are present there are other sensory or nervous changes taking place in the body, and it would seem that these results would support the hypothesis of an epileptoid nature of migraine. It was said above that the results of the touch experiments were negative.

It should be noted, however, that there was no increase in the amount of pressure necessary to cause a sensation when headaches were present as compared with the normal times. With the concomitant pains of the headache we should expect such an increase. The touch results agree, therefore, with those of pain in that, although not decreased, they were not increased. These results, however, will be considered in connection with all others in a later paragraph of the article.

RESULTS OF EXAMINATION OF URINE IN CASE OF MIGRAINE
BY OTTO FOLIN.

Tables II and III give the results of the two feeding experiments obtained from the patient under the influence of the uniform and practically protein free diets, which I have exclusively used for metabolism experiments with normal persons.¹ Whether the figures of the two periods taken as a whole are compared with similar experiments obtained from normal persons, or the figures of the "headache" days with those of the other days of these two experiments, we find that the results are well within the normal limits. I conclude that in the case of this patient, at least, migraine is not associated with any demonstrable metabolism disorder.

In view of the positive though unsubstantiated statements occasionally met with in the literature concerning the "metabolism in migraine," it would seem worth while to record the definite data contained in Tables II and III.

The occurrence of the headaches has been recorded by the patient in her diaries, and these were available from the beginning of 1901 until her entrance to the hospital in May, 1906. This material I have carefully collated, and from it I have been able to determine the periodicity of the headaches in relation to menstruation and to the different days of the week. During this period of five years and a half three periods need to be excluded to make the results as uniform as they can be made. These periods are: first, the period from August 18 to October 19, 1902, at which time the patient was pregnant and had a miscarriage, the result of operation for fibroid, which has been previously mentioned; the second period is that of a pregnancy from September 12, 1903, to June 10, 1904, when

¹ See this journal, 1905, xiii, pp. 45-138.

TABLE II a.
 Diet : 350 gm. starch, 350 c.c. cream, 6 gm. NaCl. Weight of subject : April 26, 94.5 lb. ; May 4, 94.7 lb.

Date 1906.	Spec n gravity.	Volume. c.c.	Total acidity.	Total nitrogen.	Urea nitrogen.	Kreatinin nitrogen.	Uric acid nitrogen.	Ammonia nitrogen.	Undetermined nitrogen.	Nitrogen.				
										Urea N. per cent.	Kreati- nin N. per cent.	Uric acid N. per cent.	Ammonia N. per cent.	Undeter- mined N. per cent.
April 26	12.0	1520	225	5.49	4.20	0.31	0.10	0.29	0.59	76.5	5.5	1.8	5.3	10.9
Headache. " 27	12.0	1080	112	3.11	1.97	0.33	0.07	0.27	0.47	63.3	10.6	2.3	8.7	15.1
" 28	12.0	1020	183	3.59	2.64	0.32	0.08	0.35	0.20	73.4	9.0	2.2	9.9	5.5
" 29	13.0	905	152	3.23	2.33	0.32	0.07	0.31	0.20	72.1	10.0	2.3	9.5	6.1
" 30	10.5	1300	125	3.60	2.60	0.31	0.07	0.30	0.32	72.2	8.5	1.8	8.5	9.0
May 1	9.0	1450	127	3.09	2.16	0.32	0.08	0.29	0.24	70.0	10.4	2.3	9.3	8.0
" 2	10.0	1190	138	2.91	2.04	0.32	0.09	0.28	0.18	70.1	11.0	3.2	9.6	6.1
" 3	11.5	1005	137	2.71	1.79	0.32	0.08	0.28	0.24	66.0	11.8	2.8	10.4	9.0

the patient was delivered of a healthy boy; the third period was that of lactation, which lasted from June 11 to August 30, 1904. It should be stated that menstruation began on August 31, but the patient nursed her child for two months longer.

TABLE II b.

Date 1906.	Total sulphur as SO ₃ .	Inorganic SO ₃ .	Ethereal SO ₃ .	Neutral SO ₃ .	Total sulphur.			
					Inorganic SO ₃ .	Ethereal SO ₃ .	Neutral SO ₃ .	
April Headache.	26	0.86	0.55	0.06	0.25	per cent. 63.5	per cent. 7.5	per cent. 29.0
	27	0.61	0.35	0.05	0.21	57.2	9.0	33.8
	28	0.66	0.39	0.06	0.21	58.8	9.8	31.4
	29	0.59	0.34	0.06	0.19	58.0	9.6	32.4
	30	0.64	0.35	0.07	0.22	55.5	10.5	34.0
May	1	0.57	0.30	0.06	0.21	52.7	11.2	36.1
	2	0.59	0.33	0.06	0.20	55.2	10.9	33.9
	3	0.59	0.32	0.07	0.20	54.2	12.1	33.7

In the accompanying table will be found the number of days on which the headaches occurred at different times during the menstrual period, and the total number of days for the same period. The number of days on which the headaches began is also indicated, in addition to the total number of days on which the headaches were present. These figures I have grouped in other ways for the menstrual month, showing the number of headaches which came on during the menstrual period, which may be counted as the time during the flow and possibly the day preceding. The menstrual flow usually lasted four days. This gives, then, a period of five days of headaches, during what we may call the menstrual period, and the rest of the month I have divided similarly. It is noticeable that in both tables there are two periods during the month in which the percentage of headaches is much greater than at other times. These periods are at and during menstruation and at the midmenstrual period. This periodicity is quite marked, particularly when the results are grouped, as they are in the second table, for three days together. This periodicity suggests the conclusion that the migraine may be due to a periodic

TABLE III a.

Diet: 350 gm. starch, 350 c.c cream, 6 gm. NaCl. Weight of subject: May 17, 94.5 lb.; May 26, 94 lb.

Date 1906.	Specific gravity.	Volume.	Total acidity.	Total nitrogen.	Urea nitrogen.	Kreatinin nitrogen.	Uric acid nitrogen.	Ammonia nitrogen.	Undetermined nitrogen.	Nitrogen.				
										Urea N. per cent.	Kreatinin N. per cent.	Uric acid N. per cent.	Ammonia N. per cent.	Undeter- mined N. per cent.
May 17	8.5	1910	92	4.67	0.29	0.16	per cent.	per cent.	per cent.	per cent.	per cent.
18	11.0	950	144	2.97	1.90	0.26	0.09	0.19	0.53	64.0	8.7	2.9	6.4	18.0
19	12.5	1105	141	3.42	2.57	0.28	0.10	0.21	0.26	75.3	8.1	2.8	6.1	7.7
20	11.0	1145	128	2.95	2.07	0.28	0.09	0.25	0.26	70.2	9.6	2.9	8.4	8.9
21	10.0	1260	151	2.82	1.80	0.29	0.09	0.26	0.38	63.9	10.4	3.1	9.2	13.4
22	9.5	1240	154	2.40	1.47	0.29	0.08	0.26	0.30	61.4	12.2	3.2	11.1	12.1
Headache.	23	10.5	146	2.86	1.86	0.26	0.10	0.27	0.37	65.0	9.2	3.4	9.4	13.0
25	9.0	1090	122	2.55	1.67	0.27	0.06	0.29	0.26	65.4	10.9	2.5	11.2	10.0

discharge similar to the discharge of a motor nature, which is found in true epilepsy. This is one of the theories which has been extensively spread and which is held by many to-day. This hypothesis is not supported by the occurrence of the headaches during the period of pregnancy. During the nine months there were twenty-five days on which headaches were present, there being 15 attacks. During

TABLE III b.

Date 1906.	Total sulphur as SO ₃ .	Inorganic SO ₃ .	Ethereal SO ₃ .	Neutral SO ₃ .	Total sulphur.		
					Inorganic SO ₃ .	Ethereal SO ₃ .	Neutral SO ₃ .
May					per cent.	per cent.	per cent.
17	0.65	0.39	0.05	0.21	60.2	7.2	32.6
18	0.60	0.35	0.07	0.18	58.5	11.6	29.9
19	0.58	0.34	0.06	0.18	59.0	10.8	30.2
20	0.54	0.31	0.06	0.17	57.0	10.6	32.4
21	0.54	0.27	0.05	0.22	48.9	10.0	41.1
22	0.51	0.27	0.06	0.18	53.4	11.3	35.3
Headache.	23	0.55	0.28	0.05	51.2	9.0	39.8
	24	0.54	0.27	0.07	51.1	12.1	36.8
	25	0.53	0.27	0.07	50.8	12.7	36.5

the preceding nine months there were 32 attacks on forty-three days, and in the nine months following pregnancy and the period of lactation there were 38 attacks which occurred and lasted over sixty-seven days.

When the results are considered from the standpoint of weekly occurrence, or rather of the occurrence on days of the week, there seems to be little difference, although there is a slight increase on Mondays and Thursdays. The hypothesis of fatigue, which has been advanced to explain the occurrence of the headaches, would, it seems to me, not explain the greater number of headaches which occur on Monday. One might expect an increase in the number of those appearing on Thursday, Friday, and Saturday, and in fact at the end of the week this should be much more marked than at any other time. It would seem likely that on Sunday, after the work of the remaining six days, there would be also a marked increase in number, but this is

TABLE IV.

Comparison of occurrence of headaches with the days of the menstrual month. The figures have been calculated on the basis of an average menstrual period of twenty-six days. Forty-four possible occurrences for each serial day.

SERIAL DAYS OF MENSTRUAL MONTH.																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
NUMBER OF HEADACHES BEGINNING EACH DAY.																											
9	17	6	6	6	8	0	4	4	5	4	2	2	7	4	9	6	5	4	2	4	3	5	3	8	4	9	9
TOTAL NUMBER OF DAYS ON WHICH HEADACHES WERE PRESENT.																											
13	21	15	10	9	4	4	4	7	6	6	3	9	9	11	11	7	6	5	4	3	5	7	11	9	11	12	

not found to be the case. The hypothesis of fatiguability, it seems to me, is therefore not supported by the data which have been obtained

TABLE V.

Comparison of occurrence of headaches with groups of days of the menstrual month. Five days in each group, with the exception of the fourth group, which includes results of six days.

PERIODS OF THE MENSTRUAL MONTH, DAYS.				
26 and 1 to 4	5 to 9	10 to 14	15 to 21	21 to 25
NUMBER OF HEADACHES BEGINNING IN EACH PERIOD.				
47	21	24	24	29
TOTAL NUMBER OF DAYS ON WHICH HEADACHES WERE PRESENT.				
71	30	38	36	43

TABLE VI.

Comparison of occurrence of headaches with three-day grouping of menstrual month. The third and seventh groups are totals for four days.

DAYS OF THE MENSTRUAL MONTH.							
26, 1, 2.	3, 4, 5.	6, 7, 8, 9.	10, 11, 12.	13, 14, 15.	16, 17, 18.	19, 20, 21, 22.	23, 24, 25.
NUMBER OF HEADACHES BEGINNING IN EACH PERIOD.							
35	20	13	11	19	11	15	21
TOTAL NUMBER OF DAYS ON WHICH HEADACHES WERE PRESENT.							
46	34	21	18	31	18	19	35

in this case. The occurrence of menstruation on the different days of the week was also calculated, and a comparison made with the headaches appearing on those days. After subtracting from the total number of headaches on the different days those associated

with menstruation, the number of headaches on different days of the week approached more nearly in number to each other. The differences, therefore, which are noted in the table between Sunday and Monday, and between Sunday, Tuesday, and Wednesday, and Monday,

TABLE VII.

Comparison of occurrence of headaches with days of the week.

NUMBER OF HEADACHES BEGINNING EACH DAY.						
Sunday.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
24	36	29	32	39	38	36
TOTAL NUMBER OF DAYS ON WHICH HEADACHES WERE PRESENT.						
41	52	50	49	48	60	53

Thursday, Friday, and Saturday, are more likely due to the occurrence of menstruation on certain days more often than on others. Table VII gives in detail the calculations obtained from the diary of the patient of the occurrence of the headaches in accordance with the days of the week.

Taking the results as a whole, several facts stand out clearly. Firstly, it is apparent that the headaches come on regularly, their appearance depending most probably upon menstruation, and upon a secondary change which takes place at the middle of the menstrual month. Secondly, the metabolism experiments show that the patient was perfectly normal so far as these chemical experiments can show the internal working of the body by means of urinary secretions. Thirdly, the blood pressure was high, and particularly so at the times when an attack was present. Fourthly, there was an evident sensory derangement which did not show itself, so far as the sensibility to touch is concerned, but was evident in a decreased pain threshold, *i. e.* at times of a headache less pressure was needed to cause pain.

From the metabolism studies the conclusions are negative, and the Scotch verdict of "not proven" may be entered. By this we mean that the studies which have so far been done do not indicate any great change, or in fact no change; that in this case the principal constit-

uents of the urine do not differ from those in people who are not troubled with headaches and are otherwise perfectly healthy.

The blood-pressure change might account for the headaches, and this hypothesis is supported by the experiments, the results of which are given above. The changes in the blood pressure may, however, be the result of not only the action of the heart and of the general vasomotor system, but they may be the result, as Sir Lauder Brunton says, of a local spasm of the vaso-constrictor muscles in the vascular system. Against this latter view, however, is the fact that the blood pressure in this case was high, not only about the head but also at the periphery of the body, namely, in the hands. A further fact against the consideration of blood-pressure increase as a causative factor in the headaches, is the fact that when these occur during menstruation, tamponing, with its resultant depletion and lowered blood pressure, neither caused the headaches to disappear nor decreased their severity. At the same time other depletion treatment (*e. g.*, purgatives) had no effect.

The results of the sensory tests are difficult to explain. An examination of the table will show that in this case, as in normal people, there is a tendency for the pain threshold to become higher as the number of experiments increase. In this case, at the times of headaches, the pain threshold was lowered. It is also a fact that in normal people the pain threshold is higher when there are other sensations from, or stimulations at other parts of the body. If this last statement can be applied to the conditions of migraine, we should expect to find at the time of the headache that the pain threshold on other parts of the body would be much higher than at other times, but the reverse, however, is the case. This change in sensory appreciation is different from what might be expected from concomitant sensations and from the general rise in pain threshold, may also be an indication of the sensory character of an epileptic condition in this disease.

This latter hypothesis is further supported by the occurrence of the headaches at approximately regular intervals, these intervals depending upon the appearance of the catamenia, the greatest number of headaches appearing during the menstrual flow and immediately before, there being then a decrease in the number for the next week and an increase at the midmenstrual period, followed by a second decrease. On the other hand, we would not say that because there is a periodicity in other functions of the body this is a sign of an epileptoid condition. The appearance of the catamenia, *e. g.*, at reg-

ular intervals may be due to some sort of a nervous discharge, but this is a perfectly normal condition, and we know that in some cases the menses occur a second time during the month, and sometimes to such a slight extent as to amount to only a stain, never becoming a regular flow.

For treatment at the hospital comparatively little was done. Once an attempt was made to lower the blood pressure by a long-continued warm bath, but owing to a misunderstanding on the part of the nurse, orders were not carried out properly, and although there was an apparent improvement at first, this did not continue, and after the bath the patient was no better than before. After leaving the hospital the patient tried the same sort of treatment, carrying it out as directed, namely, with a cold compress on head and around the neck, but no effect was noticed. At this time a bath of 102° F. was given for an hour. It would seem, therefore, that the lowering of blood pressure in itself does not bring about the cessation of the hemicrania, and it is probable that the aches are therefore not due exclusively to vasomotor changes.

Cannabis Indica in large doses had no effect upon the severity or number of headaches. At one time the patient was given phenacetine, 10 grains, and bromides, 30 grains, every four hours at the beginning of an attack, and continuing until the symptoms had disappeared. This treatment at first seemed to be efficacious, but later it lost its efficiency. Bromides alone were tried, from 30 to 60 grains every four hours, with absolutely no effect. The fact that bromides did not influence the ache would be an argument against the epileptic character of the condition.

Since leaving the hospital the patient has been put on thyroid treatment, on the chance that this might have some effect. This has now been continued for a period of three months, with a considerable amelioration in severity and a decrease in the number of headaches. The action of the thyroid extract, or dried gland, we know, is twofold (disregarding the more obscure action of the glandular secretion). In the first place, it increases the metabolism, particularly the metabolism of the proteid constituents of the body, and it also is a very effective blood-pressure lowering agent. The metabolism experiments conducted by Dr. Folin did not show any difference to a perfectly normal person, and lacking the further examination, we are not able to say that the thyroid treatment has been beneficial in producing the elimination of toxins, or by bettering in general the bodily

metabolism. When the thyroid treatment is considered in connection with the results of the prolonged warm baths, it is impossible to state definitely, and in fact it seems doubtful, that the whole effect of the thyroid treatment has been due to the lowering of blood pressure. This thyroid treatment has been given by Dr. W. A. Lane, of Milton, Mass., to whom I am indebted for these last few notes.

Summarizing the results and conclusions, I would say that the great necessity at the present time in the treatment of migraine was rather a study of the physiological conditions of the patient to determine the changes which are concomitant with the migraine rather than attempting so much experimentation on the therapeutic side. Moebius gives a list of drugs which have been found successful in individual cases, but he seems to know comparatively little of what is going on in the body beyond the knowledge of the headache. It seems probable, to me at least, that these hemicranias or migraines (rather than migraine) may be brought about, or due in individual cases, to a great variety of disturbances. Headache in itself is usually only a symptom of some physiological change in other parts of the body, and in the case of migraine I am inclined to think that it is only an exaggeration of what most of us call headache. If it is an exaggeration, and if it is only a symptom of some profound temporary or permanent physiological change, we should expect and we should find, as we do, that treatment for eye-strain, for dyspepsia, for constipation, for diseases of the nose and throat, for middle-ear disease, for uterine growths and displacements, etc., would bring about a cessation of the ache. The occurrence of the headaches at the menstrual period in the patient with whom we have worked is, I believe, only a symptom of the great bodily changes which take place at these periods, rather than the evidence of an epileptic or epileptoid constitution. The action of the thyroid in this case is difficult to understand. It seems to be doing much good. It has not changed, however, the pulse rate, and the temperature has kept normal. The blood pressure is lessened considerably, for whereas formerly she had a constant and usually hard pulse, the pulse is now quite soft. This treatment, it should be remarked, has been continued since the middle of August, and during three months the patient has had eleven attacks, which have been of less severity than at any time, with the exception of her period of pregnancy, for the past four years.

Since writing the above the patient has sent me an account of her

headache attacks from November, 1906, to April 15, 1907. She feels that the thyroid is doing her good, and thinks that during a recent period of six weeks in which she did not take this treatment she had more severe headaches than for a long time previously. The severity of the aches I have no means of judging, and the patient is the only one that has any standard (although this is not a measurable one) for their estimation in this respect. It is of interest that during the nine months of the present treatment, from August, 1906, to April, 1907, she had thirty-six headaches which extended over a period of fifty-five days. During this time there was a period of about six weeks in which no thyroid was taken, and the number may be larger than if the treatment had been continued over the whole period. This number of attacks, and the total number of days on which the aches were present are to be compared with the nine-month periods before, during, and after pregnancy. It will be seen that the number of attacks and the number of days on which the aches were apparent are greater during the last nine months than either the similar period of time before pregnancy, or the period of pregnancy, and that the numbers are only slightly less than during the nine months succeeding pregnancy and lactation. It is sure, therefore, that we have not found in the use of thyroid extract the much desired remedy, and even in view of the patient's statement regarding the difference in the severity of the attacks I am inclined to be sceptical regarding its value.

GALVANOTAXIS IN LARVÆ OF 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.]

ALTHOUGH a study of the galvanotactic reactions of organisms can aid but slightly, if at all, in furthering the more general problems of animal behavior, this subject, nevertheless, has an important bearing upon the fundamental problem of irritability, and possesses, in addition, an individual interest with respect to the polar excitation of animals by the electric current. The fact that, from the point of view of animal behavior, galvanotaxis is outside the pale of natural reaction to common stimuli, is no doubt a certain explanation of the circumstance that galvanotactic reaction has been made the subject of investigation in so small a number of animal forms. While the reactions of the Protozoa and Coelenterata to the galvanic current have received no little attention from students of animal behavior, comparatively little is known regarding the reactions of higher forms, especially the Crustacea, to this kind of stimulation.

The following account is presented in order to demonstrate some points regarding the reactions of the American lobster, *Homarus Americanus*, in the larval stages, to the galvanic current. This study was a subject of but incidental interest while more detailed investigations were in progress upon the reactions to light of the lobster larvæ. Indeed, only a beginning has been made in this study of galvanotaxis, and, although no far-reaching conclusions can be drawn from the following observations, they are presented tentatively, and with the hope that more substantial additions may be made at a later date.

Regarding the methods and technique it may be said, first, that the experiments to be reported do not include a study of orientation to a long-continued constant current but to an interrupted current.

For this reason the results may be excluded by those writers who assume that true galvanotaxis is dependent only upon a continuous and constant current. It is quite probable, however, that there is no great difference between the types of reaction as determined by interrupted or by constant currents. As Bancroft¹ (1905) has remarked, if the alternations are rapid enough (or, it might be added, the periods of stimulation long enough), the effect of the interrupted current may differ in no way from that of continuous stimulation. In the present instance the current was taken from two to four dry cells coupled in parallel circuit, and was seldom permitted to act for more than four to six seconds continuously. The exact strength of the current in the circuit was never known, but in the majority of instances it was the weakest stimulation that would produce a reaction on the part of the larvæ. Therefore the results are *qualitative* rather than *quantitative*. The larvæ were usually confined in a rectangular area, formed between two vertical glass slides which were placed parallel to and about one inch distant from each other within a crystallization dish 10 cm. in diameter. For electrodes, either the boot electrodes or merely strips of zinc wound with cotton were used. In the majority of cases the electrodes were placed about 7.5 cm. apart, at opposite ends of the rectangular compartment, while the larvæ were introduced in the rectangular area formed by the two glass slides and the sides of the dish, and between the two electrodes. The current was made, broken, or reversed by means of a rocking key.

When, in the course of experiment, the current had been sufficiently increased to bring about a reaction, the larvæ would group themselves at the positive pole, the anode. If now the current was increased beyond this threshold of stimulation, a more rapid orientation resulted, while, if the current was increased still further, the progressive orientation (in such cases as it would naturally occur) was more or less prevented by the production of a rigor that was apparently due to the excessive strength of the current. In the majority of instances, however, a few seconds of time was sufficient to allow the progressive orientation of a single larva to the positive pole. This reaction, moreover, was constant for larvæ of all stages and ages, in which respect the behavior is very different from the reaction of these same larvæ to light; since, in the latter case, as the writer

¹ BANCROFT, F. W.: University of California Publications, ii, p. 193; also Archiv für die gesammte Physiologie, 1905, cvii, pp. 535-556.

has shown elsewhere¹ (1907), the reaction may change from stage to stage, or even from day to day.²

The records of the few observations that were made in connection with the present investigation, may be presented in the form of *cases*, each one of which illustrates the reaction of the larvæ under certain conditions of body orientation with respect to the lines of electric current; for it was learned early in the course of experiment that progressive reaction to the current was entirely dependent upon the *position of the larvæ* in the water between the two electrodes. The assumption by the larvæ of the various body positions to be considered in this paper was not always under the immediate control of the experimenter; since for certain body orientations it was often necessary to wait many minutes, to watch carefully every movement of the larva, and then to make the current at the very instant the larva came into the desired position. In some cases, however, the directive influence of the light could be so manipulated that the larvæ would assume the desired body orientation, both to the light and to the lines of the current to be applied.

PRELIMINARY EXPERIMENT.

Several first-stage larvæ, about two days old and reacting positively to light, were placed in a crystallization dish 10 cm. in diameter. Electrodes, made by winding cotton about two zinc strips, were connected with two dry cells and hung over the edge of the dish so that

¹ HADLEY, P. B.: Report of the Rhode Island Commission of Inland Fisheries for 1906.

² It will be recalled that the lobster passes through a metamorphosis of three larval stages before it approximates, in the fourth stage, to the adult structural type. The larvæ of the first three stages swim aimlessly in the water by means of the feathered exopodites of the thoracic appendages, which beat the water with short, vibratory strokes. While these are the natural means of swimming, the larvæ frequently display sudden backward movements which are caused by the rapid flexions of the abdomen. This method of progress is often made use of by the fourth and later stage lobsters, after the loss of those swimming appendages characteristic of the larval stages. In addition to the foregoing, the fourth stage lobsters (the surface-swimming stage, *par excellence*) possess still another means of locomotion. In this case the swimming is accomplished by means of the appendages of the abdominal segments. With the entrance to the fifth stage this surface-swimming tendency disappears, the young lobsters go to the bottom, where they burrow among the shells and rocks, and never again leave this environment for the surface waters.

they extended a half inch or more into the salt water. The larvæ were introduced and swam more or less indefinitely about the dish, but were for the most part oriented with the head away from the light, which fell at right angles to the direction of the current. When the larvæ were swimming at right angles to a straight line connecting the two electrodes, and across the lines of the current, the current was suddenly made. Sometimes there was no visible effect, but at other times the larvæ would undergo an irregular, "jerky" progression, usually with the tail or back foremost, toward the positive electrode. Usually the current was not allowed to act for more than a few seconds. It was observed in this connection, that the more frequently the current was made, and the longer that it was in effect, the greater the number of larvæ that would gather at the anode; so that, although all the larvæ were never grouped at this pole, yet many of them were continuously in progression toward it. The other larvæ, meanwhile, would be swimming at various angles between the electrodes, or would be quite outside the influence of the current at the sides of the receptacle.

When it was attempted to analyze the conditions of stimulation that caused the progressive movement of some larvæ toward the anode, but not of others which were apparently acted upon in the same manner and by the same stimulus, it appeared that the character of the body orientation within the lines of force was an important factor. The cases now to be presented illustrate the reactions of larvæ of any stage or age as determined by the body orientation, and by the direction of the galvanic current; and represent the typical results of repeated trials.

Case 1: *Fig. 1.* — In this case a first stage larva was placed in the rectangular compartment formed by the glass slides placed vertically in the crystallization dish, as previously described. The light was then so manipulated that the larvæ became oriented with the back below, the longitudinal axis of the body parallel to a straight line connecting the two electrodes, and the head toward the anode. Then the current was made. Under these conditions of orientation, the larva underwent no appreciable change in position, save a slight extension of the abdomen. No increase in stimulation was able to force the larva, so oriented, either toward the positive or toward the negative pole. Sometimes, if the current was excessively strong, a rigor was produced, but never progression in any direction. When the current was broken, the larva oriented immediately to the light influence.

Case 2. — In this instance the larva was oriented, lying on its back, with the longitudinal axis of the body parallel to a straight line connecting the two electrodes, and with the head directed toward the cathode. When the current was made, the first apparent effect upon the larva was a strong flexion of the abdomen. This reaction was followed by a succession of rapid abdominal extensions and contractions. As a result of these movements (which will be considered more in detail later), the larva approached the anode. When the current was broken, the larva oriented, as in Case 1, to the directive influence of the light.

Case 3: *Fig. 2.* — The larva was oriented on one side, lying with the longitudinal axis of the body parallel to a straight line connecting the two elec-

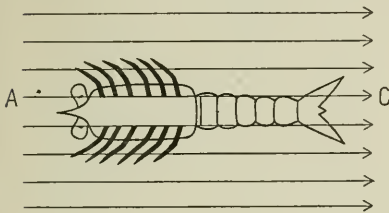


FIGURE 1.

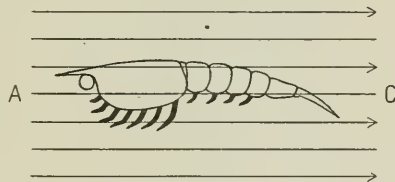


FIGURE 2.

trodes, and with the head toward the anode. Under these conditions of orientation, when the current was made, there first resulted an extension of the abdomen. This was sometimes, but not often, followed by a contraction. No change in the body orientation, however, nor any progressive orientation toward either the anode or the cathode was obtained while the larva was in this position.

Cases 4 and 5. — In these two cases, which may well be considered together, the larvæ were oriented with the back uppermost, with the longitudinal axis of the body parallel to a straight line connecting the two electrodes, and with the head directed toward the anode (Case 4), or toward the cathode (Case 5). The results, upon making the current when the larvæ were so oriented, were the same as in Cases 1 and 2, respectively: that is, there was no progression toward the anode in any case when the head was directed toward this pole,¹ but, on the other hand, progression toward the anode almost invariably resulted when the head was directed toward the cathode or negative pole.

Case 6: *Fig. 3.* — In this case the larva was oriented with the back above, and with the longitudinal axis of the body at right angles to a straight line connecting the two electrodes. When the current was made, no change in the body position of the larva took place, although a momentary extension of the abdomen, or a slight flexion of the appendages on the side of the body toward the anode, sometimes resulted.

¹ See exceptions to this rule noted under Case 10, p. 45.

Case 7: *Fig. 4.* — In this instance the larva was oriented on one side, lying with the longitudinal axis of the body at right angles to a straight line connecting the two electrodes, and with the back directed toward the cathode. When the current was made, under these conditions of orientation, the first noticeable effect was the greater extension of the abdomen of the larva, a slight flexion of the appendages on the side toward the anode, and, if the current became excessively strong, a rigor which prevented all movement. When the current was broken, the rigor was relaxed, only to

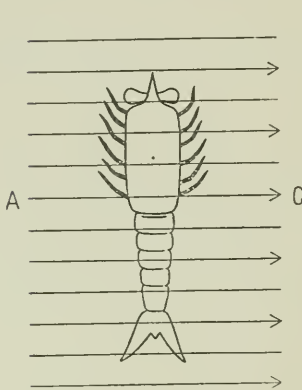


FIGURE 3.

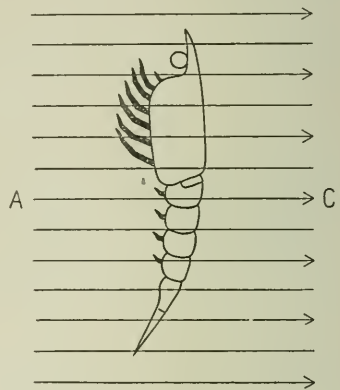


FIGURE 4.

appear again with each make of an excessively strong current. When the current was finally broken, the larva oriented to the directive influence of the light, and resumed the normal swimming position.

Case 8. — The larva was oriented on one side, lying with its longitudinal body axis at right angles to a straight line connecting the two electrodes, and with its back directed toward the anode (converse of Case 7, Fig. 4). When the current was made, the first noticeable effect was a further extension of the abdomen, followed immediately by a contraction; and then occurred a succession of abdominal extensions and contractions which were continued until the larva had, by these movements, arrived at the anode.

Case 9. — In this instance the larva was oriented with its back to the bottom, and with its longitudinal axis of the body at right angles to a straight line connecting the two electrodes. When the current was then made, no progressive orientation resulted, either toward the anode or toward the cathode. Frequently, however, the making of the current caused momentary and slight extensions of the abdomen, and in the appendages on the anodal side of the body; but these movements subsided as soon as the current was broken, and the larva at once regained the normal swimming position.

Case 10: *Fig. 5.* — In the following instances, the larvæ were oriented on the side, and lying obliquely to a straight line connecting the two electrodes. Under these conditions of orientation, the following results were usually observed: If the larva held such a position that the head was directed toward the anode, usually no reaction would occur; but if the head was directed toward the cathode, then progression toward the anode would usually take place. There were, however, certain exceptions to this general rule: First, if the larva was oriented as shown in *Fig. 5, A* (although the head was directed more fully to the anode than to the cathode), progression toward the anode usually resulted. But if the larva was oriented as shown in *Fig. 5, E'* (although the head was directed more fully to the cathode than to the anode), progression toward the anode would not take place. We may recapitulate in the following table the types of reaction which were found to take place when the larvæ were oriented on the side, and with the longitudinal axis of the body holding various relations to a straight line connecting the two electrodes. The positions designated *A, A', B, B', etc.*, are those noted in *Fig. 5.*



FIGURE 5

Following table the types of reaction which were found to take place when the larvæ were oriented on the side, and with the longitudinal axis of the body holding various relations to a straight line connecting the two electrodes. The positions designated *A, A', B, B', etc.*, are those noted in *Fig. 5.*

Orientation	Resulting Movement.
X	Progression toward anode
A	Progression toward anode
B	No change, or progression to anode
C	No change
D	No change
E	No change
Y	No change
E'	No change
D'	No change, or progression to anode
C'	Progression toward anode
B'	Progression toward anode
A'	Progression toward anode

Case 11. — We may conclude this consideration of the reactions of the lobsters to the galvanic current with an observation upon the behavior of the fourth stage larva. In this case a glass dish, 14 cm. in diameter, was employed. A cylindrical zinc roll, covered with cotton, and placed

upright near one side of the dish was used for one electrode, while for the other electrode a cotton covered zinc strip, hung over the rim of the glass dish, was employed. When several fourth stage lobsters were placed in the dish, the general course of swimming, when no current was active, was in a circle about the zinc roll electrode. Whenever one of the lobsters, swimming in the direction mentioned above, attempted to cross the region traversed by the current, there resulted, by means of the abdominal flexions, a sudden backward jump, which carried the lobster out of the region of the current. Four or five attempts to cross the lines of the current were sometimes made before the lobster pursued some other course. When, however, the lobster swam slowly, as if fatigued, into the lines of the current, he did not invariably "pull out" backward, but would often turn the head toward the cathode, and, by means of the characteristic abdominal flexions, approach the anode. If the current was made while the lobster was between the poles, and oriented with the tail to the anode, then there usually occurred a sudden darting motion backward to the positive electrode.

THE METHOD OF ORIENTATION.

Whether the larval lobsters react to the weaker constant current, maintained for long periods of time, we are not yet able to state. It is known, however, that they undergo a definite progressive orientation at the anode when stimulated for brief periods of time by the current from two cells. The mechanics of this reaction were quite different from those of the progressive orientation to light. In the latter case the orientation of the lobster larvæ was brought about by the movement of the exopodites or swimming branches of the thoracic appendages; no part either of the body orientation or of the progressive orientation was produced by the rapid and alternate contraction and extension of the abdomen, — unless it were in instances of very sudden light stimulation. In such cases the larvæ might suddenly dart backward, but this manner of locomotion was not common, and was not continued for long periods. Moreover, a gradual increase in the intensity of light never resulted in bringing about movements of this kind. In the case of galvanic stimulations, on the other hand, whether the strength of the current was increased gradually to the maximum, or whether the current was introduced suddenly, the manner of reaction usually, if not invariably, involved only the successive and oft-repeated extension and contraction of the abdomen. While this resultant reaction invariably took place if the

body orientation of the larvæ was favorable, it may be said that the galvanic current was seldom, perhaps never, the direct cause of body orientation.

DISCUSSION.

The results of these experiments naturally lead to a consideration of several interesting problems. Of these, the one of chief importance, perhaps, is that of polar excitation by the galvanic current, and the possibility of applying Pflüger's¹ (1859) law² to this type of reaction. Since the investigations of Kühne³ (1864) many investigators have given evidence to show that in the Protozoa, under galvanic stimulation, the excitation or contraction occurs at the anode, thereby representing a type of behavior which is little in accord with Pflüger's law of polar excitation. This view, however, is not substantiated by Jennings⁴ (1904), and is disputed by Bancroft⁵ (1905) and others, who attempt to demonstrate that, in the case of Paramecium, at least, the peculiar effect of the electric current is (except in the case of strong currents) confined to the cathode, and that it is the anode which is in the unstimulated or the less stimulated state. Therefore Bancroft⁵ (1905) concludes that the behavior of Paramecium under galvanic stimulation is quite in accordance with the requirements of Pflüger's law. As to the direction of movement of the organism under galvanic stimulation, we have somewhat contradictory evidence. Loeb and Budgett⁶ (1897) observed that Paramecia in weak salt solutions swam backward toward the anode. Jennings⁴ (1904) and Bancroft⁵ (1905), on the other hand, determined that the progression was usually toward the cathode, but that in strong currents it might be toward the anode. Several observers have described swimming toward the anode among the Crustacea. Nagel⁷ (1892,

¹ PFLÜGER, E.: Untersuchungen über die Physiologie des Electrotonus, Berlin, A. Hirschwald, 1859.

² PFLÜGER'S law of polar stimulation states that, during the passage of the galvanic current, the cathode is in a state of increased excitability, while the region of the anode is in a condition of diminished excitability.

³ KUHNE, W.: Untersuchungen über das Protoplasma, Leipzig, 1864, Englemann.

⁴ JENNINGS, H. S.: Journal of comparative neurology and psychology, 1904, xiv, pp. 442-514.

⁵ *Ibid.*

⁶ LOEB, J., and BUDGETT, S. P.: Archiv für die gesammte Physiologie, 1897, lxxv, pp. 518-535.

⁷ NAGEL, F. W.: Archiv für die gesammte Physiologie, 1892, li, pp. 624-631; also 1895, lix, pp. 603-642.

p. 629) reported that Cyclops and Asellus (1895, p. 633) swam toward the anode. Hermann¹ (1885) learned that tadpoles and salmon embryos were depressed by a descending or homodromic current, but were stimulated by an ascending or antidromic current. Blazius and Schweizer² (1892, p. 518) found that the crayfish was definitely galvanotactic, and went toward the anode. These investigators also showed that the descending current caused a depression of the reflexes, while the ascending current caused an increased irritability. Loeb and Maxwell³ (1896) also experimented with the crayfish, palemonetes, and other crustaceans, and found that the orientation was usually at the anode. These investigations, however, assumed that neither the stimulating effect of the ascending current nor the depressing effect of the descending current was observed when currents of medium intensity were used. In addition to these reports of galvanotaxis in the crayfish, which, because of its similarity in structure to the lobster, is of especial interest in the present consideration, Miller⁴ (1907) has given the results of a careful study of galvanotropism in this form, with reference to the points of present interest. This investigator states that the orientation of the crayfish is produced as a result of the stimulation of both the central and the peripheral nervous systems; furthermore, that, while the descending current depresses the irritability of the central nervous system, the ascending current leads to a greater stimulation. Orientation is usually at the anode.

It may be of advantage now to consider to what extent the behavior of the lobster larvæ agree with requirements of Pflüger's law of polar stimulation; and, furthermore, to observe the influence of ascending and descending currents upon the reaction of the lobster larvæ. Here, obviously enough, we are dealing with an organism whose propelling mechanism (for the galvanotactic reactions at least) is limited in position to one end of the body proper, and in which it is not difficult to determine whether the excitation is at the anodal or cathodal extremity. We have already noted in the previous pages that there seldom occurred a contraction at the negative pole of the

¹ HERMANN, L.: *Archiv für die gesammte Physiologie*, 1885, xxxvii, pp. 457-460.

² BLAZIUS, E., and SCHWEIZER, F.: *Archiv für die gesammte Physiologie*, 1892, liii, pp. 493-543.

³ LOEB, J., and MAXWELL, S. S.: *Archiv für die gesammte Physiologie*, 1896, lxiii, pp. 121-144.

⁴ MILLER, F. W.: *Journal of physiology*, 1907, xxxv, pp. 215-229.

animal, regardless of what its body orientation might be. This was shown, not only by the fact that when the "tail" was directed toward the cathode there was no contraction, with consequent orientation, but also by the fact that observation of the head appendages seldom revealed a sign of excitation either at making, breaking, or while the current was operative, if the head was directed toward the cathode.

We find, moreover, that when a fourth stage larva was fastened to a cotton-covered block and placed in the water so that the longitudinal axis of the body was at right angles to, and within the line of, the current which passed through the body transversely, then the making of the current caused a flexion of the appendages *only on the anodal side of the body* of the larva; furthermore, that breaking the current merely interrupted the tetanic contraction of the appendages on the anodal side.

With the current used, it was not observed that the limbs on the cathodal side of the body underwent extension as mentioned by Loeb and Maxwell¹ (1896) and Miller² (1907, p. 217) for the crayfish. Regarding the general behavior of the crayfish under galvanic stimulation, the report of Blasius and Schweizer³ (1893) indicates a close relation between *Astacus* and *Homarus*. To quote:

"Im absteigenden Strom (bei plötzlichem Schlusse und beispielsweise 15 MA pro qdm) bleibt er nach einer heftigen Schliessungszuckung ruhig sitzen und streckt seinen Schwanz fächerförmig aus. Trifft ihn dagegen der aufsteigende Strom, so schwimmt er mit Aufbietung aller Kräfte rückwärts, wobei er manchmal in wenigen Sekunden die ganze Länge (an 70 cm.) des Troges durchheilt. Um zu entscheiden, ob das Rückwärtsschwimmen nicht bloss eine Folge der Schliessung des aufsteigenden Stromes sei, stellten wir folgende Versuchsreihe an. Wir hielten den Krebs mit der Hand am Boden des Troges noch einige Sekunden über Schluss des Stromes (7-9 MA pro qdm) fest und liessen ihn dann sachte los, er bewegte sich sogleich rückwärts, bei schwächeren Strömen nur kriechend, bei stärkeren schnell und mit heftigen Schwimmbewegungen des Schwanzes."

But it appears that in the experience of these investigators currents of different strengths produced different effects, for they continue:

"Experimentirt man mit grosser Vorsicht, so zeigen auch die Krebse deutliche Erscheinungen von Electrotropismus. Man muss dazu den aufstei-

¹ MILLER, F. W.: *Journal of physiology*, 1907, xxxv, pp. 215-229.

² *Ibid.*

³ *Ibid.*

genden Strom sehr schwach (bei oder unter 4 MA pro qdm) verwenden und langsam ansteigen lassen. Dann gehen die Thiere häufig nicht wie bei stärkeren Strömen rückwärts, sondern drehen sich langsam um und bleiben im absteigenden Strom liegen, oder kriechen vorwärts."

These facts have been substantiated for adult specimens of *Astacus fluviatilis* by Miller¹ (1907), who found that the crayfish when oriented with head to the anode and stimulated by a weak current moved forward toward the positive pole; and that if the crayfish was not facing the anode at the make of the current, it would assume such a body orientation, and then move toward the anode as before. Miller states, furthermore, that when a stronger descending current is employed, the crayfish moves more rapidly toward the anode. But if the current is excessively strong, rigor is produced. If, however, a strong ascending current is passed through the crayfish, it moves rapidly backward by means of the repeated flexions of the abdomen, without having assumed the antidromic (head toward the anode) position. Miller states, further, that *Astacus* occasionally became oriented at the cathode, but attributes this irregularity to the influence of the products of electrolysis.

Owing to the great similarity in body form which exists between the adults of *Astacus* and *Homarus*, it is probable that a comparison of the reactions also would show many points of agreement. At the present time, however, no experiments have been attempted with the adults of *Homarus*. When we compare with these results the type of reaction found in the larvæ of *Homarus*, however, there are many points in common. In the latter case the descending current was found seldom to stimulate the larvæ to activity and sometimes even to depress the irritability. Ascending currents, on the other hand, invariably caused increased irritability, and consequent progressive orientation, by means of rapid flexions of the abdomen, to the positive pole or anode. Moreover, when the current passed transversely through the body of the larvæ, it was only on the anodal side that the muscular flexions were observable; and these were apparent in the contraction of even the abductor muscles in the dactyls of the claws. Regarding the production of a body orientation by means of the galvanic current, one point was particularly noteworthy: Observations on the reactions of lobsters of all stages and ages (at least to the seventh stage), failed to reveal the fact that any application of the current ever brought about a body orientation, as Miller¹ (1907, p. 217)

¹ MILLER, F. W.: Journal of physiology, 1907, xxxv. pp. 215-229.

has indicated was the case for the adults of *Astacus*. It may yet appear, however, that other modifications of the current, properly applied, will cause such an orientation for the larvæ and early adolescent stages of *Homarus*. It can only be said at this moment, that both the early stage larvæ (having the exopodites or swimming appendages) and the fourth and later stage lobsters (without the exopodites) undergo progressive orientation to the galvanic current in the same manner; and that this reaction is not in accord with the requirements of Pflüger's law.

There still remains one important point which has, unfortunately, not been included in the present investigation, but which the writer hopes to determine at a later date: namely, the influence of currents of different intensities upon the action of the anodal and cathodal exopodites of lobster larvæ of the first three stages.

SUMMARY.

1. Larval and early adolescent lobsters reacted in a very definite manner to the galvanic current by gathering at the anode.

2. Reaction to the current usually took place only when the larvæ were oriented with the tail or the back turned wholly or partly toward the anode (Fig. 5: *A, A', B', C, D', X*). When the body orientation, with respect to the lines of the current, was as shown by *B, C, D, E, E', Y*, no progressive orientation, in the majority of cases, took place.

3. The mechanics by which the progressive movement was brought about were the rapid extensions and contractions of the abdomen. These movements, which were produced by the ascending current only, served to impel the larvæ backward to the positive pole. The descending current did not have this effect, though it sometimes produced rigor.

4. Although the ascending electric current caused a progressive orientation to the anode if the longitudinal axis of the body came into proper relation with the lines of the current, yet the current was apparently never itself instrumental in determining the body orientation of the lobsters.

5. The reaction of the larval and early adolescent lobsters to the galvanic current does not accord with the requirements of Pflüger's law of polar stimulation and contraction.

The present work was done at the Biological Laboratory of Brown University and at the Wickford Experiment Station of the Rhode Island Fish Commission. Thanks are due Dr. A. D. Mead for the opportunity of carrying on this investigation, and to Dr. H. E. Walter for assistance during the preparation of the present paper.

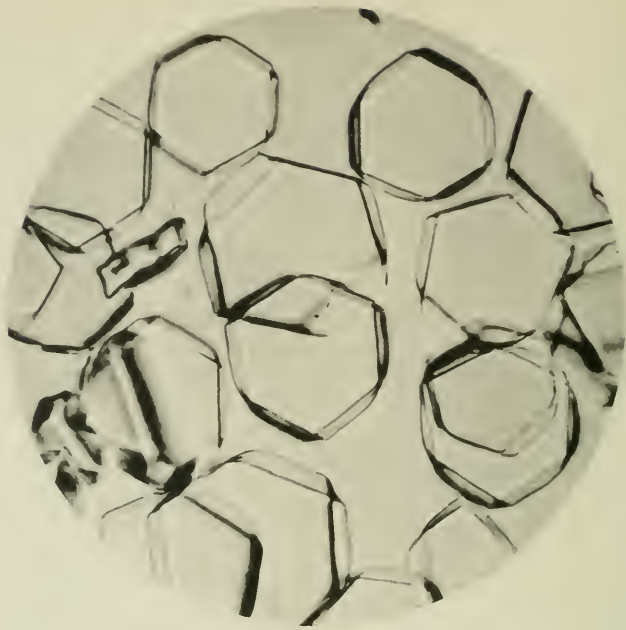


FIG. 1.

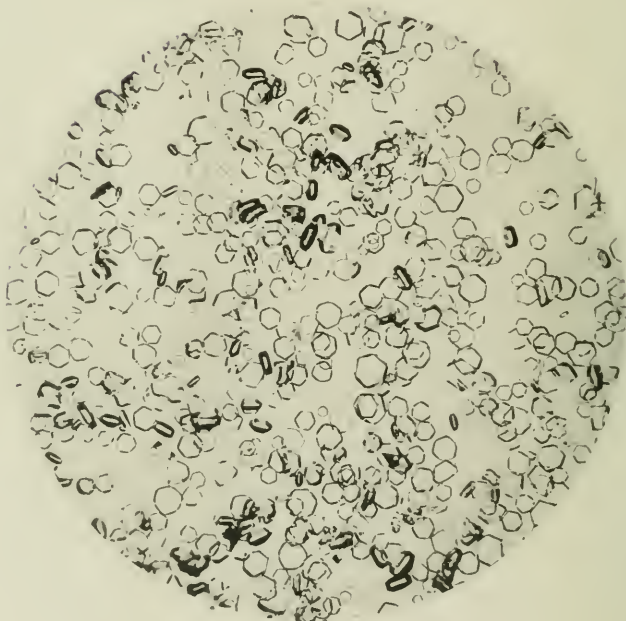


FIG. 2.

HYDROLYSIS OF EXCELSIN.¹

BY THOMAS B. OSBORNE AND S. H. CLAPP.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

THE greater part of the protein substance of the Brazil-nut (*Bertholletia excelsa*) consists of the globulin excelsin which may be obtained in beautiful hexagonal crystals when the protein separates slowly from solution. These crystals have no effect on polarized light, and, as Maschke² states, "undoubtedly belong to the regular system." The same form would result if an octahedron were cut parallel to two opposite faces. As it is possible to obtain a large quantity of this globulin in a perfectly homogeneous crystallized condition, and as excelsin is also precipitated by ammonium sulphate,³ between comparatively narrow limits, the opportunity is presented of obtaining a protein preparation which offers a better guarantee of chemical individuality than do amorphous preparations of other proteins.

The results of this hydrolysis of excelsin, like that of crystallized oxyhæmoglobin of the horse blood made by Abderhalden,⁴ shows that excelsin yields as many amino acids as most of the other chemically less well defined protein preparations.

In making our preparation of excelsin for this hydrolysis great care was taken to obtain a product which consisted wholly of perfectly formed crystals. In the preparation of this excelsin we received valuable assistance from Mr. I. F. Harris, for which we here wish to express our thanks.

The character of the material used for the hydrolysis is best shown by the following microphotographs, for which we thank Prof. E. T. Reichert, of the University of Pennsylvania.

¹ The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

² MASCHKE : Journal für praktische Chemie, 1858, lxxiv, p. 436.

³ Cf. OSBORNE and HARRIS : This journal, 1905, xiii, p. 436.

⁴ ABDERHALDEN : Zeitschrift für physiologische Chemie, 1903, xxxvii, p. 484.

The oil-free meal of the Brazil-nut was extracted with 3 per cent ammonium sulphate solution, heated to 50°, and the perfectly clear extract dialyzed until the greater part of the dissolved excelsin was deposited as crystals. These were then washed thoroughly with dilute sodium chloride solution and then with dilute alcohol which was gradually increased in strength up to absolute alcohol. The preparation was then dried over sulphuric acid.

Of the perfectly crystallized excelsin thus prepared 500 gm., equal to 450 gm. water and ash-free, were suspended in a mixture of 500 c.c. of water and 500 c.c. of hydrochloric acid of specific gravity 1.19, and warmed in a bath of boiling water for two and one-half hours. The heating was then continued in a bath of oil and the hydrolysis solution boiled for eighteen hours.

The esterification of the hydrochlorides of the amino acids and the liberation and extraction of the free esters were then executed in the manner often described. The dark-colored ether extracts were dried in the usual manner with potassium carbonate and anhydrous sodium sulphate.

The aqueous layer was freed from inorganic salts in the usual way, and the esterification repeated. As the yield of ester was considerable, the whole process was again repeated, but this last treatment yielded very little of ether-soluble ester. After distilling off the ether on the water-bath the esters were fractioned under diminished pressure as follows:

Fraction	Temp. of bath up to	Pressure	Weight
I	51°	10 mm.	43.55 gm.
II	90°	10 "	32.77 "
III	105°	0.47 "	91.65 "
IV	130°	0.40 "	59.57 "
V	170°	0.38 "	56.83 "
Total			284.37 gm.

The undistilled residue weighed 132 gm.

Fraction I. — This fraction consisted largely of alcohol and ether. It yielded, after esterification with alcohol and hydrochloric acid, 2.98 gm. of glycooll ester hydrochloride, equivalent to 1.33 gm. of glycooll. The melting-point was 144°.

Nitrogen, 0.4044 gm. subst., required 4.19 c.c. 5/7 N HCl.

Chlorine, 0.4158 gm. subst., gave 0.4265 gm. AgCl.

Calculated for $C_4H_{10}O_2NCl = N$ 10.04; Cl 25.45 per cent.

Found = N 10.36; Cl 25.34 " "

The filtrate from the glycocoll, after freeing from chlorine, yielded 1.75 gm. of alanine.

Fraction II.— This fraction was saponified with boiling water, evaporated to dryness under reduced pressure, and the proline extracted with boiling absolute alcohol. The part insoluble in alcohol, esterified in the usual way, yielded 2.59 gm. of glycocoll ester hydrochloride of melting-point 144° , equivalent to 1.39 gm. of glycocoll.

The filtrate from the glycocoll was freed from chlorine and submitted to a systematic fractional crystallization. There were obtained 3.23 gm. of substance having the percentage composition of amino-valerianic acid and 8.74 gm. of alanine.

Carbon and hydrogen, 0.1717 gm. subst., gave 0.3238 gm. CO_2 and 0.1470 gm. H_2O .

Calculated for $\text{C}_5\text{H}_{11}\text{O}_2\text{N} = \text{C } 51.28$; $\text{H } 9.40$ per cent.

Found = $\text{C } 51.43$; $\text{H } 9.51$ " "

The needles of the alanine decomposed at 290° and gave the following analysis:

Carbon and hydrogen, 0.1425 gm. subst., gave 0.2118 gm. CO_2 and 0.1033 gm. H_2O .

Calculated for $\text{C}_3\text{H}_7\text{O}_2\text{N} = \text{C } 40.45$; $\text{H } 7.86$ per cent.

Found = $\text{C } 40.54$; $\text{H } 8.05$ " "

Fraction III.— This fraction was boiled with eight volumes of water for six hours, when the solution no longer reacted alkaline to litmus. The slightly colored solution was then evaporated to dryness under reduced pressure and the proline extracted with boiling absolute alcohol. From the part remaining undissolved there were obtained 39.15 gm. of leucine. The substance decomposed at about 298° .

Carbon and hydrogen, 0.1854 gm. subst., gave 0.3746 gm. CO_2 and 0.1647 gm. H_2O .

Calculated for $\text{C}_6\text{H}_{13}\text{O}_2\text{N} = \text{C } 54.96$; $\text{H } 9.92$ per cent.

Found = $\text{C } 55.10$; $\text{H } 9.87$ " "

The substance in the filtrate appeared to consist, to a large extent, of amino-valerianic acid, but its isolation from this fraction was so difficult that we were able to obtain only 0.98 gm. of substance which appeared to be essentially homogeneous.

Carbon and hydrogen, 0.2155 gm. subst., gave 0.4032 gm. CO₂ and 0.1862 gm. H₂O.

Calculated for C₅H₁₁O₂N = C 51.28; H 9.40 per cent.

Found = C 51.03; H 9.60 " "

The remainder of the preparation of crude amino-valerianic acid weighed 2.57 gm.¹

On recrystallizing from dilute alcohol it gave figures agreeing fairly well with the calculated.

Carbon and hydrogen, 0.1522 gm. subst., gave 0.2840 gm. CO₂ and 0.1286 gm. H₂O.

Calculated for C₅H₁₁O₂N = C 51.28; H 9.40 per cent.

Found = C 50.88; H 9.38 " "

We were, however, unable to arrive at a preparation of perfectly homogeneous appearance either by fractional crystallization of the copper salt or by racemizing. It is probable that the isolation of this substance was rendered more than usually difficult by the presence, in this fraction, of a slight amount of aspartic ester, which, as is well known, is not converted smoothly to the free acid on boiling with water.²

The alcohol soluble portions of fractions II and III consisted essentially of proline, of which there were obtained 16.42 gm. For separation of the active from the racemized, the copper salt was employed and 3.70 gm. of air-dried racemic proline copper and 17.52 gm. of the lævo salt, dried at 110°, was obtained in the usual way.

Water, 0.3902 gm. subst., lost 0.0429 gm. H₂O at 110°.

Copper, 0.1564 gm. subst., gave 0.0379 gm. CuO.

Calculated for C₁₀H₁₆O₄N₂Cu · 2 H₂O = H₂O 10.99; Cu 19.41 per cent.

Found = H₂O 10.99; Cu 19.36 " "

The phenylhydantoine of the lævo-proline melted at 143°.

Carbon and hydrogen, 0.2297 gm. subst., gave 0.5586 gm. CO₂ and 0.1174 gm. H₂O.

Calculated for C₁₂H₁₂O₂N₂ = C 66.67; H 5.56 per cent.

Found = C 66.32; H 5.67 " "

¹ We have included this weight in calculating the percentage of this substance yielded by excelsin.

² FISCHER, E.: Berichte der deutschen chemischen Gesellschaft, 1901, xxxiv p. 433.

Fraction IV. — From this fraction the ester of phenylalanine was shaken out with ether and saponified by warming with strong hydrochloric acid. There were isolated 6.74 gm. of phenylalanine as the hydrochloride.

Carbon and hydrogen, 0.2331 gm. subst., gave 0.5576 gm. CO₂ and 0.1431 gm. H₂O.

Calculated for C₉H₁₁O₂N = C 65.45 ; H 6.66 per cent.

Found = C 65.24 ; H 6.82 “ “

The aqueous layer after saponifying with baryta yielded 7.77 gm. of aspartic acid as the barium salt.

Carbon and hydrogen, 0.2159 gm. subst., gave 0.2868 gm. CO₂ and 0.1119 gm. H₂O.

Nitrogen, 0.2947 gm. subst., required 3.15 c.c. 5/7 N HCl.

Calculated for C₄H₇O₄N = C 36.09 ; H 5.26 ; N 10.53 per cent.

Found = C 36.22 ; H 5.75 ; N 10.68 “ “

The filtrate from barium aspartate, freed from barium, was saturated with hydrochloric acid gas. It separated no glutaminic acid hydrochloride at 0°. After freeing from chlorine, the remainder of the aspartic acid was separated as the copper salt. There were obtained 11.75 gm. of pure copper-aspartate, equivalent to 5.68 gm. of aspartic acid.

Nitrogen, 0.4321 gm. subst., required 2.28 c.c. 5/7 N HCl.

Copper, 0.4192 gm. subst., gave 0.1222 gm. CuO.

Calculated for C₄H₅O₄N Cu 4½ H₂O = Cu 23.07 ; N 5.08 per cent.

Found = Cu 23.29 ; N 5.27 “ “

In the filtrate from copper-aspartate nothing definite was isolated.

Fraction V. — This fraction was treated precisely as the foregoing. There were obtained 9.26 gm. of phenylalanine as the hydrochloride, 10.32 gm. of glutaminic acid as the barium salt and 9.41 gm. as the hydrochloride.

The glutaminic acid hydrochloride was converted to the free acid, which decomposed at 202°–203°.

Carbon and hydrogen, 0.2214 gm. subst., gave 0.3301 gm. CO₂ and 0.1265 gm. H₂O.

Calculated for C₅H₉O₄N = C 40.81 ; H 6.12 per cent.

Found = C 40.66 ; H 6.35 “ “

The filtrate from glutaminic acid hydrochloride yielded further 8.03 gm. of pure copper-aspartate, equivalent to 3.88 gm. of aspartic acid.

Serine appeared to be present in the filtrate from the copper-aspartate, but it was not isolated in a state of purity.

THE RESIDUE AFTER DISTILLATION.

The residue remaining in the bulb after the distillation of the esters weighed 132 gm. It was dissolved in boiling alcohol and, after cooling, filtered from the insoluble (wt. = 4.70 gm.). The filtrate was evaporated to dryness and saponified by warming with excess of baryta for eight hours. There were obtained 23.25 gm. of glutaminic acid hydrochloride, equivalent to 18.62 gm. of glutaminic acid. The free acid decomposed at $202^{\circ} - 203^{\circ}$.

Carbon and hydrogen, 0.2983 gm. subst., gave 0.4455 gm. CO_2 and 0.1739 gm. H_2O .

Calculated for $\text{C}_5\text{H}_9\text{O}_4\text{N} = \text{C } 40.81$; $\text{H } 6.12$ per cent.

Found = $\text{C } 40.73$; $\text{H } 6.47$ " "

This makes the total yield of glutaminic acid obtained by the ester method 38.32 gm. or 8.52 per cent of excelsin, which falls considerably below the 12.94 per cent obtained by Osborne and Gilbert¹ with the direct method.

CYSTINE.

Although excelsin contains about the same amount of total sulphur as gliadin, the amount of sulphur obtained as sulphide on boiling excelsin with strong sodium hydroxide solution is only one half that similarly obtained from gliadin.²

This smaller proportion of cystine may possibly explain our failure to isolate any of this substance from excelsin by the same process as that which readily yielded cystine when applied to gliadin.³ Cystine could not be obtained also from the crude tyrosine by means of mercuric sulphate, as in the case of glutenin.

TYROSINE.

One hundred gm. of excelsin, equal to 90.19 gm. water and ash-free excelsin, was boiled with 100 c.c. concentrated hydrochloric acid and 100 c.c. of water for six hours on the oil bath. After evaporating to a syrup and repeatedly evaporating with water under strongly

¹ OSBORNE and GILBERT: This journal, 1906, xv, p. 350.

² OSBORNE: Journal American Chemical Society, 1902, xxiv, p. 140.

³ OSBORNE and CLAPP: This journal, 1906, xvii, p. 231.

reduced pressure, the remainder of the hydrochloric acid was neutralized by an equivalent amount of sodium carbonate, as found by determining the amount of chlorine in an aliquot part of the solution. After concentrating to about 400 c.c. the substance that separated was filtered out and the filtrate further concentrated and a second separation obtained. These two separations were recrystallized from water and the product obtained dissolved in 5 per cent sulphuric acid and the solution treated with phosphotungstic acid. After removing the phosphotungstic precipitate the solution was freed from phosphotungstic and sulphuric acids and evaporated to dryness. The residue was extracted with boiling glacial acetic acid and the part that remained undissolved weighed 2.83 gm. equal to 3.03 per cent.

Nitrogen, 0.2930 gm. subst., dried at 100° , required 2.24 c.c. $5/7$ N HCl.

Calculated for $C_9H_{11}O_3N = N$ 7.73 per cent.

Found = N 7.65 " "

ARGININE.

Fifty gm. of excelsin, equal to 47.18 gm. water, and ash-free, were hydrolyzed, and the arginine determined, as directed by Kossel and Patten.¹ The solution of the arginine contained nitrogen equal to 7.49 gm. of arginine. Adding 0.072 gm. for the solubility of arginine silver in the solutions from which it was precipitated, we have 7.562 gm. arginine or 16.02 per cent.

10 c.c. solution required 4.83 c.c. $5/7$ N HCl = 0.0483 gm. N, or 2.415 gm. in 500 c.c. = 7.49 gm. arginine.

The arginine was converted into the nitrate.

Nitrogen, 0.2945 gm. subst, dried over H_2SO_4 , required 8.29 c.c. $5/7$ N HCl.

Calculated for $C_6H_{14}O_2N_4 \cdot HNO_3 \cdot \frac{1}{2} H_2O = N$ 28.46 per cent.

Found = N 28.18 " "

The arginine nitrate was then converted into arginine copper nitrate.

Water, 0.2848 gm. subst., lost 0.0264 gm. H_2O at 110° .

Copper, 0.0981 gm. subst., gave 0.0135 gm. CuO .

Calculated for $C_{12}H_{28}O_4N_8Cu (NO_3)_2 \cdot 3 H_2O = H_2O$ 9.16; CuO 10.79 per cent.

Found = H_2O 9.27; CuO 11.00 per cent.

¹ KOSSEL and PATTEN: Zeitschrift für physiologische Chemie, 1903, xxxviii, p. 39.

HISTIDINE.

The solution of the histidine, equal to 500 c.c. from 47.18 gm. of dry and ash-free excelsin contained nitrogen equal to 0.6945 gm. histidine or 1.47 per cent.

100 c.c. solution required 3.77 c.c. $5/7$ N HCl = 0.0377 gm. N in 100 c.c., or 0.1885 gm. in 500 c.c. = 0.6945 gm. histidine.

This histidine was converted into the dichloride which crystallized in the rhombohedral crystals characteristic of this salt. These decomposed at about 233°.

Chlorine, 0.0761 gm. subst., gave 0.0947 gm. AgCl.

Calculated for $C_6H_{11}O_2N_3Cl_2 = N$ 31.14 per cent.

Found = N 30.76 " "

LYSINE.

The lysine picrate obtained from 47.18 gm. of excelsin by the method of Kossel and Patten weighed 1.9884 gm. equal to 0.7725 gm. lysine picrate or 1.64 per cent.

Nitrogen, 0.3000 gm. subst., dried at 110°, required 5.60 c.c. $5/7$ N HCl (Kjeldahl-Jodlbauer).

Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_8O_7N_3 = N$ 18.70 per cent.

Found = N 18.66 " "

The results of this hydrolysis are given in the following table:

HYDROLYSIS OF EXCELSIN.			
	Per cent.		Per cent.
Glycocoll	0.60	Cystine	not found
Alanine	2.33	Oxyproline	" "
Amino-valerianic acid	1.51	Tyrosine	3.03
Leucine	8.70	Arginine	16.02
Proline	3.65	Histidine	1.47
Phenylalanine	3.55	Lysine	1.64
Aspartic acid	3.85	Ammonia	1.80
Glutaminic acid	12.94	Tryptophane	present
Serine	not found		
Total		Total	61.09

No striking feature is shown by the hydrolysis of excelsin beyond the unusually large proportion of arginine that was found.

FURTHER STUDIES OF THE PHYSICAL AND CHEMICAL RELATIONS BETWEEN FISHES AND THEIR SURROUNDING MEDIUM.

By FRANCIS B. SUMNER.

[A Contribution from the Biological Laboratory of the New York Aquarium and from the United States Fisheries Laboratory at Woods Hole, Mass.]

IN two former papers,¹ I have dealt with some of the effects upon fishes of changes in the density of their surrounding medium. It was shown that well-marked changes of weight resulted in certain cases from an increase or decrease in the salinity of the water, and that these changes, when they occurred, were such as might have been predicted on the assumption that we had to do with osmotic action through membranes approximating the "semi-permeable" type. It was shown, however, that such changes did not always ensue in cases where the laws of osmotic action alone would have led us to expect them. The phenomena were complicated by physiological and chemical factors of an obscure nature. It was shown likewise that well-marked changes in the salt content (as measured by the *chlorine* content) of the body resulted, in certain cases, from changes in the salinity of the water. The view was urged that one factor concerned in causing the death of salt water fishes in fresh water was the diffusion from their bodies of a portion of the salts present there. That the sudden decrease of osmotic pressure in passing from one medium to the other was not an adequate explanation of the fatal effects of fresh water was shown by the fact that even a very slight admixture of salt water was sufficient to diminish or completely annul these effects, even though the difference of osmotic pressure remained practically unchanged. Finally, it was shown that in the case of one fish at least (the carp) the membranes through which the abnormal medium (in this case *salt* water) acted, were those of the gills.

¹ Biological Bulletin, May, 1906; Bulletin of the Bureau of Fisheries for 1905 (separates issued May, 1906).

Further studies have been undertaken, partly with a view to amplifying some of the previous experiments, partly with a view to clearing up certain contradictions and anomalies. My thanks are due to Mr. C. H. Townsend, director of the New York Aquarium, for the abundant facilities which he placed at my disposal in carrying out the larger part of the work herein described. The later experiments were conducted at the Woods Hole laboratory of the Bureau of Fisheries, and I am indebted to the Bureau for substantial assistance. To Commissioner E. A. Brackett of Winchester, Mass., I am under obligation for making it possible for me to obtain the carp and catfish used in certain experiments described below; and finally I must acknowledge the assistance of Mr. V. N. Edwards on several mid-winter seining trips.

I. CHANGES IN WEIGHT: SEASONAL DIFFERENCES IN THE REACTIONS OBTAINED.

As stated in a former paper,¹ I was surprised and puzzled, after obtaining a uniformly consistent series of results at the New York Aquarium, during the spring of 1905, to find at Woods Hole during the following summer, that the fishes in large degree failed to give the expected responses. It was suggested, by way of explanation, that such differences of reaction were due to seasonal differences in the physiological condition of the animals. Further experiments have confirmed this suggestion.

The first two of these experiments were conducted in New York, during the month of June, 1906, when the temperature of the water in which the fishes were kept was 19° C. Some of the females from the same stock were found to contain ripe spawn at this time. The 1905 experiments in New York, on the other hand, were conducted during the months of March, April, and May, at temperatures ranging from 3° to 15°. In these earlier experiments, as I have already said, significant variations in weight were the uniform result of certain changes in the salinity of the surrounding medium.

Experiment 1. New York, June 7, 1906. — The fishes here used (*Fundulus heteroclitus*) had not taken food for a week or more prior to the experiments, and consequently any appreciable losses through defecation were out of question. Twelve fishes, after being weighed,² were placed in

¹ Bulletin Bureau of Fisheries for 1905, pp. 78-81.

² For an account of the method of procedure and the precautions taken during these weighing experiments, see *op. cit.*, pp. 71, 72.

each of the following grades of water: (1) fresh water, (2) 10 per cent sea water, (3) 25 per cent sea water, (4) New York Bay water (at this time of a density about equal to that of 50 per cent sea water, (5) sea water having a specific gravity of 1.022.

The following changes were noted at the end of twenty-four hours :

(1) Fresh.

June 7 . . . 97.0 gm.

June 8 . . . 97.2 "

Result: a gain of 0.2 per cent.

(2) 10 per cent sea water.

June 7 . . . 109.6 gm.

June 8 . . . 110.0 "

Result: a gain of 0.4 per cent.

(3) 25 per cent sea water.

June 7 . . . 104.8 gm.

June 8 . . . 103.8 "

Result: a loss of 1.0 per cent.

(4) N. Y. Bay water (control).

June 7 . . . 97.2 gm.

June 8 . . . 95.4 "

Result: a loss of 1.8 per cent.

(5) Sea water.

June 7 . . . 104.6 gm.

June 8 . . . 102.3 "

Result: a loss of 2.2 per cent.

The gains in the first two cases are scarcely beyond the limits of experimental error; in the last three cases, however, there has been an appreciable loss of weight. The lot kept in New York Bay water serves as a control, since this was the medium from which all of the fishes were taken at the commencement of the experiment. The decrease in weight here evident is such as is always observed in fasting fishes, though it is rather excessive in this case. Removal of mucus from the skin during the process of drying is doubtless responsible for a certain part of this loss. Compared with this decrease of about 2 per cent, likewise evident in the lot kept in full strength sea water, even the practically stationary condition shown in the first two cases may be regarded as implying an appreciable *relative gain*; *i. e.*, water has doubtless been absorbed, though scarcely in sufficient amount to outweigh the decrease from waste. The 1 per cent loss in the 25 per cent sea water lot shows an intermediate condition which may also be interpreted as denoting an absorption of water.

Experiment 2. New York, June 11, 1906:

(1) Fresh water: a loss of 1 per cent in one day.

(2) 10 per cent sea water: a gain of 0.7 per cent in one day.

(3) 25 per cent sea water: a loss of 0.7 per cent in one day.

(4) N. Y. Bay water: a loss of 1.9 per cent in one day.

(5) Sea water: a loss of 1.9 per cent in one day.

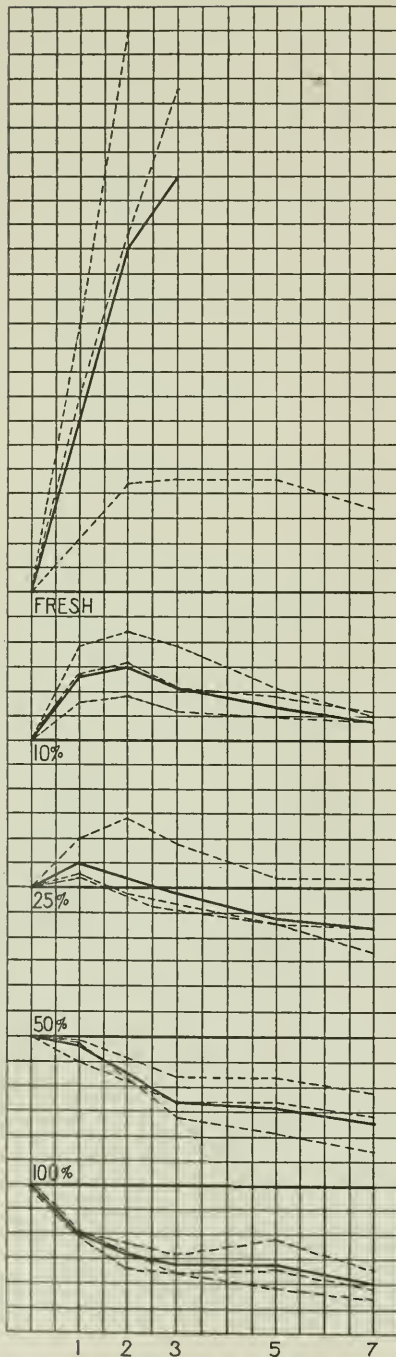


FIGURE 1.

Here, again, the smaller decrease recorded for (1) and (3) may be considered as a relative gain; while the gain in (2), on the contrary, is an absolute one though slight in amount. The results in the case of (4) and (5) are practically identical with those shown in the preceding experiment.

Such mere *relative* increase of weight in strongly hypotonic solutions is, however, in striking contrast to the very decided *positive* gains shown in the next series of experiments (3, 4, and 5). These were conducted at Woods Hole during the month of December, 1906. The fishes used were subjected to the same treatment, except that the water was kept at a temperature of 6° to 8° , by immersing the jars in the salt water of the hatchery supply. The fishes employed had not taken food for seven days or more prior to the experiments. Three series of experiments were performed, that is to say, three different lots of fishes (10 in each) were subjected to the action of each grade of water. Instead of presenting tables of figures in this case, I have thought it best to plot out the resulting changes graphically. The variations of weight undergone by each of

these lots of fishes in a given medium is indicated by a broken line; the continuous line gives the mean for the three. The ordinates represent the gain or loss, each of the divisions being equivalent to one half of one per cent of the original weight of the lot; the abscissas represent the time intervals, viz., 1, 2, 3, 5, and 7 days.

These diagrams speak for themselves. The similarity of form among the three members of each set should dispose of any doubt as to their significance. As in the preceding experiment, the fishes kept in their own natural medium (in this case, full strength sea water) serve as a control lot.¹ The less abrupt decline of the curve for the 50 per cent lot is probably of no significance, since even a relative gain here seems out of question, this mixture being isotonic or slightly hypertonic to the body fluids of the fish.² The form of the curves for the 10 per cent and 25 per cent lots is instructive. The ascent in no case continues beyond the end of the second day, after which a decline commences. This decline is certainly in part due to loss through waste as in (4) and (5), in part, doubtless, to the giving up of some of the water previously absorbed. This, at all events, is what we should expect on the assumption that the phenomena were osmotic, the limiting membranes of the fish being permeable to water, and (in lesser degree) to salts. There would result first a gain in weight, followed by a loss, after the establishment of a new equilibrium.³

In the case of the fishes in fresh water, the gain in weight has been relatively enormous; but this is not surprising, inasmuch as the changes were distinctly pathological. In the most favorable lot, represented by the lowest curve, the fishes began to die within five days; and in the other two cases, individuals began to sicken within two days or less after immersion in fresh water. Fishes in this condition, after being weighed, were removed from the lot, and the remainder returned to the water. Owing to this cause, it was neces-

¹ In the case of one of the three lots in sea water, the decline during the first day is not precisely known, owing to a very obvious error in recording the weight. Accordingly, I have assumed that the loss was identical with that shown by both of the other two lots, *i. e.*, 1 per cent. This is the only alteration which has been made in the original records.

² BOTTAZZI, in *Archives italiennes de biologie*, t. 28, 1897; FREDERICQ, in *Archives de biologie*, t. 20, 1904; GARREY, in *Biological bulletin*, viii, 1905.

³ It is not maintained, of course, that complete osmotic equilibrium is here attained, in the sense that the bodily fluids of the fish become isotonic with the surrounding medium. This is far from being true. For a rather extended discussion of this subject, see *Bulletin Bureau of Fisheries* for 1905, pp. 74, 104-106.

sary to discontinue two of the three fresh water sets long before the expiration of the seven days. It is instructive to note that, in the single case in which a sufficient number of the fishes lived long enough to determine this, a *decline* in weight commenced after five days.

Except in the case of the fresh water lots, only a single death occurred among all the 150 fishes used in the experiments, the remainder appearing to be in perfect health throughout. The results here obtained agree well with those from Experiments 41 to 56 of my former series, and support the conclusions which I then offered in explanation of them. Like the earlier experiments, the present ones were conducted at a time of year when the water temperature was low. Both sets of experiments, on the contrary, stand in marked contrast to those carried out in summer, both in New York and at Woods Hole. From the latter it was concluded that for some reason this osmotic absorption of water is, in some fishes, at least, greatly diminished during the summer months. Whether this difference is due directly to the effect of increased temperature, or due to the altered physiological state of the fishes at this time of year, I have not fully determined, though the following experiments afford some evidence on the subject:

Experiment 6. Woods Hole, December 20, 1906.—In this case the jars containing the fishes were brought into a warm room, the water rising to about 24° C. This is about the maximum temperature reached by the local sea water in August. At the end of about twenty hours the following changes in weight were determined:

- (1) Fresh water: a gain of 6.7 per cent (2 fishes dead, others sick).
- (2) 10 per cent sea water: a gain of 3.3 per cent (one dead).
- (3) 25 per cent sea water: a gain of 2.1 per cent (one dead).
- (4) 50 per cent sea water: a loss of 0.5 per cent (one dead, one sick).
- (5) Pure sea water: a loss of 4.9 per cent (fishes apparently well).

On the evening of the second day a number of fishes were dead in each jar, and on the following day, nearly all were dead.

Experiment 7. Woods Hole, March 1, 1907.—Here the fishes used were previously kept for two days in a tub of sea water, in the room in which the experiment was performed. The change from a temperature of 4° to one of about 21° resulted, as might have been expected, in a number of deaths, even before the weight determinations were commenced. The changes of weight and the fate of the fishes, after twenty-four hours, were as follows:

- (1) Fresh water: a gain of 12.2 per cent (seven dead).
- (2) 10 per cent sea water: a gain of 7.9 per cent (one dead).

- (3) 25 per cent sea water: a gain of 5.3 per cent (all apparently well).
- (4) 50 per cent sea water: a gain of 2.0 per cent (four dead).
- (5) Pure sea water: a loss of 0.8 per cent (one dead).

In both of the preceding experiments the conditions were such that the phenomena are to be regarded as distinctly pathological. The great increase of weight in some of the lots is doubtless due in part to the fact that *dead* fishes were included, and these have been found to imbibe water freely in solutions of any concentration employed. In the (2), (3), and (4) lots of Experiment 6, however, it is recorded that in each case a single individual had just died, so that practically all of the absorption of water must have occurred in living fishes. Again in the (3) lot (Experiment 7), an increase of 5.3 per cent occurred, although all of the fishes were still in apparent health. The gain of weight undergone by the fishes in 50 per cent sea water (relative in one case, absolute in the other) is a circumstance which does not accord with what would have been expected on theoretical grounds, but too much stress must not be laid on these figures.

These experiments alone do not justify us in concluding that changes in temperature, as distinguished from seasonal changes, may not affect the osmotic relations between fishes and their medium. New experiments should be performed in which a healthy condition of the fishes has been insured by a more gradual raising of the temperature.

II. EXPERIMENTS WITH WATER OF VERY LOW SALINITY, CANE SUGAR, AND PURE NaCl SOLUTIONS.

The rather surprising fact has been already emphasized by me that the addition of very small amounts of sea water renders fresh water perfectly innocuous to certain salt water fishes which would otherwise succumb in it. The conclusion was reached that these fishes withstood a decrease in the salinity of their surrounding medium, up to a point where the percentage of salts in the latter approximately equalled that of their own bodies. The proportion of chlorine in *Fundulus heteroclitus* is normally about 0.180 per cent of the entire weight of the fish. This is about the proportion of Cl in 10 per cent sea water; and it was concluded from earlier experiments that this fish could not tolerate with impunity a much greater dilution. It has been found during the present winter, however, that *Fun-*

dulus heteroclitus at this season may live indefinitely in a medium containing only 1 per cent of sea water; while control lots kept in pure fresh water died within a few days. One lot of twelve fishes lived in 1 per cent sea water for fifty-four days, after which the experiment was discontinued. During this period only a single death occurred. In the meantime three different lots of fishes, taken from the same original stock, were placed in pure fresh water, the temperature and other conditions being the same. In all cases the fishes began to die after an interval of from less than a day to two days; and in the only lot which was kept for so long (I had another object in view at the time) all were dead at the end of three days. In another pair of experiments none died out of a lot of ten kept for six days in 1 per cent sea water, while all of a control lot placed in pure fresh water died within the period. Twelve fishes were kept for twenty-one days in 3 per cent sea water, no deaths occurring during that period, though one was removed owing to sickness.

The above is a statement of all of the experiments of the sort performed at this time. No contradictory results were encountered. The process of dilution might profitably have been carried farther, but this was not the main object of my research at the time. Here, as in the case of the weighing experiments, the differences between the present results and those recorded in my former paper are certainly due in part, at least, to differences of temperature or of season. During April, May, and early June, 1906, a considerable number of similar experiments were performed at the New York Aquarium, the fishes being kept in jars of water at the temperature of the surrounding air. This, during the period of the experiments, ranged from 18° to 29°, the mean being about 23°. Fresh water, 1, 2, 3, 4, 5, 7½, 10, 25, 50± (*i. e.*, New York Bay water), 75, and 100 per cent sea water, were used, as likewise sea water strengthened by the addition of commercial sea salt. None of the grades of water below 7½ per cent were found to keep the fishes in a healthy condition. The 1 per cent lots (four in all) began to die after an interval of from one to seven days, and the majority died within less than a week. It must be remembered, however, that at such high temperatures fishes do not fare so well in limited volumes of water as they do when the temperature is lower. In the present series, for example, there were a few deaths even among the fishes in 25, 50, and 75 per cent sea water, although the second of these was the medium from which the entire lot were taken. In sea water strengthened with commercial sea salt, so as to

have one and a half times its natural salinity, many of the fishes died within the first few days ; while in water of twice the natural salinity, in one case, all died during the first day, in another, nine died during the first three days. In the Woods Hole series in January and February, on the other hand, twelve fishes were kept for three weeks in water having a salinity between one and a half and two times that of sea water, without the occurrence of a single death.

The question why the death of certain salt water fishes in fresh water is prevented by the admixture of a very small proportion of sea water has been subjected to experimental inquiry. Although other evidence pointed strongly to the conclusion that the effect of the sea water was not primarily osmotic, it was thought worth while to substitute various amounts of cane sugar, sufficient to make the water isotonic with 1, 10, 50 and 100 per cent sea water respectively. The depression of the freezing point for the local sea water has been shown by Garrey (op. cit.) to be 1.82° C. This implies an osmotic pressure very nearly equal to that of a gram-molecular sugar solution. Accordingly, solutions of cane sugar were made as follows: $\frac{m}{1}$ (= 342 gm. per litre), $\frac{m}{2}$, $\frac{m}{10}$ and $\frac{m}{100}$.¹ These were prepared with ordinary fresh water, as it did not seem necessary to use distilled water for the purpose. Two litres of each solution were placed in a jar, the latter being immersed in running water and thus kept at a temperature of 4° to 7° C. Ten medium-sized specimens of *F. heteroclitus* were used in each jar. The experiments with the second and fourth solutions were repeated, so that altogether six different trials were made. The fishes were found to die in every one of the solutions after an interval of from one to eight days, none surviving for a longer period.² Death occurred somewhat earlier in the most dilute and in the most concentrated of these solutions ; less rapidly in the two intermediate ones. On the whole we may say that the life of the fishes was prolonged little if any beyond that of those in pure water.³ The eyes of those dying in the gram-molecular solution were very noticeably

¹ This last would of course have an osmotic pressure somewhat *less* than that of 1 per cent sea water, since the ingredients of the latter, being "electrolytes," would undergo a relatively greater dissociation as a result of dilution.

² In one or two cases the experiment was discontinued before the last one was actually dead.

³ At the time this series was performed, LOEB'S interesting experiments with the crustacean *Gammarus* were unknown to me. The similarity between the results is evident. Cf. J. LOEB, in *Archiv für die gesammte Physiologie*, 1903, p. 394; translated in "University of California Publications."

sunken into their sockets, denoting an osmotic extraction of water; and no doubt a marked decrease in weight would have been revealed by weighing. This sinking in of the eyes has also been observed in the case of fishes dying in artificially strengthened sea water, and in lesser degree in fishes dying (from poison) in ordinary sea water. For some reason this effect seems to be much more pronounced in sugar solutions than in salt water mixtures having the same osmotic pressures. In the $\frac{m}{1}$ and $\frac{m}{2}$ solutions the fishes were found to keep at the surface throughout, this being obviously due to the high specific gravity of these liquids.

As already pointed out, the normal sugar solution had an osmotic pressure about equal to that of the local sea water. The osmotic pressure of a semi-normal solution, on the other hand, corresponds roughly to that of the blood of marine teleosts. Death occurred, however, even in this nearly isotonic medium. It was next necessary to determine whether these solutions were *toxic* in any sense besides their lack of certain necessary ingredients. This was tested in the following manner:

(1) A solution was prepared containing equal parts of sea water and normal sugar solution. Such a solution would obviously be almost isotonic with ordinary sea water, but would contain only a half the usual amount of salts. In one experiment the fishes died during a period of from five to twelve days. In a second case nine out of ten died during a period of from two to eleven days. The remaining one lived for twenty-three days, when the experiment was discontinued.

(2) A mixture containing 9 parts of normal sugar solution to one part of sea water was used. In the first case the fishes all died in from two to three days; in the second, the majority were dead within two days, after which they were not observed. In this and the preceding experiment the solution, although isotonic with sea water, was of course strongly hypertonic to the fish. It is intelligible, therefore, that the eyes of the latter were deeply sunken in as in the case of fishes dying in a normal sugar solution.

(3) A $\frac{m}{2}$ sugar solution was prepared in 1 per cent sea water. At the end of eleven days seven of the fishes were dead, and the remaining three were sick.

(4) This was intended as a repetition of the last, but, owing to an accident, sufficient sea water was spilled in to raise the proportion to about 8 per cent (as determined by a Cl test). In this case no

deaths occurred until the end of nineteen days; and only 7 were dead after twenty-three days, when the experiment was discontinued. This experiment was repeated, the mixture, in the second trial, containing 10 per cent of sea water. The first fish died after three days, and eight were dead after fourteen days, when they were thrown out.

In these last three experiments the fishes kept at the surface of the water and were sluggish throughout. In many instances the eyes of those which died were sunken in somewhat, which is surprising, considering that the medium was, according to generally accepted statements, not far from isotonic with the body fluids of the fish. This subject demands further investigation.

(5) An $\frac{m}{4}$ sugar solution was prepared in 10 per cent sea water. The fishes all appeared in perfect health for fourteen days, after which the experiment had to be given up.

(6) Equal parts of $\frac{m}{6}$ and 20 per cent sea water were mixed, making a solution containing $\frac{m}{6}$ sugar in a deci-normal concentration, and 10 per cent of the salts commonly present in sea water. At the end of six days the water was found to be very turbid, as if some sort of fermentation were in progress. It was consequently replaced by a fresh solution. The fishes, nevertheless, appeared in perfect health, and no deaths occurred until the expiration of twenty-six days, although the water had long been so foul that the survival of the fishes was astonishing. I have little doubt that by a frequent renewal of this solution they could have been kept in good health indefinitely.

(7) Equal parts of $\frac{m}{6}$ sugar and 2 per cent sea water were mixed; the resulting concentration of sugar being centi-normal, and that of sea water being 1 per cent. As in the 6th set, the water here became very turbid. In spite of this fact, however, only two deaths occurred within the first twenty-three days.

From these experiments it appears that sugar in semi-normal concentration, or in greater concentration, exerts some positively harmful effect upon the fishes. We can conclude, therefore, that their death in the pure sugar solutions of the experiments discussed above, was not due wholly to the lack of salts. Furthermore, the death of these fishes was not due primarily to osmotic action, since, of the two strongest solutions used, one was isotonic with sea water, the other (approximately) with the body fluids of the fishes. Osmosis was apparently *one* factor in the death of the fishes in the normal, and even the semi-normal (?) sugar solutions, however, since there were here visible evidences of an extraction of water. On the other hand, sugar,

up to an $\frac{m}{4}$ concentration, does not appear to be harmful. Accordingly, we may conclude that, while sugar cannot be substituted for the salts of sea water, it has no harmful effect, when associated with the latter, provided that it is not used in too high a concentration, the limit probably lying somewhere between $\frac{m}{4}$ and $\frac{m}{2}$.

It was next sought to determine whether the chief ingredient of sea salt, sodium chloride, was alone adequate to maintain *F. heteroclitus* in a normal condition of health. If such slight additions of sea water served to annul the fatal effects of fresh water, would equivalent amounts of pure NaCl have the same effect? Or does NaCl, either in this low concentration, or in stronger ones, have the toxic effect that has been frequently ascribed to it? Solutions were prepared as follows:

(1) Three-tenths gram¹ of chemically pure NaCl per litre of distilled water. The fishes, ten in number, were first rinsed in fresh water before being introduced into this mixture, and the solution was changed at the end of eleven days. The jar was kept at a temperature of about 5° by immersion in a tank of running sea water.² During twenty-eight days after the commencement of the experiment, nine deaths had occurred.

(2) NaCl in the proportion of 3 gm. per litre was here used, this giving the same percentage of chlorine as in 10 per cent sea water. No sickness developed in the course of twenty-eight days, and the fishes were all active at the end of this time.

(3) NaCl in the proportion of 15 gm. per litre. Result: one fish dead and another sick during the twenty-eight days; the others seemingly well.

(4) NaCl in the proportion of 30 gm. per litre, this mixture containing the same proportion of Cl as ordinary sea water, though the amount of NaCl was of course somewhat greater here. The fishes died in from two to eleven days. In a repetition of this same experiment the entire lot died within five days.

(5) Three-tenths grams of NaCl per litre was added to ordinary "tap water," which was likewise used in the remainder of the series. This experiment was performed twice. In the first case the fishes lived in apparent health for three days, and then the majority died

¹ This gives a solution having the same amount of *chlorine* as in 1 per cent sea water, though of course a somewhat greater amount of NaCl, since there is another chloride (MgCl₂) present in sea water.

² The treatment was the same throughout the ensuing series.

rather suddenly. It is possible that some other influence operated in this case, for, in a repetition of the same experiment, none of the fishes showed any ill effects after twenty-eight days.

(6) Three grams NaCl per litre: in one case the fishes were all well at the end of sixteen days, when the experiment was discontinued; in a second case they appeared perfectly well at the end of twenty-eight days.

(7) Fifteen grams per litre: no harmful effects were manifested for nineteen days, after which many of the fishes fell prey to a peculiar disease. Only two deaths occurred, however, within twenty-eight days.

(8) Thirty grams per litre. In this case 1 per cent of ordinary sea water was added. The fishes all died during a period of from two to fifteen days.

Out of the eleven lots of fishes kept in these solutions, therefore, four showed no abnormal symptoms whatever, up to the close of the experiment (with one exception, twenty-eight days). In one other case only two fishes sickened; and in still another there were only two deaths, though almost the entire lot appeared to be unhealthy. In the remaining five lots every fish, with a single exception, died during the period of the experiment. It must be stated that these solutions were only changed once, this being done eleven days after the commencement of the experiment. The fatal effect of the strongest of these solutions seems unquestionable, since the results in three cases are in full agreement. For the $\frac{3}{10}$ gm. and the 15 gm. per litre mixtures the results are conflicting. In the 3 gm. per litre solution, on the other hand, neither lot was harmed. It must be acknowledged, however, that these experiments would have to be extended through a much longer period than I have carried them, before we should be justified in making a dogmatic denial of the (ultimate) poisonous effect of pure sodium chloride, even at these concentrations. It may, for example, be suggested that the tissues of the fishes themselves contain a considerable reserve supply of those substances necessary to neutralize the poisonous effects of the NaCl.

In addition to such a toxic effect, however, the sodium chloride certainly has a potent *anti-toxic* effect, since, even in solutions which proved fatal, the rate of death was usually much lower than in pure fresh water. *In the aggregate, these experiments may be held to prove, therefore, that pure sodium chloride, in certain proportions, has nearly (if not quite) the same efficacy in counteracting the fatal influence of*

fresh water upon Fundulus heteroclitus as does the combination of salts contained in sea water. My previous experiments have abundantly proved, I think, that the action of this salt is not an osmotic but a chemical one.

III. THE TOXICITY OF CERTAIN POISONS AS AFFECTED BY THE SALINITY AND OSMOTIC PRESSURE OF THE MEDIUM.¹

The method employed in the earlier of the following experiments was first of all to determine the proportion of a given poison which was just sufficient to kill the fishes in their normal medium, in the course of twenty-four hours. A proportion of the substance somewhat smaller than this was then added to each of the following grades of water: (1) fresh water, (2) 10 per cent sea water, (3) New York Bay water (at this time, having a density of 1.011, or rather less than half that of pure sea water), (4) nearly full strength sea water (specific gravity = 1.022, this being the strongest available at the time).

Poisons were chosen which differed widely in their chemical composition and their physiological action, though it must be confessed that the choice was somewhat arbitrary. A few substances which are strongly poisonous to ourselves when taken internally, were found to have no effect upon the fishes when added to sea water in any proportion. Such were arsenious oxide and lead acetate; the former, doubtless, because of its insolubility, the latter because it formed an immediate precipitate of lead sulphate. In a later experiment with catfish, it was found that mercurous chloride was not soluble enough to have any effect upon the fishes, while ether, up to a strength of 1 per cent resulted in a narcosis which was only temporary. Such cases have, therefore, been omitted from the following discussion.

The tables given below record only the *number of deaths* in each medium at the end of a certain period. The fact that in many instances the fishes were all living in a given medium at the time the record was made must not, of course, be interpreted as meaning that they were not affected by the poison. Indeed, in certain cases they were already sick, and their death was noted some hours later.

¹ The results obtained by SOLLMANN (This journal, May 1, 1906) were overlooked by me until after the completion of this paper. So far as our territory has overlapped, there seems to be little disagreement either as to facts or interpretations.

The tables thus represent the *relative* death rate in the different media, and as such serve present purposes perfectly well.

The first of these series of experiments was conducted at the New York Aquarium, in May and June, 1906, the water temperature

SERIES 1.—POISON EXPERIMENTS.

Poison.	Concentration.	Fresh water.	10 per cent sea water.	N. Y. Bay water.	Sea water.
CuSO ₄	per litre. 0.0125 gm.	10	8	0	3
CuCl ₂	0.0125 "	10	10	0	1
HgCl ₂	0.00125 "	7	10	0	0
KCN	0.00125 gm.	4	0	1	0
KCN	0.00187 "	7	1	2	2
HCN	0.001 c.c.	4	0	0	0
Chloral hydrate . . .	1.5 gm.	7	1	0	2
Cocaine hydrochlorate	0.04 gm.	5	0	5	7
Cocaine hydrochlorate	0.0175 "	10	2	2	3
Strychnia sulphate . .	0.00375 "	1	0	2	1
Strychnia sulphate . .	0.005 "	5	3	2	9

being about 14° C. at the time. Ten small specimens of *Fundulus heteroclitus* (one and a half to three inches long) were placed in each solution and the record of deaths taken at the end of twenty-four hours.

In respect to the results tabulated, it will be seen that the poisons used may be divided into three groups. Considering first the group consisting of CuSO₄, CuCl₂, and HgCl₂, we are struck by the fact that nearly all of the fishes have died in the fresh water¹ and in the 10 per cent sea water, while few or no deaths have occurred in the New York Bay water or in pure sea water. In the case of the second group, comprising KCN, HCN, and chloral hydrate, it is only in the fresh water that we find a high death rate, while in the third group consisting of cocaine and strychnine, the heaviest death rates

¹ The fatal effects of pure fresh water alone are seldom manifested as rapidly as this.

appear to have been shown in fresh water on the one hand, and full-strength sea water on the other. Inspection shows us also that in the case of the first two groups the results have been pretty uniform within the group. In the third group, on the other hand, a great variability is evident, and the significance of the figures is extremely doubtful.

It must be conceded at once that the experiments are too few in number to permit us to base any far-reaching conclusions regarding the effect of any one poison. In the aggregate, however, and especially when viewed in connection with later experiments, they may be regarded as highly significant. The most obvious interpretation of all these results is, of course, the hypothesis that we have to do with a *summation of harmful effects*, that is, the harmful effect of the poison, *plus* the harmful effect of the change of medium. In support of this view it might be pointed out that the aggregate percentage of deaths in the fresh water was sixty-four; that in the 10 per cent sea water thirty-two; that in pure sea water twenty-five; while in the normal medium, the New York Bay water, the death rate was lowest, being about 13 per cent. Is not this series graded in accordance with the "naturalness" of the medium to the fish? Plausible as this explanation seems, I am convinced that, in the case of some of the poisons, at least, it is not the correct one. To begin with, it must be urged that we have no right to lump together the results of all three of these "groups" of poisons, since their effects have been obviously different. In the second place, experiments to be described below show clearly that the lowest death rate is not necessarily in the more *natural* medium, but that quite the reverse may be true. I shall, accordingly, postpone a general discussion of these results until after the later experiments have been described.

The next series to be described ("Series 2") does little except confirm some of the previous results, though it was undertaken with a different object in view. The fishes used here, instead of all being transferred directly from the New York Bay water to the medium containing the poison, were in each case habituated to the medium in question by remaining in it for three days before the poison was introduced. In this way time was allowed for a recovery from the osmotic shock, before the fishes were subjected to the poison. It will be seen at once that the results for the copper salts were practically identical with those in the preceding series. In the HgCl_2 solution, on the other hand, the number of deaths in the fresh water is much smaller

than before, though this result is perhaps accidental. In the first table, however, it will be seen that the number of deaths from this poison is likewise smaller in the fresh water than in the 10 per cent sea water. As regards the strychnine, it is difficult to draw any conclusions, since even the results given in the first table were not in harmony with one another. Thus far, however, the results for the copper solutions have been strikingly uniform. The death rates in fresh water and in 10 per cent sea water have been nearly equal, and in both cases nearly or quite 100 per cent; while in the other two media the percentage of deaths has been insignificant.

SERIES 2.—POISON EXPERIMENTS.

Poison.	Concentration.	Fresh water.	10 per cent sea water.	N. Y. Bay water.	Sea water.
	per litre.				
CuSO ₄	0.0125 gm.	10	8	0	0
CuCl ₂	0.0125 "	10	9	0	0
HgCl ₂	0.00125 "	2	7	0	0
Strychnia sulphate .	0.005 "	7	8	8	8

The question as to whether the "naturalness" of the medium was the factor chiefly concerned in determining the death rate of the fishes, under the influence of poisons, was next put to a crucial test. For this purpose it was necessary to obtain a small, rather hardy, fresh water fish. My choice, at this time, was limited to one species, and unfortunately this was obtainable in very small numbers. This fish was *Umbrina limi*, a member of the order *Haplomi*, and known variously as "mud minnow," "dog fish," and "rock fish." This is typically a fresh water species, and, so far as I know, is never found elsewhere. The poison employed was CuCl₂ in varying proportions; the media were fresh water and a mixture of one part sea water (specific gravity 1.022) with two parts fresh. The latter had, accordingly, an osmotic pressure approximate to that of the blood of some fresh water fishes as determined by Fredericq. The results obtained have been arranged in tabular form (Series 3). If we exclude the case of the lowest percentage of copper, with which no test was made in the brackish water, and the highest percentage of copper, with which we have no test in the fresh water (and thus no basis for comparison), we find that seven out of twelve

fishes died in the fresh water mixtures and that not one of fourteen fishes died in the brackish. These results, it is true, are based upon a very small number of fishes, but their truth is strengthened by the fact that they represent several independent experiments, none of which gave results contradictory to the main conclusion, namely, that *copper chloride is more deadly to this fresh water fish while in its own medium than when placed in strongly brackish water.* Moreover, further evidence of this same sort is not wanting, as will next be shown.

SERIES 3.—POISON EXPERIMENTS.

Concentration of CuCl_2 .	Fresh water.		One-third sea water.	
	Living.	Dead.	Living.	Dead.
per litre. 0.00312 gm.	[2]	[0]
0.00417 "	1	2	1	0
0.00833 "	3	3	7	0
0.0125 "	2	0
0.01667 "	1	2	4	0
0.025 "	[1]	[1]
Totals	5[7]	7	14[15]	0[1]

Experiments were later performed with two other species of fresh water fishes, one of the catfishes (*Ameiurus sp.*), and the mirror carp (*Cyprinus carpio, var. specularis*). As in the preceding case, my supply of these fishes was unfortunately very limited. Small individuals were used, measuring, in the case of the catfishes, one and one-half to three inches, and in the case of the carp, one to six inches. With a few exceptions, three individuals were used in each trial. In the present series the record of deaths was not made at the end of any definite period, but the fishes were observed as frequently as possible, and the experiment regarded as closed when either lot was dead. In a number of cases, however, the strength of the poison was such that *both* lots died, without the time of death being noted; in other cases the dilution was so great that no deaths resulted. A complete account of these experiments should record cases of

sickness, as well as of death among the fishes, but this would have complicated the table, and it has not been thought necessary.

SERIES 4.—POISON EXPERIMENTS.

Poison.	Concentration.	Kind of fish.	Time.	Fresh water.		One-third sea water.	
				Dead.	Living.	Dead.	Living.
CuCl ₂	per litre. 0.00833 gm.	Catfish	7 hrs.	3	0	0	3
CuSO ₄	0.01 "	"	6½ "	3	0	0	3
Acetic acid	1.0 c.c.	"	½ hr.	3	0	0	3
Acetic acid	0.2 "	"	2½ hrs.	3	0	0	3
Acetic acid	0.2 "	Carp	2½ "	2	0	0	2
HgCl ₂	0.00125 gm.	"	20½ "	1	2	2	1
HgCl ₂	0.00125 "	"	3 days	0	2	0	2
KCN	0.002 "	Catfish	24 hrs.	0	3	0	3
KCN	0.004 "	"	5 "	2	1	3	0
KCN	0.00308 "	"	1 day	0	3	0	3
Chloral hydrate	1.5 "	"	24 hrs.	0	3	0	2
Chloral hydrate	2.0 "	"	24 "	0	3	2	1
Chloral hydrate	5.0 "	"	7 "	3	0	3	0
Strychnia sulphate ¹	0.008 "	"	2 days	3	0	2	1
Alcohol	5.0 c.c.	"	21 hrs.	3	0	0	3
Alcohol	10.0 "	"	33½ "	0	3	2	1

¹ The fishes subjected to the strychnine solution manifested an extraordinary state of hyper-excitability. They "jumped" convulsively upon the slightest provocation, a light tap upon the floor, or the clapping of my hands over the jar being a sufficient stimulus. Even a loud shout, uttered near enough to them, provoked this response.

These experiments were conducted at Woods Hole during August, 1906. The solutions were allowed to follow the air temperature, no full record of which was kept. It probably did not vary beyond the limits 18° and 23°. In the table (series 4) it will be seen that

copper chloride, copper sulphate, and acetic acid have given this uniform result: *the fishes in fresh water have all died, those in 33 per cent sea water have all survived, during the period of the experiment.* It is true that only twenty-eight fishes were used with those substances, but the fact that five independent experiments agree in this way argues strongly for their significance. With the other poisons used in this series, the results are not at all decisive. In some cases an equal rate in the two media has been manifested; in others a slight preponderance in favor of one or the other. It would be necessary to make further experiments before forming any conclusions as to the action of these poisons. Such inconclusive results do not, however, affect the significance of the experiments with the salts of copper and with acetic acid; there being strong evidence that these substances are less fatal to fresh water fishes in brackish water of a certain strength than in their natural medium. Or we may express the same fact in another way by saying that a certain proportion of the salts of sea water tends to neutralize the fatal effects of certain poisons. Thus stated, the fact falls into line with a considerable range of other facts which have been discussed in recent physiological literature, namely, the effect of certain substances in antagonizing the poisonous effect of other substances in solution. Loeb has probably contributed most to our knowledge of this subject.

In the present case the anti-toxic effect of the sea salts could most readily be explained by assuming that the poison underwent an immediate neutralization outside of the body of the fish, as the result of some chemical reaction between itself and the ingredients of sea water. And, indeed, such an explanation could not be denied without a consideration of all the components of sea water and their possible reactions with each of the poisons in question. In fact, it is quite likely that this explanation is the correct one in some cases. For example, the alkalinity of sea water is sufficient to neutralize an appreciable proportion of the acetic acid used in the foregoing experiments.¹ That this is not the probable action of the sea water in the case of some other poisons, however, is rendered probable by the fact (*cf.* series 1 and 2 of the poison experiments) that copper sulphate, copper chloride, and mercuric

¹ According to DITTMAR (Challenger Report) the proportion of CaCO_3 or MgCO_3 (however the carbon dioxide be combined) present in sea water is in the neighborhood of 1 : 10,000. The strength of the acetic acid used by me in these experiments ranged from 1 : 5,000 to 1 : 1,000.

chloride proved nearly or quite as fatal in 10 per cent sea water as in fresh water. This we should not expect on the assumption that the poison was neutralized by undergoing some reaction with the salts of the sea water, since the latter or at least the more important among them would, even at this dilution, be present in quantities vastly in excess of the copper or mercury salts.¹ In order to fully dispose of this possibility, however, as well as to throw further light upon the phenomena involved, I tried the effect of substituting for sea water a solution differing widely in its chemical and physical properties, and agreeing only in maintaining the osmotic pressure of the medium at a comparatively high level. For this purpose cane sugar was used.

With this in view, ten specimens of *Fundulus heteroclitus* were put into two litres of each of the following media: sea water, $\frac{m}{2}$ sugar solution mixed in fresh water, and pure fresh water. Cupric chloride was added in the proportion of 1 : 100,000. The fishes in the fresh water were all dead at a time (five to nine hours) when none were dead in either of the other solutions. Those in the sugar solution, however, died (thirteen to twenty-four hours) before any of those in the salt water; the latter finally dying between one and a half and three days after the commencement of the experiment. This experiment was repeated, with results which were practically identical. The absolute length of the intervals were somewhat different, but the same order was followed. It is plain, therefore, that sugar had, in considerable measure, the same effect as sea water in neutralizing the poisonous action of the copper chloride; but it is likewise plain that its effectiveness was not so great.

In three experiments acetic acid was used instead of copper chloride, but with quite different results, since the sugar had no influence in protecting the fishes from the effects of this acid. The strengths of acid used were respectively 1 : 10,000, 1 : 2,000, and 1 : 1,000. In the first case no deaths had taken place after an interval of about nineteen hours, and the experiment was discontinued. In the other two cases the majority of the fishes in the fresh water and in the sugar solutions died before those in the salt water began to be affected, though the latter succumbed some hours later. The lot in semi-normal sugar died at least as soon as those in fresh water.²

¹ Even the bromides and carbonates, at this dilution, would be present in proportions of about the same magnitude as the poisons themselves.

² In one case sooner, in the other slightly more tardily.

In the case of acetic acid, therefore, such a sugar solution seems to have no anti-toxic effect.

A few experiments were performed in which *fresh water* fishes were subjected to poisons, in conjunction with solutions of cane sugar. Unhappily, as in the previous experiments, my stock of these fishes was extremely limited; but the results are none the less of high

SERIES 5.—POISON EXPERIMENTS.

Poison.	Concentration.	Kind of fish.	Time.	Fresh water.		Sugar solution. ¹	
				Dead.	Living.	Dead.	Living.
CuCl ₂ . .	per litre 0.0067 gm.	Catfish	11½ hrs.	2	0	0	2
CuCl ₂ . .	0.01 "	"	12 "	1	0	0	1
CuCl ₂ . .	0.005 "	"	11 "	2	0	2	0
CuCl ₂ . .	0.01 "	"	few "	3	0	3	0
CuCl ₂ . .	0.01 "	Carp	4 "	3	0	0	3
CuCl ₂ . .	0.01 "	"	12 "	1	0	1	0
CuCl ₂ . .	0.01 "	Umbra	1 day	3	0	2	1
Acetic acid.	0.2 c.c.	Carp	2 hrs.	2	0	2	1
Acetic acid.	0.2 "	Catfish	2 "	1	1	0	2
Acetic acid.	0.2 "	Umbra	2 days	1	2	3	0
Acetic acid.	1.0 "	"	2 hrs.	3	0	3	0

¹ $\frac{1}{4}$ solution in the first, second, third, fifth, and sixth cases; $\frac{1}{3}$ in the fourth case, 10 per cent solution in the remainder.

interest when considered in connection with those already discussed. These experiments are presented in tabular form (Series 5). It is obvious that here, as in the case of *Fundulus*, the sugar has had no certain effect upon the rate of death in acetic acid. Turning to the copper salt, however, we find that in three cases out of seven *all* of the fishes died in the fresh water and *none* in the sugar solution. In one case the number of deaths was *greater* in the fresh water, while in the three remaining cases all of the fish were dead in *both* media at the time of observation, though it is far from certain that they died simultaneously. These experiments surely have considerable confirmatory value, in spite of the small numbers of fishes used.

To sum up the results of these experiments upon the effects of poisons in relation to changes in salinity and osmotic pressure, it must be said at once that no general statement can be made which will cover the effects of all of them. In a number of cases no certain difference in the death rate was observed between those in one medium and those in another. The toxic action of some of the poisons, however, was neutralized by the admixture of certain proportions of sea water, and this anti-toxic effect of the sea salts was found to hold even for fresh water fishes. In the case of one poison, cupric chloride, it was discovered that solutions of cane sugar had an anti-toxic effect quite similar to those of sea water. In this last case, therefore, the possibility seems to be excluded that the poison was neutralized by some chemical reaction occurring outside of the body of the fish. If we disregard for the present the likelihood of obscure biochemical reactions occurring within the protoplasm itself, two explanations here suggest themselves. It might be supposed that the presence of other substances in solution may have hindered the dissociation of the poison, and thus diminished its toxic power.¹ This possibility could be disposed of only by a definite knowledge of the behavior of the substances in question under the conditions of the experiment. Such knowledge the writer does not possess. It may be remarked, however, that nearly all of the poisons employed were used at dilutions so great that, according to the generally accepted belief, their dissociation would be practically complete, at least in pure water. Whether or not the presence of other "electrolytes" in the same solution, or of the "non-electrolyte" sugar, would appreciably diminish the extent of this dissociation, I leave to the physical chemist to decide.

There remains the view that the effect of the sea water or the sugar solution in these cases has been primarily an osmotic one. I have offered much evidence for the supposition that a fish is not separated from its surrounding medium by a constantly impervious barrier, but that a certain amount of osmosis and diffusion may occur as the result of sufficiently great changes in this medium. It has been argued also that the degree and kind of permeability of the limiting membranes of a fish is a function of the physiological condition of the animal, and may be affected by various external influences. For example, the normal medium of a fresh water fish is known to be very strongly hypotonic to its blood. These two fluids are separated by

¹ This explanation was suggested to me by Dr. CARL L. ALSBERG.

a membrane which under ordinary circumstances, is impervious to water, and thus the latter fails to enter the body. Let this membrane be damaged by a poison, however, and its normal power of resistance may be supposed to give way. Death would thus be due, in part at least, to an unchecked absorption of water. Now, I do not regard it as probable that the death of fishes in fresh water, as a consequence of poisoning with copper salts, is due entirely to the absorption of water. It seems also likely that a certain amount of the poison itself enters with the latter, and contributes to, or perhaps is mainly responsible for, their death.

Let me recall at this point certain facts relating to the behavior of salt water fishes in fresh and brackish water. In the case of *Fundulus heteroclitus*, it has been shown that a percentage of sea water too small to exert an appreciable osmotic effect suffices none the less to maintain the fish in normal relations with its surrounding medium. An osmotic absorption of water may occur, it is true, in such a diluted mixture, but within strictly physiological limits. In fresh water, on the other hand, there is invariably a breakdown in the power to resist fatal osmotic exchanges.¹ It appears accordingly that the primary effect of the fresh water is to damage the limiting epithelium, or some part of it, behaving in this respect as a poison, comparable with some of the other poisons discussed above. The actual cause of death, as I have pointed out elsewhere,² probably lies in part in the absorption of an undue amount of water by the fish, in part in the loss by diffusion of some of the necessary salts of the body.³

It will be recalled that, under the influence of some poisons, the fishes died to nearly or quite the same extent in 10 per cent sea water as in pure fresh water. I should explain this by calling to mind that even 10 per cent sea water is strongly hypotonic to the blood of the fish. It is perfectly harmless to the latter, only so long as the limiting membranes are intact; let these become freely permeable, however, and a fatal influx of water (and poison?) ensues.

¹ Compare the curves showing changes of weight, p. 64.

² *Op. cit.*, p. 105.

³ LOEB believes the death of *Gammarus* in distilled water and in sugar solutions to be due mainly or entirely to the latter cause. The extremely "water-soaked" condition of fishes dying in fresh water, however, and the great increase in weight revealed by measurement, lead me to assign an important rôle to the first of these causes, in the case of some fishes, at least. The death of those fishes which die very quickly, on the other hand, is perhaps due to yet another cause (see my former discussion of this subject cited above)

This theory that the toxic action of certain substances upon fishes is due in part to their effect upon the permeability of the limiting membranes (chiefly those of the *gills*: see below) may perhaps also account for the apparently greater mortality of the fishes in full strength sea water than in 50 per cent sea water, under the influence of certain poisons (Series 1). For it must be borne in mind that sea water is strongly *hypertonic* to the body fluids of teleost fishes living in it. If the limiting membranes become, for the time being, osmotically permeable, an extraction of water will follow, even in the normal medium of the fish.

An explanation in some respects the converse of mine would attribute the anti-toxic effects of the sea salts or the cane sugar in the preceding experiments to their affecting the *permeability of the membranes to the poisons*. Without attempting a full discussion of this subject, I will offer the following objections: (1) cane sugar has been found not to appreciably affect the permeability of the membranes to the salts of the blood, which diffuse outward nearly or quite as rapidly in solutions of this substance as in fresh water; (2) small percentages of sea water, on the other hand, which do hold in check the permeability of the membranes to the salts of the blood, have little or no anti-toxic effect upon certain poisons.

In closing this discussion, I must urge that the foregoing conclusions are offered provisionally, and with due regard to the possibility of their being overthrown by more exhaustive experiments. The writer is not in the least in sympathy with the tendency, so often manifested, to explain the most complex of natural phenomena by a few simple chemical or physical formulæ. If the principles which I have evoked operate at all in the way in which I have supposed, they operate in conjunction with other principles so obscure and complex that a complete solution of these problems is certainly very far distant.

IV. CHANGES IN THE SALT CONTENT OF THE BODY.

In my earlier work I showed that certain changes in the salinity of the surrounding medium resulted in changes in the salt content of the bodies of certain fishes. The fishes used were salt and brackish water species. In these the greatest loss of salts (as measured by chlorine determinations) was found to occur in fresh water. It was likewise shown that a small percentage of sea water served to diminish, or entirely prevent, this extraction of salts. The figures then ob-

tained led me to the belief that the changes in the salt content of body were progressive, and the following table of percentages, tained with *Fundulus diaphanus* was offered in support of this view

		Percentage of
Fresh water	{ 11 days	0.08
	{ 3 days	0.10
	{ 1 day	0.11
Brackish water (the original habitat; sp. gr. 1.002)		0.134 [or 0.14
Salt water	{ 5 days	0.14
	{ 10 days	0.15

It will be seen that we have here a perfectly graded series both respect to the salinity of the water used and the time during which the fishes remained in it. It must be pointed out, however, that each of these figures, with the exception of that for the lot from brackish water, was obtained by a single determination. Due allowance was not made at that time for the inexactness of the method employed and for the frequent capriciousness of the results. Further experience has led me to doubt the significance of such a difference as that between 0.108 and 0.112, or even that between 0.143 and 0.151, when each figure is based upon a single analysis. Furthermore, I do not think it probable that the extremely low chlorine percentage obtained for the fishes which had been in fresh water for eleven days was due to a defect in the method employed (see p. 90). I must add, however, that I do not regard *all* of these differences as being due to accident or error, but that I hold it as proved that salt or brackish water fishes do lose a portion of their salts when placed in fresh water, and as probable that brackish water or fresh water fishes gain in their salt content when placed in full strength sea water. I believe the table given below to be sufficient ground for the first half of this assertion.

Before entering into a consideration of these figures, it is important that I should discuss rather fully the method employed, since the significance of the former depends so largely upon the latter. With a few exceptions, two fishes² were taken for each analysis. They were in all cases first left for a period of from five to fifteen minutes in fresh water, in order to remove any salt water that might be held by the mucus of the surface or in the gill chambers. The fishes were then carefully dried with a clean towel, after which the entire viscera

¹ *Op. cit.*, p. 92.

² Occasionally three, in a few cases one, where only one was available.

CHLORINE PERCENTAGES.

Grade of water.	One day.	Two days.	Six days.	Twelve to nineteen days.	Mean.	P. E.
Fresh water	0.132 0.139	0.144 0.161	0.148 0.135	0.141	0.143	[0.007]
1 per cent sea water	0.139 0.156	0.131 0.138	0.130 0.127	0.128	0.144	0.013
2 per cent sea water	0.160 0.153	0.147 0.182	0.164	0.169	0.167	0.011
3 per cent sea water	0.156 0.151	0.154 0.182	0.186 0.181	0.183	0.167	0.012
4 per cent sea water	0.153	0.178	0.182	0.185	0.174	0.010
5 per cent sea water	0.181	0.182	0.184	0.178	0.181	0.007
7½ per cent sea water	0.185	0.163	0.176	0.188	0.178	0.007
10 per cent sea water	0.193 0.210	0.201	0.180	0.183	0.184	0.007
25 per cent sea water	0.225 0.203	0.236 0.253	0.209	0.202	0.217	0.016
N. Y. Bay water (= 50 per cent ±)	0.203	0.244	0.233	0.202	0.217	0.016
75 per cent sea water	0.203	0.253	0.233	0.202	0.217	0.016
100 per cent sea water	0.203	0.253	0.233	0.202	0.217	0.016
1½ × sea water	0.203	0.253	0.233	0.202	0.217	0.016
2 × sea water	0.203	0.253	0.233	0.202	0.217	0.016

were removed. Thus any water contained in the alimentary canal or in the genital or urinary passages would be omitted from the analysis. The two fishes from the same medium were now weighed together and placed in a wide-mouthed bottle. Equal parts of potassium nitrate and potassium or sodium carbonate were added, in amount jointly equivalent to the weight of the two fishes. A little distilled water was poured in and the preparation set aside to be analyzed at my convenience. At that time the contents of the bottle were emptied into a porcelain crucible, the bottle also being carefully rinsed out. The crucible was placed upon a sand bath, and the water evaporated, care being taken to prevent sputtering, which may result in considerable loss of material. Upon continued heating, the preparation ignited spontaneously, or the ignition was hastened by applying a bit of burning filter paper. It was necessary that the combustion should be complete; otherwise organic compounds remained which discolored the solution and rendered the subsequent test difficult. Distilled water was poured upon the residuum in the crucible, which was dissolved by boiling. Nitric acid was then added to the solution, until it became acid in reaction. Then the excess of acid was neutralized with powdered CaCO_3 . The whole was now filtered, and the volume measured exactly. Definite portions of this solution were titrated with a standard solution of silver nitrate, potassium chromate being used as an indicator (Mohr's method). Three or more titrations were made in each case. Upon dividing the total amount of Cl indicated, by the weight of the fishes used, the percentage was of course readily computed. It is needless to say that great care was taken that the materials and the utensils used should not be contaminated with chlorides from any source. When the solution was free from organic matter, it was found that the limit of titration was tolerably clear. Indeed the results as a whole make it plain that the error in the titration process is insignificant in comparison with some unknown source of error which I shall soon discuss.

The table on p. 87 is for the most part self-explanatory, but a few points demand further elucidation. It will be seen that in a large proportion of cases the figures from two different lots of fishes are given for the same time and the same grade of water. The figure in heavy type, in each case, represents the mean of these two. In the "mean" column are given the average figures for each grade of water, computed from the results for separate days. In all cases it will be seen that two determinations were made for the longest period

(twelve to nineteen days). Where only one determination occurs in the earlier columns, the *mean* of the two in the later one has been employed in computing the general average, thus giving equal weight to each day's results. In such cases the technically irregular course has been pursued of basing the probable error of this general average upon the departure of each of the *individual* figures from their own mean, thus making full allowance for the great variability shown by them.

This high variability is one of the most striking features of the table, and may seem to the reader to wholly discredit any conclusions which I may draw from it. The difference between 0.158 and 0.192 (2 per cent sea water, last column) or between 0.147 and 0.182 (3 per cent, two days) is indeed nearly as great as that between the figures at the opposite ends of the "mean" column. The question at once arises: Do such perplexing differences as those just cited represent actual differences in the chlorine content of the fishes themselves, in spite of their having had an identical history, or do they arise from the imperfections of the method employed? I am inclined to the latter belief, though I must confess that, after much experimenting, I am unable to satisfactorily locate the source of error. It was, however, early noticed that in some of those cases in which exceptionally low figures were obtained, the mass of material used (*i. e.*, the aggregate weight of the two fishes) had been much greater than the average; while conversely, in certain cases in which the figures were exceptionally high, the mass used for analysis was small, in some cases a single fish having been taken for the purpose. An inspection of all my results revealed the fact that in twenty out of the twenty-eight cases in which two determinations were made of fishes having an identical history, the larger figure was derived from the smaller mass used for analysis, and vice versa. From a consideration of these figures, I was enabled to formulate the law that, other things equal, a difference of weight of one gram in the mass analyzed corresponded to a negative difference of 0.0022 in the percentage of chlorine. From this we must not conclude that *larger* fishes may have proportionately less chlorine. I do not think that the *size* of the fishes is the determining factor. Very low figures were obtained in certain experiments in which four or five fishes were employed, despite the fact that the fishes individually were not exceptionately large.¹ I concluded from these facts that in some

¹ The very small chlorine percentage for one lot of *Fundulus diaphanus* (fresh water eleven days) may be thus in part explained. This figure was based upon a lot of eight fishes which were incinerated together, the other figures being based upon four or less.

way the combustion of a large mass of material resulted in a greater loss of chlorine than the combustion of a small mass; and efforts were made to counteract this influence. The proportion of sodium carbonate to potassium (or sodium) nitrate was increased from 1 : 1 to 2 : 1, 4 : 1, or even, in a few cases, to 10 : 1, but with little or no effect upon the results. Covering the crucible with a loosely fitting porcelain cover was likewise without result, which is perhaps not surprising, since the vapors escaped freely, notwithstanding.

Much more accurate results could doubtless be obtained by choosing fishes of closely equal weight throughout; but even in those cases where I have done this, great discrepancies have sometimes occurred. To ensure a series of chlorine determinations sufficiently precise for a satisfactory solution of the problems under discussion, it would be necessary either to render the chemical technique more accurate, or to carry out a much greater number of analyses, and thus to allow the errors to neutralize one another. Anyone who has had experience with this sort of work will realize the enormous task implied in the second suggestion.

Having fully allowed for the inexactness of the determinations, let us consider what conclusions may nevertheless be drawn from them. Looking at the "mean" column, it will be seen that the figure for 1 per cent sea water¹ (0.144) is much smaller than that given for any of the succeeding grades of water; the difference between this figure and the next lowest being 0.023 per cent. Even in view of the very high probable errors, this difference is great enough to speak strongly for its significance. The next seven figures in the column show an ascending gradation, though it is not a uniform one. The only considerable step is that between the figure for pure sea water and that for the artificially strengthened sea water. This last difference I also regard as with little doubt a significant one. The minor differences among the figures for the grades of water between 3 per cent and pure sea water may, in view of the high probable errors, have little or no validity. *It must be borne in mind, however, that the existence of such a graded series as that shown in this column gives it a cumulative probability greater than could be inferred from the magnitude of the probable errors of the individual figures.*² The reader is not at

¹ That for fresh water is omitted from the discussion, since the series is incomplete. The figures are none the less interesting and significant.

² An examination of the masses used in each case does not justify us in applying the same explanation to these differences as has been done in another case. See p. 92.

liberty to introduce the figures for the 2, 4, 25, and 75 per cent sea water lots into this series, since they represent determinations for the longer period (twelve to nineteen days) only; and it will be shown presently that the figures in the different columns present differences of a more or less constant sort.

Granting the significance of the figures, two possible views offer themselves in explanation of these changes in the bodily salt content. The first is that they are the result of variations in the amount of water in the body consequent upon osmotic absorption or the reverse. An influx of pure water would of course lead to a diminished concentration of salts in the body as a whole, and an efflux of water would lead to an increased concentration. By reference to my former experiments, however, it will be seen that the increase of weight due to the absorption of water is at the most 3 or 4 per cent;¹ while the diminution in the salt content of the body has been, in the case of the fishes in 1 per cent sea water, about 19 per cent, as compared with the lot in New York Bay water. We are thus driven to the second view that the fishes have actually yielded up part of their bodily salts (or, in the last series, acquired an additional amount). Upon this view, the limiting membranes of the body must be in some measure permeable to these salts.

The next question which offers itself is this: Are these changes in the salt content of the body progressive, increasing from day to day, or do they cease after a short time, or do they perhaps undergo an actual reversal as time goes on? Let us take, in the first place, those series which show a diminished salt content. For this purpose we are limited to the 1, 3, 5, and 7½ per cent lots; the fresh water, 2 and 4 per cent lots, being omitted as incomplete, and the lots in waters of 10 per cent or greater concentration showing no appreciable diminution. If, then, we average the percentages obtained from each of these four lots for each day, we obtain the following result:

1 day.	2 days.	6 days.	12 to 19 days.
0.152	0.161	0.165	0.174

Here we obviously have a series of figures showing an *increasing* salinity. Before forming any conclusions as to the meaning of these figures, however, it is necessary that we should examine the data more carefully. It has already been pointed out that large masses of

¹ Exception being made for the fishes in fresh water, since they are not included in the present discussion.

the material subjected to ignition (whether due to more or to larger-sized fishes) gave as a rule smaller percentages of chlorine and vice versa. It was stated that, other things being equal, each gram of additional weight of the mass employed for analysis resulted in a decrease of 0.0022 in the chlorine percentage. Now an examination of my records led to the discovery that I had made an unconscious selection in the size of the fishes taken for analysis, picking out the larger ones in each lot first. Thus the mean weight of the mass employed (in most cases two fishes) for each day, in the four series under discussion, was :

1 day.	2 days.	6 days.	12 to 19 days.
17.8	15.7	12.3	13.4

Accordingly, an increasingly larger chlorine percentage would be expected in the first three columns. Let us make due allowance for this source of error, revising each of the three later figures by the subtraction of 0.0022 per cent of chlorine per gram weight of difference. Our series now becomes :

1 day.	2 days.	6 days.	12 to 19 days.
0.152	0.156	0.153	0.164

In other words, the apparent increase in the chlorine percentage during the first six days may perhaps be fairly attributed to this source of error in the determinations. The increase shown in the last column seems too great, however, to be thus disposed of. We must accordingly recognize the possibility, perhaps the probability, of an actual *increase* in the percentage of chlorine in these fishes, which had first suffered a considerable loss. How this could take place in solutions containing chlorides in a concentration far lower than that of the tissues themselves is hard to say, but many parallel cases could be cited from the physiology of absorption. It must be reiterated, however, that these explanations are dependent upon the truth of the premise that the salt content does undergo such a progressive increase, a fact that I do not regard as fully proved.

A few other figures were obtained from experiments which do not properly fall in the same series as the last, since they were performed under somewhat different conditions and with different ends in view. They were, however, fully confirmatory of my main thesis that considerable changes in the salt content of the body do occur. But mention may be made of a few analyses of fishes which had died in

solutions of different strengths, and which had remained in the latter for varying periods. Some fishes which had remained about twenty-four hours after death in 1 per cent sea water gave a chlorine percentage of 0.0709, or less than one half of the normal amount; while some others which had remained for an uncertain period in strengthened sea water (about one and one-half times the usual strength) gave a percentage of 0.303. Fishes which were dying or just dead, on the other hand, gave, in the majority of cases (*i. e.*, two out of three), a percentage not far different from the normal. That a free diffusion should take place after death is, of course, just what we should have expected.

V. THE RELATION OF THE GILLS TO OSMOSIS AND DIFFUSION.

In a former paper, already alluded to,¹ I have discussed some experiments which I performed, with a view to determining whether the osmosis and diffusion which seemed to have been proved, took place primarily through the gills, or whether the general integument of the body was largely concerned. For this purpose a device was constructed, by the aid of which it was possible to pass water of one sort through the gills, while the remainder of the body was bathed by water of another sort (Fig. 2). Three pairs of experiments with a hardy fresh water fish, the common carp, were described, the results of which were all in perfect harmony with one another, and were expressed as follows: "A considerable loss of weight occurred in all those cases in which salt water passed through the gills and fresh water over the body, while the weight remained practically stationary in those cases in which the conditions were reversed. . . . The three whose gills were bathed by fresh water, all remained well till the end of the experiments, while of those whose gills were bathed by salt water, two died and one sickened."

The reciprocal experiment of subjecting a salt water fish (the sea raven) to the influence of fresh water was likewise tried at that time, giving results which were somewhat inconclusive, though in no sense contradictory to those obtained with the carp. It has seemed worth while to repeat these experiments with some other salt water fish. For this purpose the tautog (*Tautoga onitis*) was employed. The experiments were conducted at the New York Aquarium in June, 1906, the temperature of the fresh and salt water being 21.5°

¹ *Op. cit.*, pp. 97-100.

and 18° respectively, and the density of the latter (New York Bay water) being 1.012, this representing a mixture containing about equal parts of sea water and fresh water. The fishes used had not taken food for two or more days, and the method of procedure was precisely the same as that adopted in the earlier experiments. In the present case two pairs of experiments were performed, *i. e.*, in

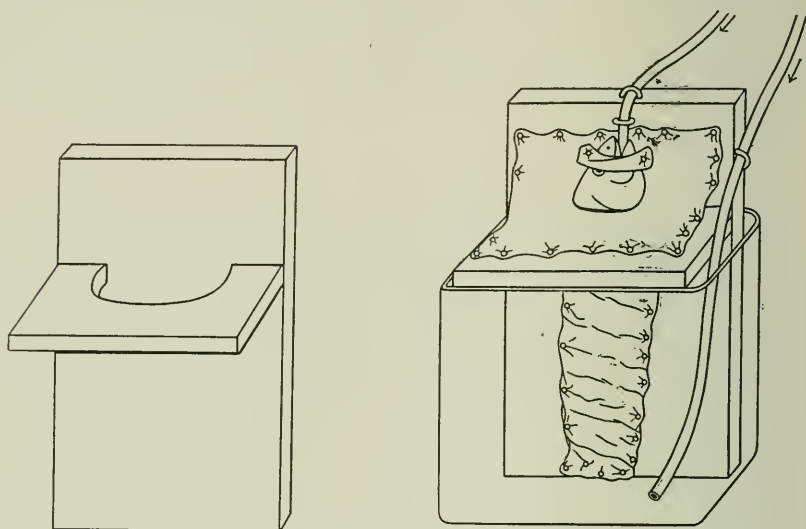


FIGURE 2.— (From the Bulletin of the Bureau of Fisheries.)

two cases fresh water was passed through the gills, while salt water bathed the body; in the other two the conditions being reversed.

The two former fishes died in from one and one-half to two and three-quarters hours. The two latter were, at the end of the same interval, in perfect health, one of them living at least three days after return to fresh water (*i. e.*, as long as observed). As regards changes of weight, it must be borne in mind that death ensued too soon to allow us to expect any appreciable increase, especially in view of the size of the fishes, which weighed from 413 to 755 gm. It must be remembered, too, that at this time of year weight changes due to osmosis have been shown to be slight or altogether wanting. In the present experiments there was in every case a slight *loss* of weight, this being in no instance as great as one per cent, and in two of the cases much less. This loss is readily accounted for by the large quantities of mucus secreted by the fishes, and removed with the

cloth in which they were wrapped. The view that the gills are the chief paths through which the fishes are affected by changes in the constitution of their surrounding medium, certainly receives some confirmation from the behavior of these tautog, even in the absence of any significant changes of weight.

VI. SUMMARY.

The more general conclusions reached as a result of the foregoing studies need not be summarized here, since they have been stated by me in two previous papers. Certain additions to and modifications of my former views may be stated as follows:

1. There exist decided seasonal differences in the osmotic phenomena displayed by certain fishes in response to changes in the constitution of their medium. These differences may perhaps be due directly to differences in temperature, but are more probably due to seasonal variations in the physiological condition of the animals.

2. It has been found that for *Fundulus heteroclitus*, during the winter months, at least, the dilution of their normal medium, sea water, can be carried much farther than was originally stated by me. Even 1 per cent of sea water, added to ordinary fresh water, suffices to keep the fish in a normal condition for considerable periods, perhaps indefinitely, while pure fresh water is invariably fatal, commonly within a few days.

3. That the value of this slight admixture of sea water did not depend upon its raising the osmotic pressure of the medium was shown (if such proof was necessary) by substituting various proportions of cane sugar. In no case did this prevent the death of the fishes, which ensued almost, or quite, as soon as would have occurred in pure fresh water. It was found, on the other hand, that sugar itself, up to a concentration of $\frac{m}{4}$, appeared to be quite harmless to the fishes, if a small percentage of sea water were present. Normal and semi-normal solutions were fatal, however, even though the former was approximately isotonic with sea water, and the latter with the blood of the fish.

4. Chemically pure sodium chloride, in certain proportions, even when dissolved in distilled water, kept the fishes in apparent health for four weeks (*i. e.*, until the experiment was discontinued). Some of the solutions, however, proved fatal, though even in these cases death did not generally occur so soon as in pure fresh water. The

necessary something which these salt water fishes miss in fresh water seems, therefore, to be chiefly sodium chloride, and of this astonishingly small quantities suffice for the physiological needs of the body.

5. The relative toxicity of various poisons in different media was studied. It was found that a number of these acted more rapidly in fresh water than in sea water. Even to fresh water fishes, two of these poisons, at least (CuCl_2 and acetic acid), were found to be less rapidly fatal in diluted sea water than in the more natural medium of the fish. And finally, copper chloride was found to be less fatal to certain fresh water fishes when acting in strong sugar solutions than when mixed with their normal medium.

6. A very extensive series of chlorine determinations was conducted with a view to determining the changes in the salt content of the body resulting from changes in the concentration of the external medium. These results are in part vitiated by certain unknown sources of error, but they nevertheless show clearly what I earlier announced, that the salt content of a fish's body may undergo considerable changes in response to certain changes in the water surrounding it. These later determinations, however, cast doubt upon certain minor conclusions reached during my former work.

7. The view that the phenomena of diffusion and osmosis implied in the preceding discussion have their seat primarily in the gills of the fish, was confirmed by some experiments with the tautog, these being reciprocal to certain experiments with the carp upon which I have already reported.

THE INFLUENCE OF SOME MEDICINAL AGENTS ON THE ELIMINATION OF URIC ACID AND CREATININ.

BY ELBERT W. ROCKWOOD AND CLARENCE VAN EPPS.

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ALTHOUGH the effects of various drugs upon the elimination of uric acid have been studied for a considerable time there is no general agreement as to the action of many of them.¹ Recently Macleod and Haskins² have published the results of experimental work, from which they conclude that the administration of sodium bicarbonate, or sodium citrate, until the urine reacts alkaline with litmus, causes an increased excretion of the endogenous purins, including uric acid. Their conclusions are so much at variance with results which we had previously obtained, that we have extended our experiments so as to include the effects of the above-mentioned salts.

The subjects (men) were kept on a constant diet, in some cases purin-free,³ in others with a constant amount of purins (those of potatoes and theobromin). In the twenty-four-hour urine were determined the uric acid, nitrogen, phosphoric acid, and, in several of the series, the creatinin. Folin and Shaffer's method was employed for determining the uric acid,⁴ Folin's for the creatinin,⁵ Kjeldahl's for the nitrogen, and titration with uranium acetate for the phos-

¹ The literature is reviewed by SCHREIBER, *Die Harnsäure*, Stuttgart, 1899, and more recently by MCCRUDDEN, *Uric acid*, Boston, 1905; also in part by WEBER, *Ergebnisse der Physiologie, biochemische Abtheilung*, 1904, pp. 266, 274.

² MACLEOD and HASKINS: *Journal of biological chemistry*, 1906, ii, p. 231.

³ Numerous analyses giving the purin content of food stuffs are given by WALKER HALL, *The purin bodies*, Philadelphia, 1903.

⁴ FOLIN and SHAFFER: *Zeitschrift für physiologische Chemie*, 1901, xxxii, p. 552.

⁵ FOLIN: *Ibid.*, 1904, xli, p. 223.

phoric acid. Usually each series was divided into a fore period, to learn the endogenous elimination, a drug period, and an after period, when only the original food was taken. Some typical protocols are given, with the averages of these, and of other unreported series. None of the subjects were engaged in hard muscular labor.

Series I. — Subject A, male, age 46, weight 120 pounds. The daily diet consisted of :

Milk	900 c.c.	Wheat crackers . . .	200 gm.
Eggs	100 gm.	Wheat bread	25 gm.
Butter	40 gm.	Strawberries	75 gm.

TABLE I.

Date.	Conditions.	Volume.	Reaction.	P ₂ O ₅ .	Nitrogen.	Uric acid.	Creatinin.
April. 12	Endogenous	c.c. 740	Acid	gm. 1.36	gm. 10.50	gm. 0.286	gm.
13	"	900	"	2.64	9.46	0.293
14	"	810	"	2.78	12.17	0.313
15	"	810	"	2.80	12.23	0.324	0.91
16	"	715	"	2.38	10.74	0.270	0.91
17	"	665	"	2.74	11.27	0.277	1.02
	Average, endogenous period	2.45	11.06	0.294	0.94
18	2 gm. lithium carbonate	1360	Alkaline	1.84	12.17	0.283	0.92
19	2 gm. lithium carbonate	1080	Faintly alkaline	1.90	11.08	0.250	1.18
20	2 gm. lithium carbonate	1190	Faintly acid	2.04	11.04	0.230	1.14
	Average, lithium period	1.93	11.43	0.254	1.08
21	Endogenous	710	Acid	2.45	11.44	0.277	0.89
22	"	700	"	2.97	12.31	0.269	1.05
23	"	682	"	2.56	11.60	0.387	1.06
	Average, endogenous period	2.66	11.78	0.311	1.00

The depressant effects of the lithium carbonate upon the general physical condition were noticeable; there was some loss of muscular control, the muscles of the lower limbs being affected, and those of the eye so much so that the power of accommodation was nearly lost.

While nitrogenous metabolism appears to be unchanged, the uric acid steadily decreased during the lithium period. The excretion of phos-

phoric acid also is less than that of the preceding or following period. The rise in creatinin is too slight to be attributed to the drug.

Series II. Subject A. The diet was free from purin except for that contained in the cocoa which was taken for breakfast. It has been repeatedly

TABLE II.

Date.	Conditions.	Volume.	Reaction.	P ₂ O ₅	Nitro- gen.	Uric acid	Crea- tinin.
Dec. 3	Endogenous	c.c. 1020	Acid	gm. 2.99	gm. 8.98	gm. 0.239	gm.
4	"	715	"	2.29	0.252	0.93
5	"	690	"	1.88	9.01	0.303	0.93
6	"	725	"	1.89	9.29	0.266	0.90
7	"	900	"	1.78	9.37	0.259	0.86
8	Average, endoge- nous period	2.17	9.16	0.264	0.91
9	10 gm. sodium bicarbonate	1160	Alkaline	1.58	8.64	0.311	0.93
10	10 gm. sodium bicarbonate	1020	"	1.66	7.88	0.231	0.76
11	10 gm. sodium bicarbonate	1120	"	1.85	8.11	0.253	0.90
12	10 gm. sodium bicarbonate	940	"	2.06	8.50	0.259	0.93
13	10 gm. sodium bicarbonate	1180	"	1.98	8.88	0.290	1.05
14	15 gm. sodium bicarbonate	1170	"	2.26	10.93	0.302	1.06
15	8 gm. sodium bicarbonate	1140	"	1.92	9.14	0.310	0.97
16	8 gm. sodium bicarbonate	1150	"	2.23	9.65	0.285	1.02
	Average, bicar- bonate period	1.94	8.97	0.280	0.95
17	Endogenous	1020	Acid	2.44	10.29	0.246
18	"	1075	"	1.93	5.39	0.240	0.86
	"	660	"	2.24	9.32	0.229	0.94
	Average, endoge- nous period	2.20	8.33	0.238	0.90

shown that the methyl purins do not increase the output of uric acid. Fauvel presented evidence of the fact recently;¹ the earlier literature is reviewed by McCrudden.² In addition it was shown to be true of the subject A, the data being given under Series VI. of this paper. The diet contained:

¹ FAUVEL: *Comptes rendus de l'Academie des Sciences*, 1906, cxli, p. 1428.

² MCCRUDDEN: *Loc. cit.* pp. 165-166.

Milk	900 c.c.	Wheat crackers . . .	200 gm.
Cocoa, prepared with milk	450 c.c.	Butter	40 gm.
Eggs	100 gm.	Cheese	20 gm.
Wheat bread	100 gm.	Apples	50 gm.

As before, there was a slight diminution of the phosphoric acid. The uric acid rose somewhat, but the rise is too insignificant to be attributed to the specific action of the bicarbonate. The creatinin remains nearly constant.

Series III. Subject A. The food was the same as in Series II.

TABLE III.

Date.	Conditions.	Volume.	Reaction.	P ₂ O ₅ .	Nitrogen.	Uric acid.	Creatinin.
Nov.		c.c.		gm.	gm.	gm.	gm.
19	Endogenous	915	Acid	2.36	10.85	0.290	0.89
20	"	850	"	2.09	8.81	0.300	0.89
21	"	850	"	2.12	9.41	0.294	0.96
22	Average, endogenous	2.19	9.69	0.295	0.92
23	5 gm. sodium citrate	790	Faintly acid	2.04	10.14	0.292	0.92
24	15 gm. sodium citrate	910	Alkaline	1.89	9.93	0.300	0.89
	25 gm. sodium citrate	1350	"	2.08	9.85	0.334	1.00
	Average, citrate period	2.00	9.97	0.309	0.94
25	Endogenous	940	Acid	1.98	9.72	0.318	0.86
26	"	1010	"	2.27	9.52	0.276	0.90
	Average, endogenous	2.13	9.62	0.297	0.88

While the uric acid was slightly increased, the additional amount is so little that it is to be regarded as within the limits of error of animal experimentation. The same is true of the creatinin.

Series IV. Subject B, male, age, 30; weight, 120 pounds. The daily diet consisted of:

Shredded wheat biscuit .	30 gm.	Butter	50 gm.
Sugar	30 gm.	Potatoes	375 gm.
Eggs	200 gm.	Milk	350 c.c.
Wheat bread	100 gm.	Cream	30 c.c.

Only purin-free food had been taken for five days previously.

TABLE IV.

Date.	Conditions.	Volume:	Reaction.	P ₂ O ₅	Nitro- gen.	Uric acid.	Crea- tinin.
Nov.		c.c.		gm.	gm.	gm.	gm.
5	Endogenous	800	Acid	2.37	10.90	0.357	1.01
6	"	1220	"	1.98	9.13	0.303	1.12
	Average, fore- period	2.18	10.02	0.330	1.07
7	120 grains sodium citrate	650	Neutral	2.03	9.45	0.359
8	240 grains sodium citrate	1000	Alkaline	2.20	7.96	0.253	0.92
9	240 grains sodium citrate	1310	"	1.84	9.89	0.341	1.12
10	120 grains sodium citrate	1270	"	1.88	9.30	0.362	1.10
	Average, citrate period	1.99	9.15	0.329	1.05
11	Endogenous	900	Neutral	1.73	9.17	0.284	1.18
12	"	870	"	1.98	9.91	0.308	1.13
13	"	1080	"	1.53	9.18	1.05
14	"	850	Acid	1.43	9.10	0.347	0.99
15	"	730	"	1.55	8.54	0.307	0.92
16	"	1000	"	1.94	9.04	0.321	0.90
	Average, endoge- nous	1.69	9.16	0.313	1.03
17	60 grains sodium bicarbonate	790	Alkaline	1.96	9.02	0.324	1.00
18	180 grains sodium bicarbonate	1310	"	2.31	9.22	0.372
19	180 grains sodium bicarbonate	1470	"	2.10	8.39	0.346
20	120 grains sodium bicarbonate	1200	"	1.64	8.79	0.322	0.96
21	180 grains sodium bicarbonate	820	"	1.90	9.36	0.161	1.13
22	60 grains sodium bicarbonate	850	"	1.76	8.78	0.263	1.06
23	180 grains sodium bicarbonate	900	"	1.29	7.32	0.158	0.96
	Average, bicar- bonate period	1.85	8.70	0.278	1.02
24	Endogenous	860	Neutral	1.55	9.58	0.296	1.08
25	"	1060	Faintly alkaline	2.09	8.60	0.260	0.98
26	"	530	" "	1.65	7.60	0.268	0.89
	Average, endoge- nous	1.76	8.59	0.275	0.98

Neither the bicarbonate nor the citrate of sodium caused marked variation in the excreted uric acid or the other urinary constituents which were determined.

Series V. Subject "Nephros;" male, age, 28; weight 155 pounds. The subject suffered from chronic albuminuria. The quantity of albumin in

TABLE V.

Date.	Conditions.	Volume.	P ₂ O ₅ .	Nitrogen.	Uric acid.
Jan. 13	Endogenous	c.c. 1100	gm. 4.22	gm. 10.49	gm. 0.393
14	"	950	1.64	12.50	0.400
20	"	1075	1.83	9.93
21	"	1050	1.40	12.17	0.302
22	"	0.385
23	"	900	2.16	10.60	0.472
24	"	950	2.11	11.07	0.434
	Average, endogenous	2.23	11.13	0.398
25	20 grains aspirin	850	0.265
26	30 grains aspirin	750	0.388
27	10 grains aspirin	1250	2.44	12.93	0.480
28	30 grains aspirin	1350	2.34	11.76	0.338
29	30 grains aspirin	750	2.36	7.54	0.348
	Average, aspirin period	2.38	10.74	0.364
30	Endogenous	900	1.69	0.393
31	"	950	2.87	13.20	0.472
Feb. 1	"	1100	2.45	10.37	0.394
2	" (two bottles of beer)	(0.553) ¹
3	"	1300	2.55	13.86	0.448
	Average endogenous, four days	2.39	12.48	0.427
4	30 grains lithium carbonate	1350	2.03	12.95	0.334
5	30 " " "	1550	1.91	12.91	0.273
6	30 " " "	1100	1.79	10.13	0.355
	Average, lithium carbonate period	1.0	12.00	0.321
7	Endogenous	1050	2.1	10.72	0.367

¹ Not included in the average.

the urine was small but constant, though the general health was good. No renal lesions could be positively determined. Before testing the urine the albumin was removed by boiling and filtration after acidification. The food contained no purin compounds, but the selection otherwise was left largely to the choice of the subject.

TABLE VI.

Date.	Conditions.	Volume.	P ₂ O ₅ .	Nitrogen.	Uric acid.	Creatinin.
May		c.c.	gm.	gm.	gm.	gm.
10	Endogenous	910	2.16	9.33	0.244	1.08
11	"	920	2.79	10.31	0.283	1.03
12	"	710	2.37	9.53	0.280	1.07
	Average endogenous	2.44	9.72	0.269	1.06
13	2 gm. aspirin	790	3.03	10.73	0.276	1.06
14	3 " "	565	3.25	9.74	0.522	0.89
15	Endogenous	700	2.00	10.18	0.183	1.07
16	2 gm. aspirin	750	2.74	10.56	0.334	1.07
17	2 " "	810	2.48	10.71	0.364	1.08
18	3 " "	785	2.57	11.09	0.473	1.05
	Average, aspirin period, five days	2.82	10.95	0.394	1.03
19	Endogenous	733	2.50	10.41	0.180	1.09
20	"	1020	2.90	11.62	0.250	1.12
	Average, endogenous	2.70	11.01	0.215	1.11
21	2 gm. sodium salicylate	1015	2.00	9.51	0.209	1.01
22	" " "	648	2.38	9.82	0.437	0.98
23	" " "	655	2.48	10.35	0.342	0.98
24	" " "	865	2.79	11.79	0.287	1.01
25	" " "	790	2.97	11.01	0.319	0.96
26	" " "	770	2.62	10.18	0.383	0.99
27	" " "	835	2.92	10.94	0.379	1.05
	Average, salicylate period	2.59	10.51	0.339	1.00
28	2 gm. sodium salicylate with 50 gm. chocolate	850	2.64	9.25	0.304	0.94
29	As on May 28	770	2.73	8.21	0.293	1.03
	Average chocolate period	2.69	8.73	0.299	0.99

The lithium carbonate acted as with Subject A, decreasing the output of uric acid and phosphoric acid without materially changing the nitrogen. No unpleasant symptoms were experienced. Aspirin, the acetic acid ester of salicylic acid, caused no increase in the uric acid eliminated, and this is noteworthy in view of the results with both of the other subjects as well as of those observed by other experimenters.¹ Whether the pathological condition referred to had any influence in preventing the usual rise in eliminated uric acid cannot be regarded as settled. The effect of alcohol in increasing the output of uric acid has been pointed out by Beebe;² it is very noticeable on one day with this subject.

Series VI. Subject A. The diet was the same as in Series I until the last two days, when 50 gm. of sweet chocolate were added. Here the

TABLE VII.
AVERAGES. SUBJECT A.

Date.	Dura- tion.	Conditions.	P ₂ O ₅ .	Nitro- gen.	Uric acid.	Crea- tinin.
April. 11-17	days. 6	Endogenous	gm. 2.45	gm. 11.06	gm. 0.294	gm. 0.94
18-20	3	Lithium carbonate	1.93	11.43	0.254	1.08
21-23	3	Endogenous	2.66	11.78	0.311	1.00
May. 10-12	3	Endogenous	2.44	9.72	0.269	1.06
13-14	2	Aspirin	3.14	10.24	0.399	0.98
15	1	No drug	2.00	10.18	0.183	1.07
16-18	3	Aspirin	2.60	10.79	0.390	1.07
19-20	2	No drug	2.70	11.01	0.215	1.11
21-27	7	Sodium salicylate	2.59	10.51	0.339	1.00
28-29	2	Salicylate + chocolate	2.69	8.73	0.299	0.99
Nov. 19-21	3	Endogenous	2.19	9.69	0.295	0.92
22-24	3	Sodium citrate	2.00	9.97	0.309	0.94
25-26	2	Endogenous	2.13	9.62	0.297	0.88
Dec. 3-7	5	Endogenous (Indigestion).	2.15	9.16	0.264	0.91
8-15	8	Sodium bicarbonate	1.98	8.97	0.280	0.95
16-18	3	Endogenous	2.20	8.33	0.238	0.90

¹ Cf. McCrudden: *Loc. cit.*, pp. 175, 245, and Weber: *Loc. cit.*

² Beebe: This journal, 1904, xii, p. 13.

aspirin undoubtedly caused more uric acid to appear in the urine, as did the sodium salicylate. The contrast with the results in Series V is marked. The sudden fall to much less than the normal endogenous amount of uric acid with the discontinuance of the aspirin is noteworthy. The comparative constancy of the nitrogen and phosphoric acid would indicate that the increase in the uric acid is not due to the destruction of nuclein compounds. It may be from oxidation of free purin compounds in accordance with Burian's¹ observations on the action of liver-pulp upon xanthin. That the theobromin of the chocolate does not increase the urinary uric acid is evident. The creatinin appears to be unaffected by the substances taken here.

The results of the experiments may be more clearly seen in Tables VII, VIII, and IX, which are the averages given of the experiments discussed above, also of others which are not more fully presented.

TABLE VIII.
AVERAGES. SUBJECT "NEPHROS."

Date.	Duration.	Conditions.	P ₂ O ₅ .	Nitrogen.	Uric acid.
Jan. 13-24	7	Endogenous . . .	gm. 2.23	gm. 11.13	gm. 0.398
27-29	3	Aspirin	2.38	10.74	0.364
Jan.-Feb. 30-3 ¹	4	Endogenous . . .	2.39	12.48	0.427
4-6	3	Lithium carbonate .	1.91	12.00	0.321
7	1	Endogenous . . .	2.10	10.72	0.367

¹ On Feb. 2 the subject drank two bottles of beer, and the uric acid rose to 0.553 gm. This was not included in the average.

SUMMARY.

1. When men are kept on a purin-free diet, or one where small and definite quantities of purin are present, the administration of lithium carbonate does not increase the elimination of uric acid; rather, it decreases it. The phosphoric acid is also lessened, although the amount of nitrogen is not affected.

2. Other compounds which make the urine alkaline, sodium citrate, sodium bicarbonate, and potassium acetate, even in large doses, do not increase the eliminated uric acid.

¹ BURIAN: Zeitschrift für physiologische Chemie, 1905, xliii, p. 497.

TABLE IX.
Averages. Subject B.

Date.	Dura- tion.	Conditions.	P ₂ O ₅ .	Nitro- gen.	Uric acid.	Crea- tinin.
Nov. 15-22, '05	days. 8	Endogenous	gm.	gm.	gm. 0.344	gm.
Jan. 28-31, '06	4	Endogenous	1.99	9.10	0.367
Feb. 1-4	4	Aspirin	1.64	9.23	0.405
5-12	7	Endogenous	1.77	8.12	0.293
April 7-11	5	Endogenous. . . .	1.59	8.45	0.343
12-14	3	Potassium acetate . .	0.93	5.56	0.334
20-24	5	Endogenous	0.357
25, 28, 29	3	Sodium salicylate . .	2.03	9.16	0.540	1.05
26, 27, 31- May 7	10	Endogenous	2.05	9.01	0.340	1.01
8-9	2	Colchicum	1.67	8.38	0.314	1.06
10-12	3	Endogenous	2.06	8.65	0.360	1.00
23-24	2	Endogenous	0.339
25-June 5	12	Aspirin	2.41	10.94	0.424	1.09
6-8	3	Endogenous	2.67	9.90	0.214	1.09
Nov. 5-6	2	Endogenous	2.18	10.02	0.330	1.07
7-10	4	Sodium citrate	1.99	9.15	0.329	1.05
11-16	6	Endogenous	1.69	9.16	0.313	1.03
17-23	7	Sodium bicarbonate .	1.85	8.70	0.278	1.02
24-26	3	Endogenous	1.76	8.59	0.275	0.98
Dec. 3-5	3	Endogenous	1.76	9.67	0.291
6-17	11	Sodium bicarbonate .	1.56	8.33	0.293
18-21	4	Endogenous	0.336

3. Colchicum acts like the lithium compound in decreasing, instead of increasing, the output of uric acid.

4. Both the sodium salt and the acetic acid ester (aspirin) of salicylic acid cause a marked increase in the urinary uric acid. In general the uric acid increases and decreases with the salicylic acid

compound administered. After the cessation of this administration the excreted acid falls much below the usual endogenous amount, then slowly rises to normal. In one case, where the subject had albuminuria, the aspirin did not increase the eliminated uric acid; but too positive conclusions cannot be drawn from this without further data.

5. In agreement with the observations of other investigators, the addition of chocolate to the food was not found to raise the amount of uric acid in the urine.

6. Creatinin was proved to be remarkably constant in quantity when these drugs were taken,—as much so as was found to be the case by Folin¹ with a meat-free diet.

As for the discrepancies between our results and those of Macleod and Haskins, we can only explain them on the ground of their experiments being of shorter duration, and their averages, consequently, less conclusive. It can be seen, not only from our data, but also from those previously obtained by one of us,² that while the average excretion, under constant conditions, shows very little variation with the same individual, the amounts excreted from day to day are subject to considerable fluctuations. This is true even when a fixed diet is maintained daily throughout the experiment.

¹ FOLIN: This journal, xiii, pp. 85, 86.

² ROCKWOOD: This journal, 1904, xii, pp. 38-54.

THE BLOOD PRESSURES OF BIRDS AND THEIR MODIFICATION BY DRUGS.

BY OSCAR RIDDLE AND S. A. MATTHEWS.

[From the Hull Laboratories of Zoölogy and Biochemistry, University of Chicago.]

IT has recently been shown by one of us¹ that the daily changes in the blood pressures of birds are able, acting through the nutrition, to modify the growth and differentiation of the tissues of feather germs in such a way as to produce marked effects on the morphology of the adult feathers. Such a relation between vascular tension and structure has been observed, so far as we know, nowhere else among animals. This gives a new interest to the blood pressures of birds. The question at once arises, do the effects on the feathers of the varying pressures depend upon the nature of the pressures, or on the nature of the feathers themselves? The work cited above shows that the nature of the growth and development of the feather is undoubtedly a factor, but this does not exclude the possibility that the vascular tension of birds may have something about it peculiar to birds and which enables it also to be an active factor. The latter would be conceivable if in the first place the absolute pressures in the birds were unusually high or low for warm-blooded animals; or secondly, if there were more extreme periodic variations in pressure in the birds than elsewhere among animals. A part of this paper deals with the first of these possibilities; the second can be tested easily and adequately only in birds of large size (*e. g.*, ostriches) which have not been available.

Another question which arises in connection with a consideration of the blood pressures of birds is, how do various drugs affect these pressures? This question first appealed to us because the drugs may afford us a means of testing experimentally certain points mentioned above regarding the relation of pressures to the nutrition of feather germs. It now seems that it may also have some practical or economic

¹ RIDDLE, O.: Biological bulletin, February, 1907, xii, pp. 165-175.

importance in connection with attempts to eliminate certain defects from the plumes of the ostriches. Duerden¹ particularly is studying these abnormal feather formations with a view to discover some means of eliminating them from the ostriches of South Africa. The effects of several drugs have been studied by us and the results are given in the second part of this paper.

MEASUREMENTS OF BLOOD PRESSURES.

Very few observations have been made on the blood pressures of birds. The only ones we have been able to find in the literature were made by Volkmann² and Blake.³ These total only five measurements of arterial pressures; no venous pressures were measured by either of these authors. Since these observations — made nearly sixty years ago — were so incomplete, it seemed advisable to repeat and extend them. In the following table we summarize our own readings of arterial pressures in mm. of mercury, and for the sake of completeness we add to these the observations previously made.

Our observations were made on birds anæsthetized with ether. Volkmann and Blake operated presumably without an anæsthetic. The pressures obtained by us therefore represent relatively higher values than the figures indicate, since the anæsthetic lowers the pressure in birds as it does in mammals. We used a mercury manometer and the usual methods of taking blood pressure tracings in mammals. It will be noted that our measurements reinforce those made earlier, which, though few in number, seemed to show that the arterial pressures of birds are in magnitude essentially comparable with those of mammals. Tracings taken simultaneously in the right brachial artery (3 cm. from distal end of humerus) and in the carotid (2.5 cm. from the innominate) showed nearly equivalent pressures — 174 mm. in the former and 176 mm. in the latter. The opening of the chest cavity always produced a lowering of the arterial pressure by several millimetres. The pressures for the innominate are lower than those for the carotid, partly, at least, from this cause, since the cannula could be inserted into the innominate only after opening the thorax. A tracing, showing a pressure of 164 mm. in the carotid of a duck is reproduced in Fig. 1.

¹ DUERDEN, J. E.: *Agricultural journal*, Cape of Good Hope, May, 1906.

² VOLKMANN, A. W.: *Die Hämodynamik*, Leipzig, 1850.

³ BLAKE, J.: Quoted in VOLKMANN, p. 177.

TABLE SHOWING THE ARTERIAL PRESSURES OF BIRDS.

Bird.	Vessel.	Mm. of Hg.	Observer.
Goose . . .	Carotid artery	162	Blake.
Stork . . .	Brachial artery	161	Volkman.
Hen . . .	Brachial artery	88	Volkman.
Cock . . .	Carotid artery	171	Volkman.
Pigeon . .	Brachial artery	157	Volkman.
Duck . . .	Carotid artery	164	
Duck . . .	Carotid artery	144	
Duck . . .	Carotid artery, 6 cm. from heart	169	
Cock . . .	L. innominate-chest cavity open	140	
Cock . . .	L. innominate-chest cavity open	122	
Cock (old) .	Carotid artery	88	
Goose . . .	Carotid artery, 2½ cm. from in-		
	nominate	176	
Goose . . .	R. brachial artery	174	
Goose . . .	R. brachial artery	134	
Goose . . .	Carotid artery	129	
Goose . . .	Carotid artery	144	

We have attempted a comparison of the venous pressures of birds with those mammals. Measurements of the pressure on the parietes

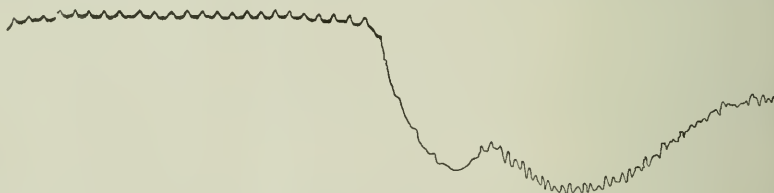


FIGURE 1. — Showing a pressure of 164 mm. Hg in the carotid artery of a duck. A trace of amyl nitrite caused a temporary fall of pressure to 100 mm.

of avian veins is attended with considerable difficulty, and this we assume to be the chief reason why the workers have hitherto neglected these, for the larger, more accessible veins of mammals. We

have succeeded in getting four trustworthy readings from the brachial veins of ducks which we place in the form of a table:

Bird.	Vessel.	Manometer.		
		H ₂ O mm.	Hg mm.	
Duck	L. brachial	88	6.5	} 3 cm. from distal end of humerus.
Duck	L. brachial	47	3.5	
Duck	L. brachial	47	3.5	
Duck	R. brachial	58	4.3	

In taking these pressures the birds were anæsthetized, placed on their backs, their necks extended (slightly stretched) in the line of the body axis, and the legs drawn backward and abducted. The wing was elevated so that the operated vein, the heart, and manometer zero were in exactly the same horizontal plane. A specially constructed manometer was used in which water was substituted for mercury. For the sake of convenience in comparison, the mercury equivalents are given in the table. The manometer was connected with a "T" tube having an internal diameter equal to that of the vein. During the readings deep anæsthesia was avoided. The respiratory movements had considerable influence on the tracings. Compression of the chest with the hand always raised the pressure. It was noticed also that by lightly touching a feather anywhere on the body, one always produced a marked rise of pressure. The effect on the venous pressure of opening the chest wall was not studied by us.

A comparison of the pressures obtained in the brachial artery of the duck, with those obtained by others in the brachial artery of mammals, shows that the two are very similar. Burton-Opitz¹ found this pressure to be 3.7 mm. Hg in one dog and 4.2 mm. in another. Jacobsen² found a pressure of 4.1 mm. in the brachial vein of a sheep. In a branch of the brachial he found a pressure of 9 mm. Hg. The tracing of a venous pressure is shown in Fig. 2.

It would be extremely desirable to determine experimentally the capillary pressure in birds; unfortunately the methods thus far devised

¹ BURTON-OPITZ, R.: This journal, 1903, ix, pp. 198-214.

² JACOBSEN: From Tigerstedt, Text-book of physiology, 1906, p. 233, New York and London.

for taking such pressures are too crude to be of much use. From the results obtained above, however, we are justified in concluding that the capillary pressure in birds is equal to that in mammals,¹ or about 30 mm. Hg. This would seem to follow necessarily from the demonstration of equivalent arterial and venous pressures in the two groups. If then the pressure in the fluid immediately in contact with the cells is the same in birds and in mammals, this would give a negative answer to the first question we asked ourselves. That is to say, the blood pressure in birds is not exceptionally high for warm-blooded animals, and therefore we may not expect the pressure, merely because of its magnitude, to produce unusual effects. The daily fluctuations of this pressure may, however, be more or less considerable than those of mammals; we do not know.

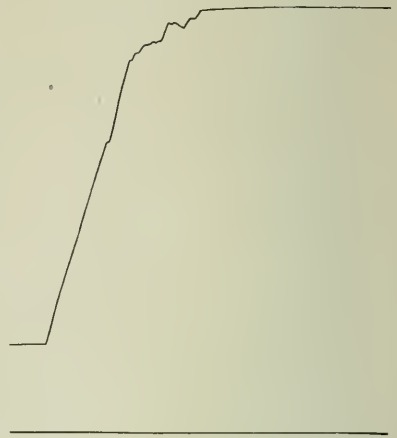


FIGURE 2.—The pressure in the v. brachialis of a goose, = 6.5 mm. Hg, recorded by a water manometer. The pressure in the carotid of this bird was 144 mm. Hg.

THE EFFECTS OF DRUGS ON THE ARTERIAL PRESSURES OF BIRDS.

We have not attempted an exhaustive study of the effects of drugs on the blood pressures of birds. It has been our purpose to determine the effects in birds of a considerable number of drugs which have strong and well-known effects on the pressures of mammals. We herewith give an account of the action of digitalis, squills, barium chloride, barium chloride with calcium chloride, magnesium sulphate, ergot, and amyl nitrite. The action of certain organic acids has been studied, but it does not seem advisable to discuss these results at this time. It can be said of all the drugs here reported upon, that their action in birds is essentially the same as in mammals.

Digitalis. — With small doses ($\frac{1}{2}$ c.c.) of the fluid extract of digitalis, well diluted, it was easy to produce the rise of blood pressure, which

¹ v. KRIES, N.: Berichte der Sächs. Gesellschaft der Wissenschaften; Math-Phys. Kl., 1875, pp. 148-160. †

characterizes the first stage of its action. Fig. 3 shows only a very slight slowing of the heart, and no changes in the ventricular contractions; but the rise in blood pressure is very pronounced, equaling 56 mm. Hg. While little or nothing is shown by the blood pressure about the changes in the ventricular contractions, yet it is fair to assume that the same changes take place here as in mammals, *i. e.*, an increased ventricular contraction, and consequently an increased output per beat as well as per unit of time.



FIGURE 3.—Action of digitalis (first stage) in a goose. A very weak solution was used and two injections made, 1 c.c. and 2 c.c.

This marked rise of blood pressure clearly indicates a vascular contraction, exhibiting itself chiefly on the arterioles of the splanchnic area, as in strophanthus poisoning. This vascular contraction may be brought about by the drug acting on two separate parts of the nervous system: first, it may stimulate the vasomotor centre in the medulla oblongata; or, second, it may stimulate the peripheral ganglia cells with which the vasomotor nerves make connection. In the case of

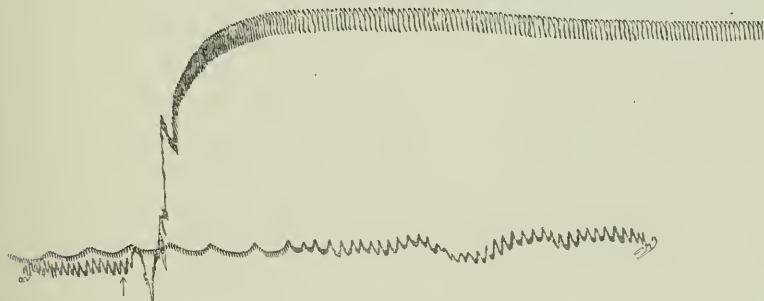


FIGURE 4.—The rise in pressure produced in the carotid of a duck by the injection of 1 c.c. of $\frac{1}{100}$ BaCl₂. The normal pressure was 169 mm. Hg.

digitalis and its congeners, the stimulation probably takes place both centrally and peripherally. If this interpretation of the action of digitalis on fowls is correct, the blood supply to the skin, and consequently its nutrition, should be increased by this drug.

Squills.—A fatal dose of squills caused a marked slowing of the rate of the heart, accompanied by a corresponding fall in the blood pressure. The slowing of the heart is due to vagus stimulation both

centrally and peripherally, and the fall in blood pressure is simply a result of the extreme slowing of the heart.

Barium chloride.—Like digitalis and squills, barium chloride ($\frac{m}{100}$ in doses of 1 c.c.) has a similar effect upon the intravascular pressure of fowls and mammals. Figs. 4 and 5 show the marked rise in the blood pressure produced by the intravenous injection of 1 c.c. $\frac{m}{100}$ barium chloride. Barium chloride seems to influence the contractile tissue in the walls of the blood vessels in the same way that it does

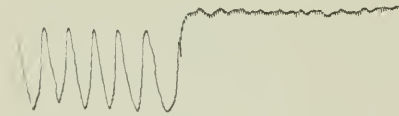


FIGURE 5.—Effect of 1 c.c. of $\frac{m}{100}$ barium chloride given several minutes after the injection of ergot into vein of rooster.



FIGURE 6.—The tracing shows a fall from 130 mm. to 50 mm. pressure in the brachial artery of a goose. Between the arrows 2 c.c. of 25 per cent magnesium sulphate were injected.

striated and non-striated muscle when directly applied; that is, it increases the irritability and contractility of the muscle. This action, so far as known, is direct on the muscle, and not necessarily through the intervention of any nervous mechanism.

Barium chloride and calcium chloride.—Barium chloride with calcium chloride in the quantity to make an $\frac{m}{100}$ solution of each does not give the characteristic barium rise in blood pressure. As is well known, calcium antagonizes barium on all contractile tissues. This antagonism was effective in these experiments, notwithstanding the fact that calcium itself causes a moderate rise in blood pressure.

Magnesium sulphate.—Magnesium sulphate ($\frac{m}{2}$) in 1 c.c. doses injected intravenously produces its characteristic anæsthetic effects, together with marked slowing and weakening of the heart, accompanied by a fall of blood pressure. The respiration is affected earlier than the heart. This is its characteristic effect upon mammals. Its very strong action is shown in Fig. 6.

Ergot.—In the fowl, as in mammals, ergot causes a primary fall of blood pressure, followed by a slow and continuous rise to 10–15 mm. Hg above normal. The primary fall is unexplained. The succeeding moderate rise in blood pressure is generally accredited to a constriction of the arterioles, brought about by a direct action of the ergot upon certain non-striated muscular tissues, including the heart

and blood vessels. While this does not preclude the theory that ergot acts upon the vessels through some nervous mechanism, investigators are at such variance on this point as to preclude its further discussion in this connection.

In the case of fowls, however, ergot poisoning generally takes a form indicative of a lack of nutrition, especially in those parts in which the vessels are small or capillary-like. This is shown by gangrene of the comb, wattles, tip of the tongue, and even the tips of the wings. The spurs may suffer a similar change. This is supposed to be due to a continued contraction of

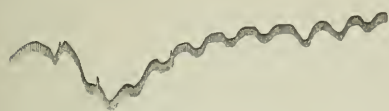


FIGURE 7. — Effects of ergot on the pressure in the left innominate of a rooster. Normal pressure, 122 mm.

the capillaries in these parts to such a degree as to shut off their blood supply. If this be true, the supply to the skin, and consequently to growing feathers, might easily be reduced by the presence of ergot in the blood of the fowl. In Fig. 7 the effects of ergot are shown by a tracing.

Amyl nitrite. — When amyl nitrite is inhaled by fowls, an immediate and pronounced fall of blood pressure occurs. A simultaneous and extensive vaso-dilatation can easily be observed in the small vessels of the nearly transparent patagium of the wing. An increased redness of the comb, wattles, and of naked patches of skin on all parts of the body, reveals a general dilatation of the superficial vessels. This dilatation of the arterioles is undoubtedly due to the direct action of the drug on the contractile tissue in the walls of the vessels. This effect of amyl nitrite on the calibre of the small vessels of the skin and viscera has been observed in several genera of birds, and has been proved to occur in chicks of all ages. The effect of amyl nitrite on the blood pressure in a duck is shown in Fig. 1. In this case a fall of two fifths of the total pressure was produced by a mere trace of the vapor. It is not difficult to maintain the pressure at only one fifth of the normal pressure for considerable periods of time; the pressure can, of course, be brought to zero immediately if the dose is increased.

The action of this drug has been studied with particular care since certain experimental results mentioned in the introduction of this paper are based on its power to diminish the vascular tension in growing feather germs.

Magnesium sulphate and amyl nitrite are certainly very reliable

and efficient drugs for lowering the vascular pressure of birds, while digitalis — in proper amounts — and barium chloride have proved to be very efficient in the maintenance of a high vascular tension. It would seem possible for the ostrich farmer to dose his birds with either of the two latter drugs, and thus improve the nutrition of the plumes¹ during their period of growth.

SUMMARY.

The results to which we would call especial attention are the following :

1. The arterial pressures of birds, as the few observations of Blake and Volkmann seemed to show, are of practically the same magnitude as those of mammals.

2. The venous pressures in the wings of birds are closely comparable in magnitude with the venous pressures in the fore-limbs of mammals.

3. The capillary pressure in birds is probably the equivalent of that in mammals.

4. The effects of the blood pressure in birds on any developing structure would seem, so far as our observations go, to indicate that these effects depend upon the structures themselves rather than upon the nature of the pressures. We do not know, however, what the magnitude of the daily variations in these pressures is.

5. A considerable variety of drugs affect the blood pressures of birds as they do those of mammals.

6. These observations indicate that the presence in the blood of digitalis, or barium chloride, aids the nutrition of the superficial structures of birds, while on the other hand, the presence of amyl nitrite, magnesium sulphate, or ergot, interferes with the nutrition of the skin and its appendages.

¹ The ostrich plumes suffer a depreciation of from 20 to 50 per cent of their value by the frequent appearance in them of certain defects. It has been shown by one of us (R) that similar defects occur in all birds, and, further, that they represent the stunted growth which occurs under the poor nutritive conditions brought about by a nightly lowering of the blood pressure. The plumes of the South African, Florida, and California ostriches are all thus more or less affected.

HYDROLYSIS OF HORDEIN.¹

By THOMAS B. OSBORNE AND S. H. CLAPP.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

THE seeds of barley, like those of other cereals, contain a relatively considerable amount of protein soluble in alcohol of from 70 to 80 per cent by volume. An investigation of this seed, which was made some years ago in this laboratory, gave no evidence of the presence of more than one protein soluble in alcohol.² Extensive fractionations of this protein yielded products of the same composition and properties, and it was therefore proposed to call it hordein. The composition of hordein as shown by closely agreeing analyses of a large number of different preparations is: C 54.29; H 6.80; N 17.21; S 0.83; O 20.87 per cent.

The preparation of hordein used for this hydrolysis was made from freshly ground barley flour³ by extracting with cold 75 per cent (by volume) alcohol. The alcoholic extract was filtered perfectly clear and concentrated to a thin syrup on a water bath at about 70°, under strongly reduced pressure. This concentrated solution was then poured into a large volume of distilled water containing much pure ice and, after washing with water, the precipitate was again dissolved in 75 per cent alcohol and its clear solution poured into several volumes of absolute alcohol. The resulting precipitate of hordein was digested with absolute alcohol until thoroughly dehydrated and the alcohol removed in the desiccator by sulphuric acid. A sample of the hordein thus prepared, after grinding fine and drying at 110°, was extracted for a long time with ether but yielded only traces of substance soluble therein.

¹ The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

² OSBORNE: *Journal American Chemical Society*, 1895, xvii, p. 539.

³ We are indebted to Dr. Frank Fuller of the Health Food Company of New York for this barley flour which he gave expressly for us. We wish here to express our thanks for his kind assistance.

Seven hundred grams of hordein, equal to 628.7 gm. ash and moisture free, were dissolved in a mixture of 700 c.c. of hydrochloric acid of specific gravity, 1.2 and 700 c.c. of water, by warming on the water bath for eight hours. The solution was then boiled for fifteen hours in a bath of oil and the hydrolysis completed.

After concentrating somewhat under reduced pressure the hydrolysis solution was saturated with hydrochloric acid gas and allowed to stand at 0°. The yield of glutaminic acid hydrochloride, after deducting for the ammonium chloride present, was 237.16 gm., equivalent to 189.73 gm. of glutaminic acid, which, with the 24.62 gm. isolated from Fractions V and VI of the esters, makes the total of glutaminic acid obtained in this hydrolysis 214.35 gm. or 34.07 per cent of the protein, while Osborne and Gilbert¹ found 36.35 per cent by their direct determination. The free acid decomposed at about 202°–203°.

Carbon and hydrogen, 0.2768 gm. subst., gave 0.4114 gm. CO₂ and 0.1547 gm. H₂O.

Calculated for C₅H₉O₄N = C 40.82 ; H 6.12 per cent.

Found = C 40.53 ; H 6.21 “ “

The filtrate from glutaminic acid hydrochloride was concentrated very sharply, under reduced pressure, and the very thick syrup esterified with alcohol and dry hydrochloric acid gas, by the method of Emil Fischer. The free esters were then liberated, shaken out, and dried in the usual manner. As a repetition of this process materially increases the yield of ester, the aqueous layer was freed from inorganic salts and the esterification repeated. By distillation under diminished pressure, the esters were divided into the following fractions:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	65°	20 mm.	22.1 gm.
II	85°	9 “	38.4 “
III	a 100°	9 “	37.1 “
	b 100°	2 “	53.4 “
	c 100°	0.48 “	46.7 “
IV	140°	0.33 “	63.5 “
V	200°	0.33 “	60.4 “
VI	210°	0.33 “	13.3 “
Total			334.9 gm.

The undistilled residue weighed 68 gm.

¹ OSBORNE and GILBERT: This journal, 1906, xv, p. 338.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	65°	20 mm.	22.1 gm.

From this fraction no glycocoll could be separated as the hydrochloride of the ethyl ester. It seemed to consist largely of leucine and alanine and was accordingly added to Fraction II.

Fraction.	Temp. of bath up to	Pressure.	Weight.
II	85°	9 mm.	38.4 gm.

The esters of this fraction were boiled with water until the alkaline reaction had ceased. After evaporating to dryness under strongly reduced pressure, the proline was extracted with boiling alcohol and the part remaining undissolved subjected to fractional crystallization from water and from water and alcohol. There were obtained from the less soluble part 4.75 gm. of leucine and 0.8 gm. of substance of perfectly homogeneous appearance, which on analysis gave figures agreeing best with a mixture of equal parts of leucine and valine.

Carbon and hydrogen, 0.1970 gm. subst., gave 0.3817 gm. CO₂ and 0.1716 gm. H₂O.

Calculated for equal molecules of leucine and valine

Found $\left\{ \begin{array}{l} = \text{C } 53.12; \text{ H } 9.66 \text{ per cent.} \\ = \text{C } 52.84; \text{ H } 9.67 \end{array} \right.$

The more soluble portion of this fraction was examined for glycocoll, but none could be isolated as the hydrochloride of the ethyl ester.

By systematic fractional crystallization there were obtained 2.72 gm. of alanine and 0.80 gm. of amino-valerianic acid. The alanine decomposed at about 290°.

Carbon and hydrogen, I, 0.1418 gm. subst., gave 0.2114 gm. CO₂ and 0.0972 gm. H₂O.

II, 0.1538 gm. subst., gave 0.2268 gm. CO₂ and 0.1094 gm. H₂O.

Calculated for C₃H₇O₂N = C 40.45; H 7.86 per cent.

Found = $\left\{ \begin{array}{l} \text{I, C } 40.66; \text{ H } 7.61 \text{ per cent.} \\ \text{II, C } 40.22; \text{ H } 7.90 \text{ " "} \end{array} \right.$

The valine gave the following analysis:

Carbon and hydrogen, 0.1351 gm. subst., gave 0.2546 gm. CO₂ and 0.1165 gm. H₂O.

Calculated for C₅H₁₁O₂N = C 51.28; H 9.40 per cent.

Found = C 51.40; H 9.58 " "

Dissolved in 20 per cent hydrochloric acid (a) $\frac{20^\circ}{D} = +26.95^\circ$.

Fraction.	Temp. of bath up to	Pressure.	Weight.
III	100°	0.48 mm.	137.2 gm.

This fraction consisted mainly of the ester of *a* proline. It was saponified by boiling with water to the cessation of the alkaline reaction and the solution rapidly evaporated to dryness under strongly reduced pressure. The part remaining undissolved in absolute alcohol consisted almost wholly of leucine. The yield was 30.89 gm.

Carbon and hydrogen, 0.1946 gm. subst., gave 0.3930 gm. CO₂ and 0.1746 gm. H₂O.

Calculated for C₆H₁₃O₂N = C 54.96; H 9.92 per cent.

Found = C 55.08; H 9.97 " "

The substance decomposed at about 298°.

The alcohol solutions of the proline of Fractions II and III were united. After concentrating under reduced pressure and precipitating with ether, the substance was obtained as a white crystalline mass, which when carefully dried weighed 86.32 gm. On redissolving in absolute alcohol, the proline separated in the characteristic prisms, melting at 200°-205°.

Carbon and hydrogen, 0.2737 gm. subst., gave 0.5235 gm. CO₂ and 0.1981 gm. H₂O.

Calculated for C₅H₉O₂N = C 52.18; H 7.83 per cent.

Found = C 52.16; H 8.04 " "

For the strict identification, the substance was converted into the copper-salt and by boiling the latter with alcohol, the *lævo* separated from the racemic.

The insoluble copper-salt of *lævo*-proline crystallized from water in the characteristic plates containing two molecules of water-of-crystallization.

Water, 0.3447 gm. subst. (air-dried), lost 0.0377 gm. H₂O at 110°.

Calculated for C₁₀H₁₆O₄N₂Cu · 2H₂O = H₂O 10.99 per cent.

Found = H₂O 10.94 " "

Copper, 0.3026 gm. subst., gave 0.0817 gm. CuO.

Calculated for C₁₀H₁₆O₄N₂Cu = Cu 21.81 per cent.

Found = Cu 21.57 " "

The phenylhydantoin of the *lævo*-proline melted at 143°.

Carbon and hydrogen, 0.2668 gm. subst., gave 0.6547 gm. CO₂ and 0.1313 gm. H₂O.

Calculated for C₁₂H₁₂O₂N₂ = C 66.67; H 5.57 per cent.

Found = C 66.92; H 5.47 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
IV	140°	0.33 mm.	63.5 gm.

From this fraction the ester of phenylalanine was removed with ether in the usual way. The weight of the hydrochloride of phenylalanine obtained was 16.36 gm.

The free acid decomposed at about 280° on rapid heating.

Carbon and hydrogen, 0.1489 gm. subst., gave 0.3583 gm. CO₂ and 0.0875 gm. H₂O.

Calculated for C₉H₁₁O₂N = C 65.45; H 6.66 per cent.

Found = C 65.63; H 6.53 " "

The phenylisocyanate derivative melted at 177°-178° (uncorr.).

Carbon and hydrogen, 0.1126 gm. subst., gave 0.2796 gm. CO₂ and 0.0609 gm. H₂O.

Calculated for C₁₆H₁₆O₃N₂ = C 67.60; H 5.63 per cent.

Found = C 67.72; H 6.01 " "

The aqueous layer was saponified by warming with an excess of baryta. After prolonged standing no barium aspartate had separated. The barium was accordingly removed with sulphuric acid and after concentration the solution was saturated with hydrochloric acid gas.

The precipitate that separated at 0° weighed 3.98 gm. and consisted of very nearly pure phenylalanine hydrochloride. The filtrate from phenylalanine was examined for aspartic acid; but efforts to isolate this substance, either as the copper or barium salt or in the form of the free acid, failed.

Fraction.	Temperature of bath up to	Pressure.	Weight.
V	200°	0.33 mm.	60.4 gm.
VI	210°	0.33 mm.	13.3 gm.

From this fraction there were isolated in the usual way 14.97 gm. of phenylalanine as the hydrochloride. The remainder of the fraction consisted mainly of glutaminic acid, which was isolated partly as the barium salt and partly in the form of the hydrochloride. The yield was 24.62 gm. of the free acid.

The substance decomposed at about 202°–203°.

Carbon and hydrogen, 0.1933 gm. subst., gave 0.2878 gm. CO₂ and 0.1035 gm. H₂O.

Calculated for C₅H₉O₄N = C 40.81; H 6.12 per cent.

Found = C 40.61; H 5.94 “ “

. The filtrate from glutaminic acid hydrochloride yielded no definite substance. It did not seem to contain an appreciable quantity of aspartic acid, while neither here nor in the filtrate from phenylalanine hydrochloride of Fraction IV could preparations be obtained which gave evidence of the presence of serine. In the filtrates from glutaminic acid hydrochloride of Fractions V and VI, after removal of the chlorine and subsequent conversion to the copper-salts, there was obtained, after slow concentration over sulphuric acid, a small quantity of copper-salt crystallizing in needle prisms, very unlike copper aspartate and of much greater solubility in water and which was possibly the copper-salt of a new amino acid. Unfortunately the amount of this substance was too small to warrant further investigation.

TYROSINE.

A quantity of hordein equal to 246 gm. ash and moisture free, was boiled with three times its weight of sulphuric acid and six times its weight of water for eight hours, and after removing the sulphuric acid with an equivalent quantity of barium hydroxide the tyrosine was separated by concentration and cooling. The crude tyrosine was dissolved in 5 per cent sulphuric acid and the solution treated with phosphotungstic acid. After removing the phosphotungstate precipitate the solution was freed from phosphotungstic and sulphuric acids with barium hydroxide and from barium with an equivalent quantity of sulphuric acid and 4.14 gm. of tyrosine separated by crystallization equal to 1.67 per cent.

Nitrogen, 0.2333 gm. subst., required 1.81 c.c. 5/7 N-HCl.

Calculated for C₉H₁₁O₃N = N 7.73 per cent.

Found = N 7.76 “ “

The precipitate produced by phosphotungstic acid in the solution of the crude tyrosine was decomposed with baryta, and after removing the barium, the solution was concentrated and the precipitation with phosphotungstic acid repeated until the Millon's reaction had ceased.

The filtrate from barium phosphotungstate, freed from barium with sulphuric acid, separated sparingly very thin prisms of brilliant mother-of-pearl lustre, which proved identical with a substance recently isolated by us¹ by a similar method from gliadin, and which we feel justified in regarding as a dipeptide of proline and phenylalanine. When heated side by side with a pure preparation from gliadin, the substance decomposed simultaneously with the latter at about 249° (uncorr.), and when mixed the decomposition point was not lowered.

HISTIDINE.

Fifty grams of hordein, equal to 44.91 gm. ash and moisture free, were hydrolyzed and the bases determined according to the method of Kossel and Patten. The solution of the histidine was made up to 500 c.c. and found to contain nitrogen equal to 0.5770 gm. of histidine or 1.28 per cent.

Nitrogen, 100 c.c. solution required 3.13 c.c. 5/7 N—HCl = 0.0313 gm. N = 0.1565 gm. N in 500 c.c. = 0.5770 gm. histidine = 1.28 per cent.

The histidine was converted into the dichloride which decomposed at 232°–233°.

Chlorine, 0.0770 gm. subst., gave 0.0963 gm. AgCl.

Calculated for $C_6H_{11}O_2N_3Cl_2 = Cl$ 31.14 per cent.

Found = Cl 30.92 “ “

ARGININE.

The solution of the arginine was made up to 1000 c.c. and found to contain nitrogen equal to 0.972 gm. arginine or 2.16 per cent.

Nitrogen, 100 c.c. solution required 2.9 c.c. 5/7 N—HCl = 0.0290 gm. N = 0.2900 gm. N in 1000 c.c. = 0.9 gm. arginine. Adding 0.072 gm. for solubility of arginine silver = 0.972 gm. = 2.16 per cent.

The arginine was converted into the copper nitrate double salt.

Water, 0.1069 gm. subst., air dry, lost 0.0099 gm. H₂O.

Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2 \cdot 3 H_2O = H_2O$ 9.16 per cent.

Found = H₂O 9.26 “ “

Copper, 0.0962 gm. subst., dried at 100°, gave 0.0143 gm. CuO.

Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2 = Cu$ 11.87 per cent.

Found = Cu 11.87 “ “

¹ OSBORNE and CLAPP: This journal, 1907, xviii, p. 123.

LYSINE.

No lysine picrate could be obtained by the usual method, which confirms the recent observation of Brown,¹ who likewise failed to detect lysine in this protein.

The results of this hydrolysis were as follows :

	Per cent.		Per cent.
Glycocoll	0.00	Cystine	undetermined
Alanine	0.43	Tyrosine	1.67
Valine	0.13	Oxyproline	undetermined
Leucine	5.67	Arginine	2.16
Proline	13.73	Histidine	1.28
Phenylalanine	5.03	Lysine	0.00
Aspartic acid	not isolated	Ammonia	4.87
Glutamic acid	36.35	Tryptophane	<u>present</u>
Serine	not isolated	Total	71.32

This hydrolysis shows that hordein is characterized by marked differences in the proportion of its decomposition products when contrasted with the other proteins that have been thus far analyzed. Like the other alcohol soluble proteins, it yields no lysine, relatively little histidine and arginine, and much ammonia. The very large proportion of glutamic acid is practically the same as that obtained from gliadin.

The most marked feature, however, is presented by the very large proportion of proline, which greatly exceeds that yet obtained from any other protein, being practically twice as much as the relatively large quantity yielded by gliadin.

¹ BROWN: Transactions of the Guinness Research Laboratory, 1906, i, pt. ii, p. 229.

A STUDY OF RELATIVE RHYTHMICITY AND CONDUCTIVITY IN VARIOUS REGIONS OF THE AURICLES OF THE MAMMALIAN HEART.

BY JOSEPH ERLANGER AND JULIAN R. BLACKMAN.

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

INTRODUCTION.

EXCISION from the perfused rabbit's and cat's heart of that region of the right auricle which is included between the mouths of the venæ cavæ results, according to the experiments of Langendorff and Lehmann,¹ in permanent stoppage of the auricles and in slowing of the rate of the ventricles, sometimes preceded by a transitory stoppage of the ventricles. After such a cut the auricles do not respond even to the contractions of the ventricles. These authors therefore conclude, and later experiments performed by Lehmann² seem to confirm them in this conclusion, that the auricles, outside of the region of the great veins, are not spontaneously rhythmical.

When these experiments were brought to our attention, we could not understand why the auricles did not respond to ventricular contractions at least. It has been definitely established that impulses travel from auricles to ventricles, and in the reverse direction through the auriculo-ventricular bundle, and through that only.³ Therefore a cut which severs the auricles from the region of the great veins, and which does not intersect the auriculo-ventricular bundle, should not, we thought, prevent impulses of ventricular origin from stimulating the auricles unless, and this seemed rather remote, such a cut annuls the irritability of the auricles. Inspection of a heart or two convinced us that a cut similar to the one practised by Langendorff and Lehmann

¹ LANGENDORFF and LEHMANN: *Archiv für die gesammte Physiologie*, 1906, cxii, p. 352.

² LEHMANN: *Archiv für die gesammte Physiologie*, 1906, cxii, p. 522.

³ ERLANGER: *Journal of experimental medicine*, 1906, viii. p. 8. Here references to earlier publications will be found.

could not well have intersected the auriculo-ventricular bundle, and we were therefore still further from a satisfactory explanation of their results. For these reasons we undertook to repeat the experiments of Langendorff and Lehmann. From the very beginning our results differed from those of the authors mentioned, and in our effort to harmonize our results with theirs our preliminary tests have grown into an investigation of some magnitude.

EXPERIMENTS ON THE DOG'S HEART IN SITU.

(a) **Methods.** — In view of the successful results obtained by one of us in a study of the physiology of heart block in mammals upon

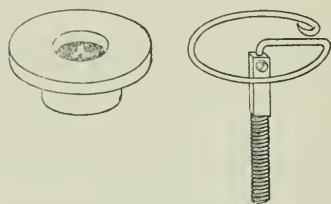


FIGURE 1. — Auricular clamp.

hearts preserving their usual relation to the organism, an attempt was first made to clamp off in the living animal the region in which the heart beat is supposed to originate. We felt that if satisfactory experiments could be performed in this way the results obtained would be more valuable, at least in their practical bearings, than any that might be gathered from experiments upon the excised, perfused heart. Therefore a clamp was devised by means of which all of the area could be clamped off in which, according to MacWilliam,¹ Adam,² and others, the heart beat originates.

This clamp was made in two pieces, — a piece to be inserted into the cavity of the auricle and a piece to be applied to the outer surface of the auricle. The former consisted of a piece of piano wire 1.7 mm. in diameter bent into the form shown in Fig. 1. This form was such that once the point had pierced the outer wall of the auricle the after-following turn of wire could be screwed into the auricle with the assurance that the point would not catch in, or again perforate, the wall of the auricle. Thus a large ring of wire could easily be inserted into the auricle through a small puncture. The hæmorrhage through this puncture was usually slight, and under any circumstances soon ceased. The ring was fastened into a brass bar, the proximal end of which was square on cross section, the far end round and threaded.

¹ MACWILLIAM: *Journal of physiology*, 1888, ix, p. 167.

² ADAM: *Archiv für die gesammte Physiologie*, 1906, cxi, p. 607.

The other essential part of the clamp consisted virtually of a brass plate, with a diameter slightly greater than that of the wire ring. This plate was perforated at its middle point by a square hole into which fitted the square rod. When the plate was placed on the rod, the threaded portion of the latter projected above the former. By driving a nut on this part of the rod the ring could be drawn up against the surface of the plate. The intervening tissue which could thus be crushed was not a complete circle, owing to the fact that the wire ring was imperfect where the point of the ring did not quite reach the opposite turn of the wire. Here there was a space of about 2 mm., through which the wall of the auricle might be slipped while inserting the ring into it.

In order to place this auricular clamp upon the heart, the chest is opened in the usual way while maintaining respiration by means of a modification of the Brauer method. The pericardium is opened widely and cut down on the left side to its line of reflexion over the right auricle. The right pulmonary veins are doubly ligated close to their origin and cut. This operation gives good exposure of the entire right auricle. The point of the ring is then rested against the right auricle, at a place about midway between the mouths of the cavæ and about 3 mm. ventral to the line of reflexion of the pericardium. It is now an easy matter to force the ring into the auricle through the hole made by the point at this place. In successful experiments the position of the ring, as determined at autopsy, was found to be approximately as follows: Viewed from without, the ring passes dorsally below the line of reflexion of the pericardium, ventrally it reaches to the base of the appendage and cephalad and cordad to the mouths of the cavæ, which may be somewhat stretched over the ring to accommodate it.

(b) **Results.**—The results obtained by thus compressing the right auricular area described were, on the whole, negative. Usually at the time sudden compression was made, the heart exhibited momentary irregularities, but soon the heart beat, as well as the rate, became normal again. After thus suddenly compressing an almost complete ring of tissue, this ring was completed either by turning the clamp through an angle of 180° and again crushing, or by crushing the auricular wall in the defect with an instrument of appropriate form. Excepting a repetition of the heart irregularities, these last manipulations were without significant results.

It will be noted that the clamp crushes only the adjacent portions

of the mouths of the great veins, and the objection might be raised that negative results were obtained because the clamp failed to compress the whole of the region surrounding the openings of the cavæ into the auricle. An attempt was therefore made to meet this objection by crushing these regions with forceps, making the line crushed around the mouths of the veins continuous with the ring of tissue crushed by the clamp. The results obtained were, however, negative ;



FIGURE 2.—To show stoppage of the whole heart resulting from slight tension upon the auricles through the medium of the auricular clamp. Dog.

but we attach no significance to them owing to the uncertainties of the method and to the fact that the crush so made was not suddenly produced.

Of three experiments of this kind performed, in only one was a significant result obtained. In this experiment the ring of tissue had been crushed with the usual negative results. But it was found that certain manipulations of the tightened clamp resulted quite regularly in stoppage of the whole heart, which resembled that obtained by vagus stimulation. The duration of these stoppages was variable, but often exceeded eleven seconds. The first one or two cardiac cycles following upon the stoppages were usually long, often about twice the length of the normal cycle, and were succeeded at once by a regular beat, the rate of which was a bit more rapid than that preceding the stoppage (Fig. 2). Later in this experiment the stoppages of the whole heart were succeeded at first by a slow ventricular beat, accompanied by retrograde auricular beats, the auricles later determining a slow but normal beat of the heart.

When this result was obtained, we were at a loss for an explanation of it, but in the light of later experiments (see Experiment 35, p. 137) we are now inclined to believe that it resulted from tension, due to manipulation of the clamp, exerted either upon the region of the auricles which was determining the heart beat or upon tissue intervening between such region and the rest of the heart. Certain it is that the stoppage was not caused by any process originating in the part of

the auricular wall included in the ring, nor was it caused by stimulation of inhibitory nerve fibres, because the same result was obtained after complete atropinization of the heart. Where this region lies it remains for future experiments to determine. It is, however, justifiable to conclude from these experiments that the heart of the dog *in situ* will beat to all appearances normally after it is removed from any influence which the region of the great veins may possibly exert upon it.

EXPERIMENTS UPON THE PERFUSED HEART.

Methods and introductory remarks.—The difficulties in the way of determining upon the heart *in situ* what effect, if any, the functional separation of the region of the great veins from the rest of the heart has upon the heart beat, were, it has been seen, too great to foster the hope of a successful issue by that method. For these reasons a series of experiments on the isolated, perfused heart was inaugurated. The animals, all rabbits with the exception of one dog and one cat, were killed under ether anæsthesia by bleeding from the carotid, and the heart was then quickly excised, great care being taken not to injure it in any way. Long pieces of the cavæ were carefully dissected free and removed with the heart. In most of the experiments a heavy thread on an aneurism needle was passed through the left auricle, entering by way of the inferior vena cava and leaving by the superior. Usually this was done after the heart had been excised and suspended, occasionally before excision. The perfusion was conducted in the usual way. Locke's solution was used, in the earlier experiments, without dextrose. The temperature of the fluid was taken close to the cannula tied into the aorta. Every effort was made to keep temperature and pressure constant in any one part of an experiment, but this was not always possible owing to the fact that vessels were often severed during the course of an experiment. Rarely, however, and then only when one of the main trunks of the coronary system was severed, was there a sudden change in temperature or pressure. Our conclusions are not based on experiments in which this accident occurred. The temperature and pressure were as a rule maintained at a low level, so that the heart beat would be slow and therefore easily studied and analyzed. In the rare instances in which the heart fibrillated it was recovered with potassium chloride.

After excising and suspending the heart the effect was determined

of separating functionally various parts of the auricles from one another, either by means of cuts made with scissors or by crushing or by twisting.¹ The first method we consider the most satisfactory, because it accomplishes the separation most rapidly and cleanly, and therefore is associated with a minimal duration of stimulation and destruction of tissue. The results obtained by cutting into the heart tissue are, however, open to the objection that, as a consequence of the section of the smaller blood vessels, the distribution of fluid to the neighboring regions is altered from the normal. It was for this reason that crushing and twisting were resorted to. But since the results obtained by the latter methods, where tried, seem to differ in no essentials from those obtained by cutting, there can be no objection to the use in the following discussion of results obtained by all methods.

The movements of the right and left auricular appendages, occasionally of other parts of the auricles, and of the right ventricle, were recorded by means of air transmission in the usual way. In the earlier experiments continuous records could not be made owing to the lack of necessary apparatus. In later experiments these were obtained, and they not only confirm all of the earlier observations, but, in addition, provide us with most of the quantitative data.

It is here necessary to refer to two phenomena constantly occurring in these experiments as the result of operations upon the auricles, and of which it is necessary to have some understanding before satisfactory interpretation can be made of the results obtained. We refer here to the direct stimulating and inhibitory effect of the various operations.

1. Usually, while cutting or crushing or twisting auricular tissue, there occur marked irregularities and acceleration of the beat. These irregularities may possibly have influenced in some way the subsequent behavior of the heart. Thus some of the slowing of the heart rate following operations may have resulted from it.

2. In every experiment stoppage of the heart, or of parts thereof, has frequently occurred as a result of operations, or sometimes even from a mere touch upon the auricles. The cause of this phenomenon,

¹ It is taken for granted throughout this paper that the contraction wave can be conducted from auricle to ventricle and in the reverse direction through the auriculo-ventricular bundle only. A bit of auricle is therefore functionally isolated, even though it still be in connection with the ventricles, when its continuity with the rest of the auricular tissue has been severed, and provided it has no connection with the ventricles through the auriculo-ventricular bundle. This premise is justified by the experiments of HIS, HUMBLET, HERING, ERLANGER, FREDERICQ, and others, and is further amply justified by the experiments herein reported.

which Hering has termed "shock," is not known.¹ We, however, believe that our experiments shed some light upon it. We are inclined to believe, and perusal of the protocols herein quoted will justify us in this belief, that "shock" occurs mainly, if not exclusively, as the result of two procedures which are in reality parts of but a single one. (a) In the first place stoppage of the heart, "shock," occurs when, we believe, the heart is separated by cutting, crushing, or twisting from that part of it — in the case of these experiments, from that part of the auricle — which is presumably driving it. Under these circumstances the heart, removed from the influence of its pace-maker, comes to rest until, we believe, its inherent but dormant rhythmical power awakens; until, to use the suggestion of Gaskell,² the rhythm of the heart tissue develops. This is the explanation suggested by one of us³ of the phenomenon of stoppage of the ventricles of the mammalian heart which occurs when they are more or less suddenly removed from the influence of the auricles by means of compression of the auriculo-ventricular bundle. We therefore believe it to be a general law. (b) In the second place, as stated above, stoppage of the heart, or "shock," sometimes results from merely touching the auricles. We are inclined to believe that stoppage of the heart in such cases results from the fact that the part of the auricles which determines their beat has been touched or stimulated, and that this stimulation results in inhibition (?) of such part with consequent stoppage of the parts of the heart then being driven by it. Under these circumstances it is conceivable that the beat of the heart may be resumed in one of two ways, — either by the recovery of the inhibited (?) part or by the assumption of rhythmical powers by some other part of the heart.⁴ It is, of course, possible that under

¹ HERING: *Archiv für die gesammte Physiologie*, 1907, cxvi, p. 143.

² GASKELL: *SCHAFFER'S Text-book of Physiology*, 1900, ii, p. 175.

³ ERLANGER and HIRSCHFELDER: *This journal*, 1906, xv, p. 153.

⁴ We think it advisable to insert here some very suggestive notes from one of our protocols.

Experiment 20, Jan. 26, 1907. Rabbit. See p. 134. Coronary sinus region, upper part of auricular septum (this had caused transitory stoppage of auricles), and region of great veins had previously been excised, when the outer wall of the right auricle was seen to be the first part of the auricles to contract, the contraction wave spreading from this place to right appendage and to left auricle.

12.01. Cut made down through this outer wall of right auricle close to base of appendage: no disturbance in beat.

12.03½. Cut in same place deepened into vault of auricle: both auricles stopped at once, but the left soon began to beat again.

12.09. First contraction of right auricle. Shortly after, LA: RA = 1:2.

certain circumstances stimulation of any part of the auricles may inhibit (?) them, but it is, to say the least, suggestive that we rarely, if ever, have obtained stoppage ("shock") even from severe operations upon the heart, unless such operations involved the part of the heart which presumably was determining the heart beat or the beat of its parts.

Finally, we wish it to be distinctly understood that we do not maintain that the heart preserving its normal relations to the organism will react in the same way to operations of the kind specified below, as does the excised, perfused heart. Neither do we maintain that the heart removed from the organism is driven by the same mechanism as is the heart in the organism. Furthermore it is possible that the excised heart when fed with different fluids may react differently to the operations we have practised upon the auricles. We have made no effort to determine if such is the case. None the less we are inclined to believe that when it is found possible to repeat our experiments upon the heart *in situ*, results qualitatively, if not quantitatively, alike will be obtained. An account of our experiments upon the perfused heart follows.

THE INFLUENCE EXERTED UPON THE RHYTHM OF THE HEART BY THE REGION OF THE GREAT VEINS.

Results obtained by inspection. — The region of the great veins is occasionally the only part of the heart which may be seen beating when the heart is losing its rhythmical power as the result of excision or as the result of cessation of perfusion; and occasionally it is the part of the heart to first begin beating when perfusion is begun. Under the latter circumstances the beat may be seen to spread gradually from this region to other parts of the heart. This is not a new observation, it having been noted by MacWilliam,¹ Hering,² Fredericq,³ and others. The following protocol will serve to illustrate the manner in which the rhythmical power, or better, perhaps, the irritability of the heart, spreads from the region in which the beat begins.

Experiment 33. February 18, 1907. — Very young rabbit.

2.15½. Heart begins to beat in complete auriculo-ventricular block.

Region of the great veins beats most vigorously, contractions spreading

¹ MACWILLIAM: *Loc. cit.*

² HERING: *Archiv für die gesammte Physiologie*, 1900, lxxxii, p. 21.

³ FREDERICQ: *Archives internationales de physiologie*, 1906. iv. p. 57.

from there feebly into the right auricle. Left auricle cannot be seen beating.

Later. Both auricles are now beating, and auriculo-ventricular block is partial, the ventricles responding to every other auricular beat.

Later. Auriculo-ventricular rhythm 1 : 1. Now, 2.35, proceeded with experiment.

2.35. All parts of heart now beating vigorously. Region of great veins liberally excised. This cut went well down into the septum of the auricles, over into the outer wall of the left auricle, and left below¹ a fringe of outer wall of right auricle 1.5 mm. wide attached to the auriculo-ventricular junction just below the inferior cava and joining the intact coronary sinus region with the right auricle to the right of the cavæ, and above a broad band in the vault joining the right with the left auricle. Temperature, 33.5° C.; pressure, 66 mm. Hg. Whole heart stopped beating for over seventy-seven seconds. Record ends here, probably for over four minutes.

2.40. Auriculo-ventricular rhythm, 1 : 1. All parts of heart beating, auricles synchronously, ventricles slightly later than they. Rate much slower than before cut, but is gradually increasing.

2.40½. Heart beat irregular, the result of occasional retrograde ventricular extra systoles.

Results of excision of the region of the great veins. — The results of excision of the region of the great veins are not always the same. For this reason it is necessary to state just how the operation of excision was performed. While the two projecting ends of the thread in the right auricle were drawn outward so as to elevate a cone of auricular tissue, a liberal cut was made with flat scissors through the base of this cone. The amount of tissue removed in this way varied somewhat in the different experiments. But whether or not the variable results obtained are in any way dependent upon this slight variation in the amount of tissue removed we have not been able to determine. The protocol of Experiment 33 (see above) will serve to illustrate a result frequently obtained. This extract will also serve to illustrate in a general way the usual extent of the cut. In this experiment the stoppage of the whole heart following the cut lasted perhaps over four minutes. Rarely, however, has stoppage lasted quite so long as in this experiment. We are inclined to believe that in this case the prolonged stoppage of the heart is to be accounted for by the fact that the rhythmicity of the parts of the heart had not yet had time to

¹ Throughout this paper descriptions of operations upon the excised heart are given with reference to the position of the heart as suspended from the aorta.

develop to the usual power when the region of the great veins was excised. The first beat after this long pause was normal; that is, the two auricles contracted practically synchronously, the ventricles a moment later. The rate of beats then gradually increased to a constant one, which was, however, slower than that preceding the cut.

Stoppage, or at least a slowing of the whole heart, followed excision of the region of the great veins in almost every experiment in which the heart had not been mutilated before the veins were excised.

Two possible causes for the few failures to obtain stoppage or decided slowing in these experiments suggest themselves: (a) The beat may previously have originated in the great veins, but other parts of the auricles may have been quite as rhythmical as the part excised, and may at once have assumed the function of originating the heart beat; or (b) the heart at the time the cut was made may have been driven by some region located without the region removed.

If we may be permitted to reason by analogy, much may be found in support of the first explanation of stoppage. For stoppage of the ventricles, or at least an abrupt slowing of their rate, occurs in a vast majority of instances when the auriculo-ventricular bundle is suddenly compressed. It does not, however, occur invariably. The ventricles may at once begin to beat rapidly but out of cadence with the auricles. None the less we have also some evidence indicating that the second cause of stoppage mentioned above may occasionally, at least, account for the failure of stoppage to occur. Thus in one experiment, at least, stoppage was obtained by excising the outer (posterior) part of the auricular septum above the region of the coronary sinus, whereas subsequent excision of the region of the great veins was without noticeable effect. Some extracts from the protocol of this experiment are here given.

Experiment 20. January 26, 1907. — Heart of sick rabbit.

Thread passed through auricle. Perfusion begun. Heart beating normally.

Horizontal cut made through outer auricular walls, midway between the two cavæ from appendage to appendage.

Horizontal cut made into auricular septum not quite to aorta. (At autopsy it was found that this cut was close to the auriculo-ventricular junction.)

Auricles beating synchronously.

Outer edge of auricular septum above horizontal cut quickly excised;

auricles stopped a few seconds and then began to beat slowly. A few seconds later, rate approximately same as before last cut.

Region of great veins rapidly excised. Without effect upon auricles. . . .

It may be seen that the results we have obtained from excision of the region of the great veins differ materially from those of Langendorff and Lehmann.¹ In some of our experiments the heart has stopped beating, but only temporarily. The auricles as well as the ventricles have invariably recovered after such stoppage. Usually the heart beat becomes normal; not infrequently, however, the auricles and ventricles beat simultaneously or almost simultaneously. On a later page we shall discuss this phenomenon of simultaneity of beats. In this connection it might be well to quote from Langendorff and Lehmann's paper: "Eine Wiederaufnahme der Herztätigkeit scheint nur für die Kammern möglich zu sein: wenigstens gelang es uns nicht, an den sonst so lebhaft pulsierenden Herzohren irgend etwas von Pulsationen zu entdecken." In some of our experiments we often found it difficult to decide whether or not the auricles were beating, so nearly simultaneously with the ventricles did they contract. It was not, however, a difficult matter to show in such cases that the auricles were contracting. For section of the auriculo-ventricular bundle whenever performed at such a time, resulting, as it does, in complete dissociation of beats, showed that the auricles were, and therefore had been, beating at the same rate as the whole heart. Extracts from the protocol of an illustrative experiment are given below (also see Fig. 11).

Experiment 27. February 25, 1907. — Young rabbit.

Thread placed in veins before excising the heart.

Heart was suspended, and tissue adherent to auricles was cut away.

Heart began to beat slowly when perfused, and then showed the group beating, which is characteristic of the passing away of a sino-auricular block.

Thread in veins twisted. Result negative, except that later auricular and ventricular beats were synchronous. This procedure was repeated with similar results.

Chambers still beat synchronously, and it is difficult to tell whether auricles are beating.

Horizontal cut made through outer auricular walls midway between veins and from appendage to appendage.

¹ LANGENDORFF and LEHMANN: *Loc. cit.*

Record made while cutting auriculo-ventricular bundle. Before: auricles and ventricles almost exactly synchronous. After: auricular rate moderately increased, ventricular rate immediately slowed. . . .

In some, but not in all, of our experiments it would have been quite impossible to have determined without the aid of this procedure whether or not the auricles were beating. It is possible that the observations of Langendorff and Lehmann are correct, and that the differences we have observed are the result of differences in method. In this phase of the subject we have not interested ourselves. It should, however, be added here that the fact that in Langendorff and Lehmann's experiments after removal of the "sinus," the duration of the spontaneous ventricular cycle plus the extra systole and compensatory pause following it is less than twice the normal heart period, does not prove their contention that the ventricles were beating independently of the auricles. It is known that if the heart rate be reduced by cooling the sinus, for example, it may become possible to insert a ventricular extra systole between two natural ventricular contractions. A more certain method of determining whether or not the ventricles were beating automatically would have been to determine the effect upon the ventricles of stimulating the supposedly quiescent auricles.¹

Results of crushing the tissue surrounding the region of the great veins.—It was stated above that the effects of removal of parts of the heart by section might possibly be influenced by the change in the distribution of the perfusion fluid which must necessarily result from the opening of the blood vessels. An effort was made to eliminate this factor, possibly a disturbing one, by separating the region of the great veins from the heart by processes other than cutting. In this section the effects of crushing the tissue surrounding the region of the great veins will be recorded.

In two experiments the region of the great veins was clamped off momentarily in a clamp of the Gaskell type. By means of gentle tension on the thread in the right auricle a cone of auricular tissue including the great veins was drawn through the jaws of the clamp. This was then clamped off gradually at its base until the tissue was supposedly thoroughly crushed. Then the clamp was quickly opened. In one of these experiments the result obtained was a slight slowing of the whole heart, the auricular and ventricular beats later becom-

¹ ERLANGER: This journal, 1906, xvi, p. 160.

ing synchronous. In the second experiment stoppage of the whole heart was repeatedly obtained by repeated tightening of the clamp upon practically the same region of the auricle. The behavior of the heart in this experiment may be gleaned from the following protocol:

Experiment 35. February 19, 1907. — Young rabbit. Heart excised, thread passed through auricles.

10.42. While clamping region of great veins in clamp. Temperature, 33.2° C.; pressure, 40 mm. Hg. Before: heart beat normal, right auricle contracting before ventricles. As clamp is tightened, As-Vs interval shortens despite increase in heart rate. When crush is complete, the whole heart stops. Clamp was opened immediately; whole heart began to beat slowly, and by beginning of next record (10.45) has nearly attained earlier rate, but auricles and ventricles still beat synchronously.

10.45. Record started as soon as possible after changing position of drum. Rate is now fast — the change was sudden and occurred while adjusting drum. Sequence seems to be normal.

10.52½. Procedure same as 10.42. Temperature, 30° C.; pressure, 45 mm. Hg. No special beat can be seen in region of great veins. Before: heart practically the same as at opening of experiment, and sequence is normal; but again shortly after beginning to clamp, before clamp is tight and before any decided change in rate, auricles and ventricles become synchronous. Just before clamp becomes tight, heart rate slows for a few beats and heart then stops fourteen seconds, when it begins to beat slowly, the rate gradually increasing.

11.11. Clamping region of great veins again. Temperature, 32° C.; pressure, 50 mm. Hg. Before: rate rather slow, but sequence normal. While clamping: heart rate accelerated, auricles and ventricles becoming synchronous. As clamp is tightened, rate slows down more or less gradually. When clamp is tight, beats abruptly become twice as long as they were before clamping. Toward close of record rate is increasing and normal sequence is gradually returning.

11.20. More of region of great veins pulled through clamp and crushed. No beat visible in this region. Temperature, 33.2° C.; pressure, 55 mm. Hg. Before: rate practically same as before 11.11, auricles and ventricles beating synchronously. While clamping: marked acceleration of beat. When tight: stoppage for six and a half seconds, then slow beats, gradually increasing. Auricles and ventricles synchronous to end of record.

11.24. Beats much more rapid, but still are slower than at beginning of 11.20. Sequence normal.

11.40. Region of great veins can clearly be seen beating regularly and more rapidly than rest of heart.

11.59. Slight tension put on region of great veins by means of thread in right auricle : a definite partial "sino-auricular block" results, there being periods of short beats alternating with long, the latter being just a trifle shorter than two of the former. Auriculo-ventricular sequence normal. . . .

12.17. While stimulating beating venous region more or less rhythmically with induction shocks of a rate more rapid than heart beat ; result negative.

12.19. While putting tension on right auricle by means of thread in it. Sequence normal. Partial "sino-auricular block" obtained : at first, 2 : 1, then two of 3 : 1, four of 2 : 1 ; irregular alternation of 2 : 1 with 1 : 1 (Fig. 3).

12.31. Repeated : results similar. Before the heart stops beating the auricles and ventricles begin to contract synchronously, and the synchronous beat persists while the heart is beating slowly. In one trial, however (11.20), the beats were synchronous before the process of clamping was begun, and in this instance as in the others stoppage of the heart resulted.

Considerable interest attaches to this experiment for several reasons. In the first place, the region of the great veins, after having been functionally separated from the rest of the heart, was seen to be beating more rapidly than, and independently of, the rest of the heart. We have here complete block between the region of the great veins and the parts of the heart below this region. It might be added that the same phenomenon was observed in another experiment, the details of which it is unnecessary to repeat here, excepting to state that the region of the great veins was crushed off from the rest of the heart in another way. In the second place, stoppage of the heart and another presumably related phenomenon, which we shall, for the sake of brevity, call sino-auricular block, were repeatedly obtained after the region of the great veins had been functionally separated from the underlying parts of the heart.

Sino-auricular heart block.—Mention has just been made of a form of arrhythmia, resulting from tension on the auricles, termed, for the sake of convenience, sino-auricular heart block. This form of arrhythmia is characterized by a more or less regular variation of duration of beats of auricles and ventricles, the duration of the various cardiac cycles bearing, in the cases we have observed, a more or less definite aliquot relation to one another. For example, it may be seen in the protocol quoted above that the durations of beats vary and measurements show definite ratios, such, for example, as 1 : 2, 2 : 3, and 1 : 3. This is not exactly true, in that the longer cycles are usually

somewhat shorter than twice or thrice the shorter cycles (see Figs. 3, 4, 5).

The same irregularity of beat has been chanced upon by Wenckebach¹ in man and by Hering² in the perfused mammalian heart. Both of these investigators have concluded that it was the result of a partial block to the passage of the excitation wave from the place which determines the beat of the heart, presumably the counterpart of the sinus of the cold-blooded heart, to the parts of the heart driven by it. The irregularity of beat is such as is obtained when, in the cold-blooded heart, a partial block is interposed to the passage of the excitation wave through the sino-auricular junction. The sinus³ then beats regularly, and still determines the beat of auricles and ventricles, but not every impulse acts as an efficient stimulus to the lower chambers of the heart.

In the course of this investigation we have occasionally chanced upon the heart irregularity typical of, we believe, sino-auricular block. It was occasionally seen, for example, when the heart was beginning to beat as the result of beginning perfusion (see Exp. 27, p. 135). But, inadvertently, we have discovered a method by means of which it is sometimes possible to produce the phenomenon at will in the rabbit's heart. This method, which does not always succeed, — indeed it has succeeded perfectly in but two of six trials, — is as follows: A heavy thread is passed through the right auricle by way of the cavæ, and the projecting ends of the thread are twisted gently upon each other. This puts the region of the great veins under slight torsion, and sino-auricular block may then develop. In the successful experiments it was possible in this way to produce a partial sino-auricular block repeatedly. This is illustrated by the following protocol:

Experiment 25. February 2, 1907. — Young rabbit. Heart excised with long pieces of veins attached. Tissue around veins not dissected away. During most of the experiment the perfusion pressure was maintained a low level, not over 40 mm. Hg. Temperature was about 34° C.

¹ WENCKEBACH: Die Arrhythmie als Ausdruck bestimmter Funktionsstörungen des Herzens. Leipzig, 1903.

² HERING: Zeitschrift für experimentelle Pathologie und Therapie, 1906, iii, p. 511.

³ The word "sinus" where used in this paper without modification has reference to that region of the mammalian auricles which is the analogue of the sinus venosus of the cold-blooded heart, and which has been described by His under the name of *sinus reuniens* (Ref., HERING: *Loc. cit.*).

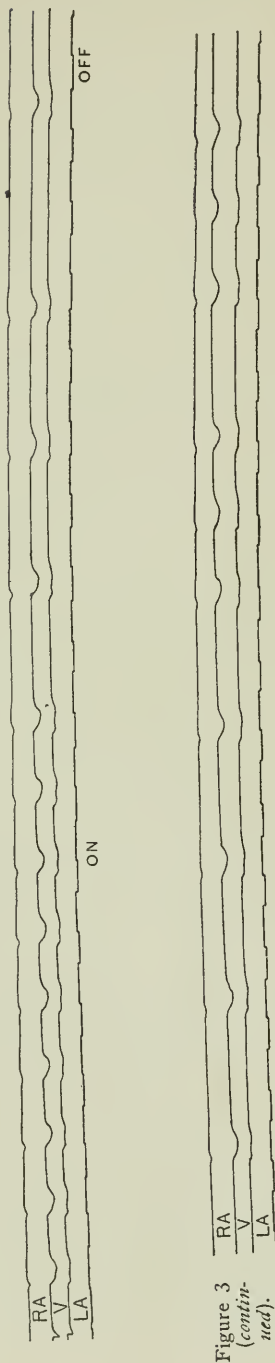


Figure 3
(continued).

FIGURE 3.—To show the production and disappearance of partial block of the sino-auricular type. At "on" tension was put on the region of the great veins, at "off" it was released. Rabbit.



FIGURE 4.—To show the production of, and different stages of recovery from, complete sino-auricular block resulting from the twisting of the region of the great veins. Before, there was a 2:1 auriculo-ventricular block. The duration of torsion is indicated by the line just below the time record. Time: Interval between two symmetrical marks is one second. Rabbit. (a) Shows the establishment of complete (?) sino-auricular block.

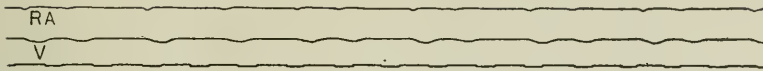


FIGURE 4*b*—Shows the change from sino-auricular block of the 2 : 1 type to block of the alternate 2 : 1, 1 : 1 type. Now, none of the auricular impulses are blocked at the auriculo-ventricular junction.

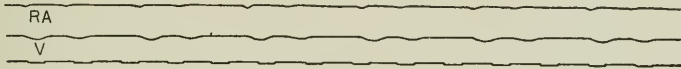


FIGURE 4*c*—Shows alternate 2 : 1 and 1 : 1 sino-auricular block.

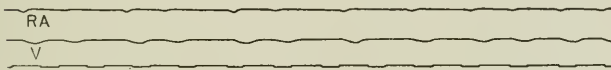


FIGURE 4*d*—Shows the return to normal sequence from an alternate 2 : 1 and 1 : 1 sino-auricular block.

1.¹ Showing an auricular irregularity possibly due to sino-auricular block. This came on immediately after thread was passed through the great veins and passed off shortly after completion of record.

2. Upon handling thread same results again obtained.

3. Upon handling thread same results again obtained.

4. Upon handling thread same results again obtained.

5. Before twisting thread: slight auriculo-ventricular block, the ventricles occasionally failing to contract with the auricles.

6. After twisting: record shows recovery from sino-auricular block of the alternate 2 : 1 and 1 : 1 type. The ventricles now respond to every auricular contraction (see Fig. 4).

7. Before twisting: slight auriculo-ventricular block, as in 5.

8. After twisting: same as 6.

9. Later: immediately after twisting the auricles are sometimes slightly accelerated. Sino-auricular block appears immediately after acceleration disappears.

10. Before twisting: same as 5.

11. After: showing recovery from slight sino-auricular block.

12. Before twisting: same as 5.

13. After severe twist: arrhythmia, which might well be the result of alternation of 2 : 1 with higher grade of sino-auricular block.

14. Later: heart beat now regular, but slower than before.

15. Before twisting: about as in 5.

¹ These numbers correspond with those of tracings, analysis of which furnishes the data for the notes.

16. After: slight or no effect.
17. Before twisting: about as in 5.
18. While twisting: negative.
19. While twisting: slowing, in all probability 2 : 1 sino-auricular block; auriculo-ventricular block disappears.
20. 19 continued: still probably 2 : 1.
21. 20 continued: at first definite alternate 2 : 1 and 1 : 1 sino-auricular block, changing into normal 1 : 1 rhythm of whole heart.
22. While twisting: before, alternating 2 : 1 and 1 : 1 auriculo-ventricular block; after, stoppage of whole heart, lasting about six seconds. Rate then gradually increases, sino-auricular block apparently complete, auriculo-ventricular sequence normal.
23. Showing complete recovery.
24. While twisting: before, same as in 22; after, stoppage of whole heart lasting six seconds, then slow to end of record, but gradually increasing, probably complete sino-auricular block; auriculo-ventricular sequence normal.
- 25-30. Showing gradual recovery to normal.

This result was repeated twice more, but after a third trial, which likewise was followed by stoppage of the heart, the heart rate did not again become normal. Subsequent twisting was without effect upon the heart beat, and so likewise was liberal excision of the region of the great veins.

In the other successful experiment (No. 26), some tracings of which are here reproduced (Fig. 5), what we believe to be sino-auricular block, similar in every way to that illustrated in the above protocol, was obtained in five successive attempts. Recovery did not occur after the sixth attempt, and then subsequent liberal excision of the region of the great veins was without effect upon the heart beat. In neither of these experiments were we able to see any part of the heart beating with a rate which was so related to that of the visibly beating auricles and ventricles that it might have been their pace-maker.

The unsuccessful attempts to obtain sino-auricular block might have been due to the operation of the same factors as were referred to in connection with a previous section (see p. 134). That such in all probability was the case is indicated by another experiment (35), extracts from the protocol will be found on page 137. In this experiment the region of the great veins, which had been separated functionally from the rest of the heart, was beating independently of, and more rapidly than, the rest of the heart. Nevertheless tension upon the region of the great veins resulted at this time in the irregularity which is typical of a partial block between the heart and

Figure 5



Figure 5 (continued)

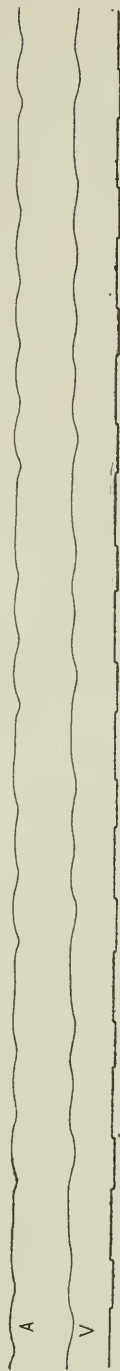


FIGURE 5. — To show a slowly disappearing partial sino-auricular block. At the beginning of the record the intervals between contractions are equal to two units. The number of two-unit cycles diminishes as the end of the record is approached. Rabbit.

its pace-maker, wherever it may be. It is quite probable that in this case the heart, after it had been removed from the influence of its most rhythmical region, fell under the influence of the next most rhythmical region, but this latter region was so situated that tension upon the cone of auricular tissue projecting through the clamp interposed a block between it and the underlying mass of the heart. This experiment indicates the possibility that parts outside of the compressed region may be quite as rhythmical as the parts compressed. In the case of the unsuccessful experiments the former may have assumed the function of pace-maker without any pause when the latter was prevented from exercising this function.

We do not desire to convey the impression that the method we have used in these experiments for the purpose of obtaining sino-auricular block is a satisfactory one. There is no doubt but that a much more certain method can be devised. Nevertheless, since this method permitted us to obtain desired results, we thought it best not to delay publication of such results pending an improvement of the method.

In this connection the question arises: Are there to be found in the various regions of the auricles structural arrangements which might in some way account for the regional differences in rhythmicity? Particularly we are interested in the question: Is there something in the architecture of the heart which will account for the phenomena of sino-auricular heart block? We might, perhaps, expect to find in the auricular region an arrangement similar to that which we know to exist at the auriculo-ventricular junction, *i. e.*, either an attenuated functional connection between sinus and auricle, or a connection, perhaps a broad one, possessing a low grade of conductivity. Such a connection might best exist at the mouths of the great veins,¹ or where the sinus reuniens joins the auricular tissue proper. On the other hand, it is conceivable that the impulse might be blocked at these places without there being provided any special blocking mechanism. If, for example, the tissue of the sinus reuniens possesses a higher grade of irritability or rhythmicity than the tissues of the auricle bordering on it, then the impulses generated in the sinus might be too feeble to pass over into the auricle with a residual efficiency sufficient to act as a stimulus to the auricle. A phenomenon, comparable in every way with the one above described, has been seen in the case of a strip of terrapin's ventricle, one end of which has been dipped in a

¹ WENCKEBACH (*Archiv für Physiologie*, 1907, p. 1) states that such a connection has been described by KEITH.

solution which will cause it gradually to assume the property of rhythmicity. The end in the solution will sometimes begin to beat, but the impulse will not, for some time at least, traverse the entire length of the strip. It will be blocked for no other reason than a functional one in that part of the strip which has a lower grade of "Reactionsfähigkeit" than the part that is determining the beat.¹ Which one, if either, of these possible causes of sino-auricular block accounts for the spontaneous development of this type of block in mammals, it remains for future work to decide.

THE RHYTHMICITY OF THE LEFT AURICLE.

For the purpose of determining the rhythmical power of the left auricle, its connection with the septum of the auricles and the right auricle was severed by means of a cut through the vault of the auricle, usually just to the left of the auricular septum. This cut, which was made with a pair of scissors, extended, as a rule, from alongside the wall of the aorta backwards and downwards to and through the auriculo-ventricular junction. In some experiments the septum was first excised and the cut in the vault was then made near the line of attachment of the septum. This cut was made gradually, occasionally, abruptly. Fredericq² has practised the same cut on the excised dog's heart with the result that when the left auricle is completely severed from the rest of the auricular tissue it continues to beat, but independently of the rest of the heart.

Our experiments performed on the rabbit's heart (in one case on the dog's heart) do not agree with those of Fredericq performed upon the dog. In all excepting three experiments out of a total of sixteen, it was found that the left auricle, when definitively separated from the rest of the auricular tissue, stopped beating, and did not exhibit the slightest evidence of rhythmical power, although it remained irritable to electrical stimulation (Fig. 6). Occasionally great difficulty was experienced in obtaining complete isolation of the left auricle, owing to the fact that it is not an easy matter to sever all of the auricular tissue alongside the aorta, unless the risk is taken of opening the aorta and thus ending the experiment. Extracts from a protocol or two will serve to illustrate the behavior of the partly

¹ GUENTHER: This journal, 1905, xiv, p. 73.

² FREDERICQ: *Loc. cit.*



FIGURE 6.—Shows at the beginning complete blocks between the right auricle and left auricle plus septum, and between the latter and the ventricles. Later (at irregularities), the left auricle was separated from the auricular septum and stopped beating at once. Rabbit.

and completely isolated left auricle, as well as the difficulties in the way of procuring complete isolation.

Experiment 30. February 9, 1907.—Young rabbit.

Heart removed with long pieces of veins attached. A piece of thin aluminum wire was inserted through the cavæ instead of thread. (After a record of many attempts to obtain sino-auricular block by exerting torsion with the aid of the wire, during which attempts the auricles and ventricles were for the most part beating synchronously, the following cuts were made.)

12.15. Auricles and ventricles still synchronous. Region of great veins liberally excised. Usual extent of cut. Difficult to tell with the eye if auricles are beating. Record shows that throughout, auricles and ventricles are synchronous; slight slowing immediately after.

(?) While inserting scissors preparatory to cutting the auriculo-ventricular bundle, there was a period during which the auricles were beating perceptibly before the ventricles. This passed off.

12.23. Auriculo-ventricular bundle cut through. Before: auricles and ventricles synchronous. After: the ventricles are beating more slowly, the auricles regularly and slightly more rapidly than before.

12.26 $\frac{1}{2}$. Cut made downward in vault to and through auriculo-ventricular junction just to right of auricular septum. Auricles previously beating practically synchronously, although the left auricle may possibly precede the right. After: no change.

12.30. Cut made downward through outer wall of auricular tissue near the base of right appendage from the edge of cut made to excise the veins to and through auriculo-ventricular junction, thus excising (still attached to auriculo-ventricular junction) a large part of the outer wall of the right auricle. Before and after: auricles beating synchronously. After: rate very slightly slower.

12.33½. Cut made upward toward aorta in vault to right of auricular septum. Before: auricles apparently synchronous. After: right auricle slightly accelerated, left auricle slowed decidedly, auriculo-auricular block complete.

12.42. Cut made downward from lower edge of cut made while excising veins (this included some of the outer wall of the left auricle) to left of septum and through auriculo-ventricular junction. This cut severed the region of the coronary sinus from the left auricle. Left auricle stopped beating.

12.44½. Left auricle gave one beat.

12.48. Left auricle has begun to beat in partial block with right auricle. Rhythm, RA : LA = 4 : 1 at first, then changes abruptly to 2 : 1 (see Fig. 7), then one of 4 : 1, one of 3 : 1, and 2 : 1 to end of record.

12.50. While stimulating the right auricle tetanically. Before: rhythm, RA : LA = 2 : 1. During: marked slowing of left auricle. Immediately after: 2 : 1. Repeated, with same results.

12.56. Showing 2 : 1 rhythm, with long RAs-LAs interval.

12.57½. While narrowing bridge of auricular tissue alongside of aorta just to right of septum. Before: rhythm, RA : LA = 2 : 1. After: left auricle stopped beating.

1.00. Left auricle began to beat in partial block with right auricle.

1.01. Same as 12.50, with practically the same result.

1.07. While crushing auricular tissue alongside aorta to right of septum : left auricle stopped beating. Before: RA : LA = 2 : 1.

1.13½. Isolated coronary sinus region can be seen beating regularly, somewhat more slowly than right auricle and independently of it and of the ventricles. Left auricle still quiescent and remained so until 1.50, when experiment was concluded.

Experiment 15. December 18, 1906. — Rabbit. Heart prepared in the usual way.

3.01. Horizontal cut made through the outer auricular walls midway between cavæ and from appendage to appendage.

3.08½. Heart beating normally. Auricular septum excised as quickly and as completely as possible, including upper part of ventricular septum. After completion of cut there was auriculo-ventricular heart block. No other abnormality.

3.11½. Region of great veins excised without disturbance of heart beat.

3.12. Cut made upward toward aorta in vault of auricle along line of septum. Left auricle contracted a few times immediately after, then stopped; right auricle continued to beat.

3.13½. Left auricle begins to beat independently of, and more slowly than, the right auricle.

3.17½. Auricles beating simultaneously.

3.45. There is a narrow fringe of external wall of auricles remaining attached to auriculo-ventricular junction, connecting right and left auricles just to the right of the septum. This cut through: left auricle stopped for a long while, then began to beat slowly and independently for some time and finally responded to every other beat of the right auricle. Later: auriculo-auricular rhythm becomes 1 : 1, the left auricle occasionally not following the right.

(About the external half of the auricular septum remains and is connected with the left auricle.)

4.12. Cut upward toward aorta deepened while RA : LA = 2 : 1. Block becomes complete.

4.20. Auricles beating 1 : 1.

4.27. Cut in vault toward aorta deepened. Left auricle stopped. Later, it began to beat. This procedure repeated until left auricle eventually stopped beating.

Experiment 24. February 1, 1907. — Rabbit. Heart prepared in the usual way.

2.35. Heart beating normally.

2.41. A small bit of vault of auricle excised over septum and alongside of aorta, thus opening both auricles.

2.51. Tissue in septum of auricles and in upper part of ventricular septum alongside of aorta crushed with clamp, one jaw each being inserted through the openings in the vault into the two auricles. Heart irregular while crushing, then auricles continue undisturbed; ventricles stopped for about one minute, and then began to beat in complete block with auricles.

2.55½. Cut made in vault of auricles just to left of septum from opening into left auricle to and through auriculo-ventricular junction. Left auricle stopped, right auricle continued to beat without interruption. Left auricle did not contract again, although the experiment was continued until 3.30.

These and many similar experiments demonstrate that complete separation of the left from the right auricle and septum as a rule (but one positive exception) results in permanent stoppage of the left auricle. There seem to be many exceptions to this rule. Thus the left auricle, as in the experiments quoted, frequently beats apparently independently of the right auricle and septum, and with a much slower rate. The picture produced is that similar to complete block at the auriculo-ventricular junction, in which the ventricles beat more slowly than, and independently of, the auricles. We do not, how-

ever, believe that in such case the left auricle is entirely uninfluenced by the right, and for the following reasons:

Not infrequently the left auricle, after a more or less prolonged stoppage following the cut isolating it, begins to beat apparently independently of the rest of the heart, but, with but one exception mentioned above, in every case the block between the auricles soon thereafter became partial, the left auricle responding to every third or second, etc. beat of the right auricle and septum (Fig. 7). And frequently, even this allorhythmia passed away, leaving as the only sign of disturbed connection between the auricles a distinct pause between their contractions, — a pause which might well be termed the RAs-LAs interval, following the custom of terming the auriculo-ventricular pause the As-Vs interval. With the one exception, then, whenever the left auricle began to beat with apparent independence, it soon fell into cadence with the right auricle or some other rhythmically beating part. We are therefore inclined to believe that the independence of the left auricle in these experiments was only apparent; that before the impulse of the right auricle or septum reached the left auricle with strength sufficient to immediately elicit a contraction, it, in some unknown way, caused the left auricle to beat without relation to the rhythm of the impulses. Whether or not such is the case, it remains for future experiments to determine.

To summarize: by narrowing the tissue connecting the left auricle with the rest of auricular tissue, it is possible to cause (a) permanent stoppage of the former without in any way affecting the rhythm of the latter; (b) complete and permanent dissociation of beats of the left auricle, where this auricle beats out of cadence with the right and more slowly than

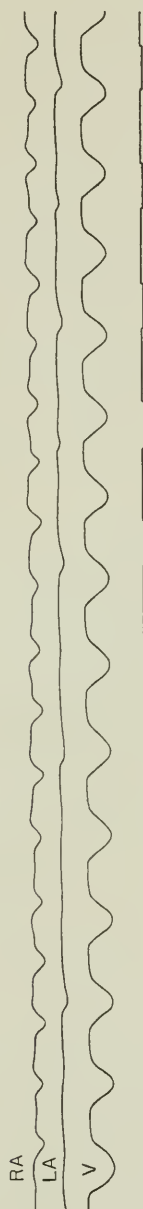


FIGURE 7. — Showing the ventricles beating independently and the left auricle responding at first to every fourth contraction of the right auricle, then to the third, and then to every second to the end of the tracing. Rabbit.

it, — it is believed that this occurs only when some functional connection still exists; (*c*) partial auriculo-auricular block, the left auricle responding to every third, second, etc. beat of the right side; and (*d*) delay in the transmission of the impulse from right to left auricle.

THE RHYTHMICITY OF THE RIGHT AURICLE COMPARED WITH THAT OF THE LEFT AURICLE AND AURICULAR SEPTUM.

For the purpose of determining the behavior of the right auricle when separated from the rest of the auricular tissue, a cut was made with scissors along the vault of the auricles just to the right of the septum from the wall of the aorta backward and downward to and through the auriculo-ventricular junction. The cut was usually so made as to leave attached to the auricular septum those parts of the right auricle surrounding the coronary sinus. Extracts from the protocols of two typical experiments performed in this way are here given.

Experiment 23. January 31, 1907. — Rabbit. Heart excised and perfused.

Thread passed through right auricle.

10.48. Horizontal cut made through outer auricular wall between cavæ parallel to auriculo-ventricular junction and extending from appendage to appendage.

10.49. Ventricles fibrillating and auricles beating irregularly. KCl passed through heart.

11.24. Heart beating normally.

11.31. Cut made downward from horizontal cut alongside of septum between it and the great veins to and through auriculo-ventricular junction: Ventricles stopped (?), auricular beat undisturbed.

11.32. Ventricles beating independently of auricles.

11.34. Cut made upward in vault of auricles between cavæ and septum from horizontal cut to as close to wall of aorta as possible. Both auricles continue to beat, the right almost twice as quickly as left and septum and apparently independently.

11.36. Region of great veins rapidly and liberally excised, cut involving only right auricle. Right auricle stopped for a few beats and then began to beat more slowly than the left.

11.37½. Each auricle and the ventricles beating independently of one another.

11.40. Ventricles responding normally to left auricle.

11.42. Block between auricles has disappeared.

11.43½. Cut to right of septum deepened toward aorta. Right auricle stopped a short while and then began to beat independently.

Experiment 36. February 23, 1907. — Old rabbit. Heart excised and perfused. Thread passed through right auricle.

11.53. Both auricles opened by a small cut in vault just above septum.

11.55. Cut made upward to right of septum from opening in vault toward aorta. No disturbance in heart beat.

11.59½. Cut made downward from opening in vault between the septum and veins to, but not through, auriculo-ventricular junction. Before: auricular sequence normal. After: rate unchanged, but left auricle seems to be following right.

12.02. Cut in vault toward aorta deepened. Interval between beats of right and left auricles is longer.

12.03½. Cut downward deepened. Before: the same as after 12.02. After: Right auricular rate is absolutely the same, left auricle and ventricles at once slow down and beat synchronously; complete block with right auricle.

12.07. Right and left auricles and ventricles are still beating independently, right auricle the faster. Left auricle now seems to be beating a trifle before the ventricles.

12.26½. Region of great veins to right of cut in vault excised. Before: ventricles follow left auricle and septum; right auricle beats faster than they, and block seems to be complete. After: left auricle and septum practically unaffected; right auricle slows at once, but its rate gradually increases, and at close of long record it is considerably slower than before, but slightly faster than left auricle and septum.

12.32. More of right auricle cut away in region of great veins. Before: right auricle and left auricle independent, right auricle beating more rapidly than before 12.26½. After: right auricle stopped beating and contracted but once during a long record. Left auricle continued beating without interruption and with somewhat increased rate.

12.34. Right auricle began to beat; two apparently independent contractions in a short record.

12.35. Right auricle is responding to every other beat of the left and later becomes independent again.

12.40. More or less rhythmic stimulation of left auricle with induction shocks. Before: LA:RA = 1:1, with long LAs-RAs interval. During: left auricular rate is increased by stimulation and LA:RA = 2:1.

12.41. Tissue crushed in vault to right of septum alongside of aorta. Before: auriculo-auricular rhythm, 1:1. After: slight irregularity, after which right auricle slows and is independent of left auricle.

12.45. Outer (posterior) wall of coronary sinus, including part of outer wall of right auricle, still attached to auricular septum cut away. Upon

touching this region left auricular rate markedly increased. After: beyond very slight acceleration of the left auricle immediately after, there was no change.

12.48. Cut made downward to and through auriculo-ventricular junction very close to the right side of the auricular septum, thus severing from the left auricle a small bit of right auricular wall forming the floor of the coronary sinus. Before: right auricle independent of left, slower and slightly irregular. After: irregularities of right auricle persist. Left auricle was at first somewhat accelerated, but at end of record it was somewhat slower than before.

These protocols show that, contrary to what happened in the case of the left auricle, the right auricle when separated from the left and septum continues to beat without any interruption whatever. Not alone does it continue to beat, but, in addition, its rate of beat is faster than that of the left side (Fig. 8). This result might have been predicted from the fact, recorded on an earlier page, that, at least in the case of the vast majority of hearts, the most rhythmical region lies in the right auricle. That the rapid rate of beat of the right auricle is largely dependent upon the presence in it of the region of the great veins is proved by the protocols. In them it is seen that when the region of the great veins is quickly excised from the right auricle while it is beating faster than, and independently of, the left and septum, it usually stops beating for some time, and then slowly assumes a rate of beat which, when fully developed, is slower than its previous rate; indeed, it may be slower than that of the left side.

When the separation of the right from the left auricle and septum is not complete, different stages of partial block may develop. That auricle which beats the more rapidly when the block is complete will, when the block becomes partial, determine the beat of the other (Figs. 9, 10). An example is given in the following protocol:

Experiment 33. — (continued from p. 132).

2.49. Cut made downward through lower outer auricular wall about 5 mm. to right of septum. Here there is a fringe of auricular wall about 1.5 mm. wide. Heart is still beating slowly but normally, excepting perhaps a rather short As-Vs interval. After: beat of whole heart at first accelerated, then slows down.

2.52. Cut made upward in vault to right of septum. Temperature and pressure same as before. There is left attached to right auricle the part of septum not removed by first cut and a small bit of the floor, and



FIGURE 8.—To show the production of complete block between the auricles. At the beginning the auricles are beating synchronously. Where the record is irregular the right auricle was being severed from the left auricle and auricular septum. Thereafter the left auricle beats more slowly than and independently of the right auricle. The ventricles are beating independently throughout. Rabbit.



FIGURE 9.—To show a 1:1 rhythm of the left and right auricles with a long I.As-RAs interval produced by narrowing the connection between the auricles after excision of the region of the great veins. When the rate of beat of the left auricle is increased by means of electrical stimulation, the rhythm becomes 2:1. The movements of the ventricles were not registering. Rabbit.

a fragment of the outer wall of, the right auricle. Cut followed by acceleration of the right auricle as well as of ventricles. Left auricle seems to be driving ventricles. Right auricle independent and after acceleration disappeared, very much slower than rest of heart, beating about once in three seconds, while the left auricle and ventricles are beating once in one and a quarter seconds.

3.00. While stimulating the left auricle more or less rhythmically. Before: condition of heart same as at close of 3.52. During: ventricles respond to every extra contraction of left auricle. The right auricular periods increase in length.

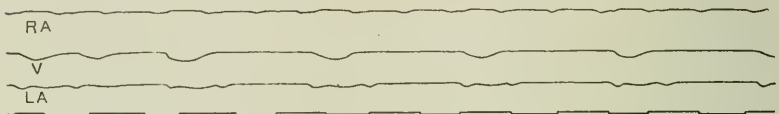


FIGURE 10.— To show partial blocks between the right auricle and the left auricle plus septum, and between the left auricle plus septum and the ventricles. The right auricle beats regularly, the left auricle failing to respond to every third, the ventricles responding to every third, beat of the right auricle. Rabbit.

3.04½. Cut made downward through vault to and through auriculo-ventricular junction to the left of septum. Before: same as before 3.00, but right auricle is now beating more rapidly, almost as rapidly as ventricle. After: left auricle is still driving ventricle, but right auricle is beating more rapidly than rest of heart.

3.06½. More or less rhythmical stimulation of right auricle. Before: left auricle is driving ventricle, right auricle beating more rapidly than rest of heart. Right auricle responds to rhythmic stimuli, but this is without effect upon rest of heart.

3.08½. Cut made upward in vault of left auricle to left of septum. Before: left auricle and ventricles are practically synchronous and regular; right auricle seems regular and more rapid than rest of heart. After: right auricle unaffected, ventricles slow at first, but soon resume almost former rate; left auricle apparently stops beating.

3.15. Long interval during which left auricle began to beat, at first driving ventricles; later, with this record, right auricle sets the pace of the whole heart. While dictating this note there was for a time 2:1 rhythm between right auricle and rest of heart. The record shows a 1:1 rhythm of the whole heart, right auricle beating first, then, after an interval, left auricle, and, a trifle later, the ventricles.

3.20. While deepening cut to right of septum toward aorta. Before: same as at end of 3.15. After: right auricular beats but little affected, possibly slightly accelerated; ventricles markedly accelerated (were nicked with scissors) even to end of long record, and now apparently drive left auricle. Right auricle in complete (?) block with rest of heart.

It will be seen that the isolated right auricle less the region of the great veins was at first beating more slowly than the left and septum, but, as the experiment progressed, the rate of the right auricle increased until it overtook and passed that of the left. The block between the auricles became partial, the left auricle responding to every other beat of the right. And still later, when the rhythm became 1:1, there was a long RAs-LAs interval.¹

To summarize: The right auricle with region of the great veins attached beats more rapidly than the left auricle plus septum. The right auricle after the excision of the venous region may beat more rapidly or more slowly than the left auricle plus septum. Since the left auricle possesses practically no rhythmicity, the conclusion is warranted that the septum and the right auricle less the venous region both possess approximately the same degrees of rhythmicity.

THE RHYTHMICITY OF THE VARIOUS PARTS OF THE RIGHT AURICLE.

It has been shown that the rapidity of beat developed by the isolated right auricle depends upon the presence in it of the region of the great veins. The question now arises: Is there left in the right auricle after the removal of the veins any special part or parts which still impart to it its rhythmical power? According to the observations of Langendorff and Lehmann, to which reference has already been made, the auricles less the region of the great veins do not possess spontaneous rhythmicity. Our observations do not support their

¹ At this place it might be well to refer to an isolated but interesting observation which shows that there may be a block even complete in the direction from a rapidly beating part to a more slowly beating or quiescent part, although the impulse induced by artificial means in the quiescent or more slowly beating part may pass over and stimulate the more rapidly beating part. The following notes illustrate this fact.

Experiment 20 (see pp. 131, 134).

12.52. Right auricle beating regularly; left auricle quiescent, but responds to electrical stimulation. Rhythm of right auricle is disturbed whenever left auricle responds to electrical stimulation. Stimulus applied to left auricle far away from right auricle. This phenomenon is perfectly clear. Left auricle is not contracting too feebly to be seen, because at any time it responds to electrical stimulation with very distinct contractions. The irregularities of right auricle are not the result of escape of stimulus to it, because it is not affected by stimulation of ventricle immediately below it.

1.07. Cut in vault to left of septum deepened. No effect on beats of auricles, but now electrical stimulation of left auricle (which responds) is without effect on right auricle. In this connection see ERLANGER: This journal, 1906, xvi, p. 175.

contention. Not alone will the right auricle beat when, under the conditions of our experiments, it is separated from the region of the great veins, but even a narrow strip consisting practically of the aortic wall of the appendage may beat when its functional connection with the rest of the heart has been destroyed. In this connection we quote the following from one of our protocols:

Experiment 23 (see p. 150).

12.16½. Left auricle is not beating, but responds to electrical stimulation.

12.19½. Auricular tissue adherent to aorta at upper end of cut to left of septum and above tricuspid valve crushed with forceps. No effect upon the beat of right auricle.

12.20½. Right auricle slightly irregular.

12.21½. Trimmed off some of tissue from edge of right auricle. No effect upon beat.

12.23½. Auricular tissue adherent to aorta crushed, the line of crush extending from cut edge of auricle into conus arteriosus. Right auricle somewhat accelerated for a while.

12.26. Remainder of outer wall of right auricle excised. No disturbance excepting momentary acceleration of beat. There is now left of right auricle only the inner wall of the appendage. This is beating vigorously and regularly.

12.29. Tissue in the auriculo-ventricular groove between appendage and right ventricle crushed from below upward almost to aorta. Right auricle stopped at once.

12.36. Right auricle is irritable to electrical stimulation.

12.51. Right auricle has begun to beat regularly but slowly and independently.

1.05. Crushed tissue in auriculo-ventricular junction under appendage to aorta, thus completing the isolation of appendage. To accomplish this it was necessary to crush some of the auricular tissue above the junction. Right auricle stopped at once and did not again contract up to the time of close of experiment at 2.04.

Experiment 10. December 13, 1906. Rabbit's heart. Perfusion. After many procedures, two of which consisted of functional separation of the right auricle from the rest of the heart and liberal excision of the great veins, the following note occurs: Upon cutting more and more tissue away from the cut edge of the right auricular appendage it beats more and more slowly and does not stop until there is left but a narrow strand.

The question is suggested by these protocols: Why does the small bit of remaining right auricular tissue stop beating? We do not

know, but we believe that it does not recover from stoppage resulting from the cut, because of deficient perfusion produced by the section of most if not of all of the important vessels supplying the part. It is possible to explain in the same way the observation of Langendorff and Lehmann, which we have confirmed, that the auricular appendage when severed from the heart ceases to beat, whereas the venous region under such circumstances continues to beat. The venous region is originating its own beat at the time it is cut away, and it continues to beat thereafter. On the other hand, the appendage, when it is cut away, is being driven by the region of the great veins or by some other part of the heart, and therefore then comes to rest. It does not resume its beat because, its circulation having been discontinued, there is no opportunity for recuperation, with development of rhythm, to occur. The appendage may continue to beat, if, instead of cutting it away from the heart, its connection with the heart be destroyed by crushing. In this case the circulation through the appendage is impaired, but is not permanently interrupted. The following extract is illustrative of this fact.

Experiment 30. Rabbit (see p. 146). Tissue had been cut from right auricle until there was left practically nothing of it but the appendage.

1.19½. With a clamp crushed right auricle alongside of aorta from cut edge of auricle up to and through conus arteriosus. Right auricle accelerated.

1.23½. With clamp crushed tissue in auriculo-ventricular junction below the right auricular appendage to a point within about 4 mm. of aorta. Appendage markedly slowed, more at first than later.

1.28½. Last crush continued so as to completely destroy all connection of auricular appendage with surrounding tissue. Right auricular appendage again slowed, at first quite markedly.

1.32. Remainder of outer wall of right auricular appendage cut away. Right auricle stopped.

1.50. Right auricle has not recovered. Ventricle and region of coronary sinus beating independently. Experiment discontinued.

These experiments would seem to indicate that the rhythmicity of the right auricle exclusive of the region of the great veins decreases steadily from left to right, but that every part of it possesses the property of spontaneous rhythmicity. It should, however, be borne in mind that the successive cuts into the right auricle progressively interfere with the circulation of the small remaining part, and that it is therefore possible that all parts of the right auricle exclusive of the

region of the great veins may be equally rhythmical. We, however, believe that the outer wall of the auricle is more rhythmical than the appendage. Illustrative of this we quote the following:

Experiment 29. February 27, 1907. Young rabbit. Perfusion. After many procedures, including separation of right auricle from septum and liberal excision of the region of the great veins.

4.37. Cut made downward along base of right auricular appendage to and through auriculo-ventricular junction, thus separating functionally all remaining part of outer auricular wall from rest of heart. Appendage slowed at once and then gradually became still slower. Outer wall of right auricle, which is still attached to the outer wall of right ventricle, beats rapidly and regularly, more rapidly than the right auricular appendage and more rapidly than remnant of septum.

Summary.—All parts of the right auricle possess the property of rhythmicity. The rhythmical power seems to diminish from right to left, but is present even in the appendage.

THE RHYTHMICITY OF THE CORONARY SINUS AND AURICULAR SEPTUM.

Practically all of our experiments throw some light upon the physiology of the auriculo-ventricular bundle. The results obtained all agree in that a cut made so as to positively intersect the auriculo-ventricular bundle at once produces complete and permanent functional separation of the ventricles from the auricles. They also throw some light upon other phases of the physiology of this structure, particularly of its auricular end. It might be well, therefore, to give here the latest description, known to us, of this end of the bundle. According to Tawara,¹ it "begins in the neighborhood of the anterior edge of the coronary vein, and then passes forwards on the right side of the auricular septum below the foramen ovale, lying close upon the auriculo-ventricular septum. . . ."

In recent years much has been written upon the influence which the auriculo-ventricular bundle exerts upon the heart beat. The view is very generally expressed by those who have interested themselves in this subject that the bundle is a highly rhythmical structure, and they imply, at least, that its rhythmical power is exceeded only by the region which normally determines the beat of the heart. The experi-

¹ ASCHOFF (for TAWARA): British medical journal, October 27, 1906, p. 1103.

mental evidence advanced in the support of this view, in so far as it concerns the mammalian heart, is not, however, convincing. It rests upon two sets of observations. In the first place, it has been found that under certain circumstances the auricles and ventricles may beat almost or quite simultaneously. This may occur following vagus inhibition¹ and during stimulation of the accelerator,² and under these circumstances simultaneity of beats may develop gradually, the A-Vs interval gradually becoming shorter, or it may appear suddenly. In the second place it is maintained by Lohmann³ and others that stimulation of the block fibres in the auriculo-ventricular junction may cause simultaneous beats of the auricles and ventricles which may persist after the cessation of stimulation. Examination of the figure reproduced by Lohmann indicates, however, that the stimulus, at least in the case of the experiment used for illustration, was applied to a place some distance removed from the bundle, and it might be added that there is no evidence that block fibres exist in the part of the heart stimulated by him.⁴

In the course of our work experiments were performed which seem to throw some light upon this subject. Attention was called in the last section to the fact that the left auricle plus auricular septum beats at about the same rate as the right auricle after excision of the region of the great veins. Sometimes one auricle, sometimes the other, beats the more rapidly. It must therefore be admitted that, under the conditions of our experiments, the parts attached to the auriculo-ventricular bundle possess a grade of rhythmicity no higher than that of the right auricle less the region of the great veins.

Other experiments were devised with the object of testing in how far the auriculo-ventricular bundle influences the rate of beat manifested by the septum. Since the bundle appears to have its origin near the anterior edge of the coronary vein, we thought it would be interesting to determine if the auricular impulse originates in the region of the coronary sinus, and if it is necessary that the auricular impulse enter the bundle at this place in order that it may be conducted into the ventricles.

¹ LOHMANN: *Archiv für Physiologie*, 1904, p. 431.

² HERING: *Zentralblatt für Physiologie*, 1905, xix, p. 129. Here similar observations made by RIHL are referred to.

³ LOHMANN: *Loc. cit.*

⁴ MACKENZIE and WENCKEBACH (*Archiv für Physiologie*, 1905, p. 235) have noted in man a form of arrhythmia which they believe is due to the assumption by the auriculo-ventricular bundle of the function of setting the pace for the whole heart.

THE EFFECT OF SEPARATION OF THE CORONARY SINUS REGION
FROM THE HEART.

Several experiments were performed to test the effect of functional separation of the coronary sinus region from the heart. Either the sinus, including the ventricular tissue immediately subjacent to it, was abruptly cut away from the heart or a cut was made downwards severing the coronary sinus region from the auricle but leaving it connected with the ventricles. In some experiments the coronary sinus was the first part of the heart operated upon. Frequently previous operations, such as separation of the right or left auricle or excision of the region of the great veins had been performed. The results obtained varied somewhat in the different experiments, but none the less they indicate that in most hearts, at least, the impulse does not originate in the region of the coronary sinus, not even after the influence of the great veins has been removed; and they prove that the presence of the anterior edge of the coronary vein is not necessary for the continuance of the conduction of auricular impulses into the ventricles. It should, however, be added that microscopic examination of the individual hearts for the purpose of determining the position in them of the auriculo-ventricular bundle, and the relation of the bundle to the cuts made are necessary before any conclusion bearing on this phase of the physiology of the bundle would be warranted.

The results of these experiments were more specifically as follows: In five or more experiments the coronary sinus was excised or cut into before the great veins were excised. In only one of these did stoppage of the auricles result. This experiment (No. 11) was unique, in that cuts into the tissue of the auricles in the vicinity of the coronary sinus frequently caused stoppage of the auricles.

Experiment 11. December 13, 1906. Rabbit. When heart had begun to beat normally, a cut was made through the external auricular wall a little above and parallel to the auriculo-ventricular junction from appendage to appendage.

Heart beating normally. Cut made into septum thus exposed so as to open coronary sinus. Beat normal.

Short cut made into auricular septum through coronary sinus parallel to auriculo-ventricular junction. Auricles stop, ventricles continue to beat; a moment later auricles following ventricles; beat later became normal.

Most of auricular septum cut out, not, however down to ventricular

septum. Followed by the same events as was last procedure. Later: ventricles stopped beating (quiescent during rest of experiment), auricles beating rapidly.

Cut out tissue posterior to coronary vein in region of sinus. Some irregularity of auricles, followed by stoppage, and later by renewal of irregular beat.

Made cut in vault of auricles toward aorta, approximately along line of septum: left auricle ceased beating for some time, the right continuing to beat irregularly; suddenly the left auricle began to beat synchronously with the right. This procedure repeated with approximately the same results, and again at:

4.23. Cut in vault made a trifle deeper. Immediate result same as before. Lower cut edge of auricular septum can be seen to beat before right appendage. . . .

4.33. Cut in vault made still deeper. Left auricle stops, septum and right auricle continue to beat.

4.38. Cut made downward through septum of auricles into ventricular septum near wall of aorta. All contractions cease at once.

4.42. Septum of auricles began to beat.

4.47. Right auricle began to beat feebly and independently of septum.

4.50. Floor of right auricle between septum and outer wall of right auricle beating synchronously with septum. This region beats more rapidly than right appendage.

5.10. Septum apparently causing beats of auricles.

5.40. Right auricle seems to follow septum occasionally. Outer wall of right auricle cut through to auriculo-ventricular junction: right auricle continues to beat; septum beating also, but very feebly.

The protocols show that the coronary sinus region was driving the right auricle plus the region of the great veins. A cut so made as to almost completely separate the region of the coronary sinus from the rest of the heart resulted in stoppage of all beating parts of the auricles, but the sinus region was the first part to resume its beat. The right auricle began to beat some minutes later, but its rate was slower than, and independent of, that of the coronary sinus and septum. The region of the great veins was subsequently excised (5.40), but unfortunately at a time when the beats of the right auricle might have been determined by the septum. The result obtained is, therefore, without significance.

The coronary sinus region was separated from the heart or severely mutilated in six other experiments after previous serious operations upon the heart. In only one of these did excision of the coro-

nary sinus region stop the auricles, and in this connection it is interesting to note that the beat of the heart had suffered no distinct alteration with the preceding excision of the region of the great veins. Extracts from the protocols of this experiment are given below.

Experiment 13. December 15, 1906. — Rabbit's heart. Perfusion.

Heart excised. Thread passed through cavæ.

Perfusion begun. Heart began to beat in block, but soon became normal. Began to fibrillate; was recovered with potassium chloride.

1.00. Heart beat normal. Horizontal cut made through external auricular walls parallel to auriculo-ventricular junction and a little above it.

1.05. Heart beat normal. Region of great veins excised. Beat normal at once.

1.07. Cut out auricular wall toward, but not quite to, coronary sinus. Beat normal.

1.08. Coronary sinus opened. Beat normal.

1.08½. Auricular septum above coronary sinus cut away. Beat normal at once.

1.09. A bit of septum central of coronary sinus excised. Beat normal at once.

1.10. Cut made into septum toward aorta parallel to auriculo-ventricular junction. Beat normal at once.

1.10½. Auricular septum above this cut excised. Beat normal at once.

1.13½. Made a cut so as to excise a part of coronary sinus (cut invaded ventricles). Auricles slowed at once, ventricles fibrillating.

1.14½. Auricles beating more rapidly again. Ventricles continue to fibrillate and then stop beating. . . .

This result is in sharp contrast with that obtained in another one (No. 12) of these experiments. In this experiment, too, excision of the great veins was without noticeable effect upon the heart beat, but so, too, was the separation of the coronary sinus region from the auricles. The protocols show that rapid excision of this region was without effect, even though the last cut was carried sufficiently anteriorly to cause block. In the remaining experiments excision of the coronary sinus likewise did not alter the beats of the auricles. The region of the great veins had previously been excised, in two of these, with transitory stoppage of the auricles.

A few experiments were so performed as to separate both the right and left auricles more or less abruptly from the auricular septum. In these the beats of the septum were sometimes seen. Their re-

lation to the beats of the other parts of the heart could therefore be studied. We quote here rather freely from the protocols of an experiment (33) which is more or less typical, and illustrates many other phenomena associated with operations of various kinds upon the auricular septum.

Experiment 33 (continued from p. 132).

3.20. Left auricle and ventricles synchronous, right auricle a trifle slower than rest of heart. Temperature, 30° C.; pressure, 60 mm. Hg.

3.30. Cut made downward through outer wall of right auricle near base of appendage, thus functionally excising most of outer wall of right auricle. Before: left auricle beats just a trifle before ventricles; right auricle more rapidly than rest of heart. After: left auricle and ventricles unaffected; right auricle at first markedly slowed, but later becomes more rapid, not so rapid, however, as before.

3.34. While stimulating left auricle more or less rhythmically. Before: left auricle still driving ventricles; right auricle seems to respond occasionally to rest of heart. Temperature, 33.2° C.; pressure, 60 mm. Hg. During: ventricles follow perfectly the extra contractions of left auricle. After: at first marked slowing of left auricle and ventricles, which are synchronous; rate gradually increases to previous one; right auricle same as before.

3.38. Tissue alongside of aorta to right of septum crushed. Before: beat same as in 3.34. After (after disengaging levers): left auricle and ventricles beating slightly more rapidly, and ventricles now definitely precede left auricle; right auricle rapid and independent.

3.41½. Temperature and pressure same as before. Left auricle and ventricles now synchronous, very slight difference in rate; right auricle same as before.

3.44. Right auricle beating rapidly and irregularly, long beats being interspersed between shorter beats, which, in most instances, have one half the length of the longer ones. Cause of irregularity cannot be discovered.

3.46. Cut in vault to left of septum deepened (remnant of auricular septum can now be seen beating before the ventricles). Before: left auricle and ventricles practically synchronous; right auricle beating rapidly and independently. After: left auricle stopped, right continued to beat slowly at first, but gradually increased to exactly preceding rate.

3.56½. Part of outer wall of right and left auricles included between cuts downward on either side of septum cleanly excised, thus exposing coronary sinus, septum still seen preceding ventricles. No effect on right auricle. Ventricles at first accelerated, but soon resumed former rate.

3.59. Temperature and pressure the same as above. Ventricles and right auricle beating regularly and independently.

4.05. Small bit cut away from outer edge of septum of auricles. Septum can still be seen preceding ventricles.

4.09. Cut made downward through septa just inside of outer wall of left ventricle. Before: conditions same as at last notes. After: right auricle unaffected; ventricles markedly slowed, but rate gradually increases till, at the end of long record, it is one half of previous rate. Floor of right auricle (coronary sinus) included between this and previous cuts can be seen beating regularly and independently of other parts, and more slowly than right auricle. . . .

Attention should be called to the fact that the rate of the beat of the septum, plus the coronary sinus, was at first more rapid than that of the right auricle minus the region of the great veins (excision of the veins had caused prolonged stoppage of the heart), but that later sometimes one part, sometimes the other, contracted more rapidly. At times (3.46 *et seq.*) the septum could be seen driving the left auricle and ventricles (the right auricle was independent). Occasionally, when the left auricle was completely severed from the septum, the left auricle and the ventricles contracted simultaneously, but their contraction was definitely seen to be preceded by the contraction of the septum. Definite proof was then obtained that the septum was driving both the left auricle and the ventricles. For, upon temporary impairment of the functional connection between auricle and septum, the former stopped beating. And later, upon separating the coronary sinus region from the rest of the auricular septum, the former continued to beat without interruption, while the ventricular rate was abruptly slowed.

Summary. — The results which have been obtained relative to the rhythmicity of the coronary sinus region are not perfectly clear. On the whole it would appear that it possesses a degree of rhythmicity which is second only to that of the right auricle after removal of the region of the great veins.

THE RHYTHMICITY OF THE AURICULAR SEPTUM WITH AND WITHOUT THE REGION OF THE CORONARY SINUS.

Earlier in this paper it was shown that the septum of the auricles, while still united with the sinus, possesses a degree of rhythmicity which may be estimated to be almost equal to that of the right

auricle after the region of the great veins has been cut away from it. Other experiments have shown that the comparatively high rhythmical power of the septum is in large part due to the presence in it of the region of the coronary sinus. Upon cutting away the region of the coronary sinus the rate of beat of the septum and of other parts of the heart being driven by it is usually slower than before (*e. g.*, Exp. 33). And when the region of the coronary sinus is functionally separated from the rest of the septal region by a cut perpendicular to the auriculo-ventricular junction and passing somewhere anterior to the coronary region, this region, in favorable cases, has been seen beating more rapidly than the parts of the septum remaining functionally attached to the heart.

These observations would imply that the septum of the auricles possesses the property of rhythmicity, but that its grade is below that of the great veins, right auricle (usually), and coronary sinus region. In the course of these experiments we have had abundant opportunity for seeing small bits of the auricular septum attached either to the ventricular septum or to the wall of the aorta, beating with great regularity and, in some experiments, independently of the remaining portions of the heart, and more rapidly than the ventricles. We have also seen such a bit of auricular tissue, attached to the septum of the ventricles, apparently determining the beat of those chambers. At this time a cut so made as to sever the functional connection of this bit of auricular septum with the ventricles resulted in permanent slowing of the ventricles, the bit of septum then beating more rapidly than, and apparently independently of, the ventricles. We have not had occasion to test the rhythmical power of the upper parts of the auricular septum, and in only one experiment has a cut into the upper part of the septum stopped the heart. This was an experiment (No. 11, p. 160) in which stoppage of the heart resulted whenever a cut was made in or near the region of the coronary sinus. However the excision and destruction of the upper and outer parts of the auricular septum and of the whole of the auricular septum between the anterior edge of the coronary vein and the aorta has been more or less successfully attempted in several experiments, always with negative results, excepting when the region in which the bundle lies was impinged upon. Then auriculo-ventricular heart block resulted under such conditions as will be considered in a subsequent section.

These results conform with the statement that the septal region possesses a low grade of rhythmicity. Its destruction does not

noticeably affect the heart beat because, we presume, the heart at the time is being driven by more rhythmical parts.

WHERE MUST A CUT BE MADE THAT WILL CERTAINLY PRODUCE
AURICULO-VENTRICULAR HEART BLOCK?

It has been mentioned that, according to Tawara, the auriculo-ventricular bundle seems to begin near the anterior edge of the coronary vein. It would be interesting to know if impulses of auricular origin must enter the bundle at that place in order that they may pass to the ventricles. If so, then a cut made through the auricular and ventricular septa through the auriculo-ventricular junction anterior to the coronary sinus should permanently block the passage of the impulse from auricles to ventricles. This we have found not to be the case; at least, it is not always the case.

A cut made downward through the auriculo-ventricular junction with scissors, the blades of which are inserted one into each of the ventricles, must of necessity pass central to the anterior lip of the coronary vein. Such a cut does not always produce auriculo-ventricular block. And in experiments of this kind the negative results are more valuable than positive results, because the cutting instrument probably injures tissue some distance on either side of its direct path. Quotations from two protocols will serve to illustrate the results obtained.

Experiment 20 (see pp. 131, 134, 155).

Made cut parallel to auriculo-ventricular junction into auricular septum not quite to aorta. Heart beat unaffected. (At autopsy it was seen that this cut was made very close to the auriculo-ventricular junction, and therefore the upper parts of the auricular septum were not involved in the succeeding cuts through the auriculo-ventricular junction.)

Now began cutting out bits of tissue with cuts traversing the auriculo-ventricular junction and perpendicular to it, beginning in the coronary sinus and going deeper and deeper toward aorta. When a cut was made about 2 mm. inside of the inner walls of the ventricles, stoppage of the ventricles occurred, the auricles continuing to beat. Soon the ventricles began to beat, and then a partial block developed, but ten minutes later the block again became complete spontaneously.

Experiment 33 (see pp. 132, 152, 163).

4.05. Small bit cut away from outer edge of septum of auricles. Septum can still be seen preceding ventricles.

4.09. Cut made downward through septa, just inside of outer wall of

left ventricle. Before: conditions same as in last notes. After: right auricle unaffected; ventricles markedly slowed, but rate gradually increases till at end of long record it is one half of previous rate.

4.15. The left auricle beats after ventricles.

4.19. Left auricle stimulated more or less rhythmically. Ventricles respond to beats of left auricle occasionally.

4.30. Cut down in septa carried about 1 mm. inside of last cut. The ventricles still precede left auricle.

4.34½. Cut in septa carried a trifle nearer to aorta. Left auricle now contracts with every third ventricular beat; rhythm soon became 1:1.

These cuts were carried nearer and nearer to the aorta, always with the same result, until the aorta was cut, thus ending the experiment. The autopsy findings were not clear because so much damage had been done to the heart tissue by the successive cuts. All that can be positively said is that the last cut, which was probably carried as close as possible to the aorta, may have produced block. In the case of the heart prepared as in our experiments a cut made perpendicular to the auriculo-ventricular junction cannot well be carried nearer than about 2 mm. to the posterior edge of the membranous septum without opening the aorta. In every trial excepting the one just quoted a cut in this position has immediately been followed by permanent auriculo-ventricular heart block.

In this connection it should be added that a cut into the auricular septum almost to the wall of the aorta parallel to, and just above the auriculo-ventricular junction, does not block the passage of impulses between auricles and ventricles (*e. g.*, Exp. 20, pp. 131, 134, 155).

DISCUSSION OF THE CAUSE OF SYNCHRONISM OF THE CONTRACTIONS OF AURICLES AND VENTRICLES.

The protocols from which extracts have been chosen have frequently contained the statement that "auricles and ventricles are beating synchronously." In this section an effort will be made to determine the cause of this synchronism. Synchronism of beats seems to have developed after one of two procedures. It appeared either as the result of some operation upon the region of the great veins, usually resulting in its functional separation from the rest of the heart, or after more or less complete separation of the auricles from the auricular septum. In the analysis of this phenomenon we shall, for the sake of accuracy, note its occurrence only in some of

those experiments in which records were made while the operations were being performed upon the heart.

In Experiment 26 the auricles and ventricles contracted synchronously for a while after the region of the great veins had been severely twisted.

In Experiment 27 the auricles and ventricles became synchronous with the first operation upon the region of the great veins, and remained so until a cut was made through the auricular and ventricular septa so as to intersect the auriculo-ventricular bundle. With this the rate of auricular beats at once increased slightly, while the ventricular rate slowed at once.

Torsion exerted upon the region of the great veins in Experiment 28 resulted only in slight slowing of the heart. This result was at first unassociated with any striking changes in the auriculo-ventricular sequence; later, however, the As-Vs interval became very much shorter than it was at the beginning of the experiment, and shortly after the excision of the great veins the auricles and ventricles were beating almost synchronously. At this time the auriculo-ventricular bundle was presumably cut as in Experiment 27. Complete auriculo-ventricular block was immediately established; the auricles continued to beat with absolutely unchanged rate, but the rate of the ventricles was somewhat accelerated.

In Experiment 29 a type of irregularity of heart beat was met with which will require further study for its elucidation. What relation, if any, this irregularity has to our problem we do not know, but neglecting it for the present, it may be said that after several operations upon the region of the great veins and after liberal excision of this region, the left auricle and ventricles were beating practically synchronously, the right auricle a moment later than they. At this time a cut was made so as to presumably intersect the auriculo-ventricular bundle. The ventricles were at once slowed, the auricles somewhat accelerated.

In Experiment 30 a wire was passed through the cavæ, and with it the region of the great veins was twisted. In the early trials, in which but light torsion was used, the rate of the heart beat immediately after was slowed and the beats of the auricles and ventricles were synchronous. After some delay, however, the sequence became normal again. These changes in sequence were not the result of changes in the rate of heart beat. It is true that at times the synchronous gradually gave way to normal beats as the slowed heart

rate approached the normal. But at other times the sequence returned to normal while the heart rate was constant. Furthermore the beats sometimes became synchronous when, after twisting the region of the great veins, the heart rate suffered no change. At times, during the course of this experiment the ventricular beat actually preceded the auricular beat. While the auricles were beating synchronously with the ventricles, the auriculo-ventricular bundle was presumably cut, with the result that the ventricular rate slowed at once, whereas the auricular rate was slightly accelerated.

Experiment 31 was performed in such a way as to throw no light upon the particular problem in hand.

Experiment 32 was performed with a heart the right auricle of which had been so crushed that it no longer contracted. The left auricle and the ventricles were beating normally, but after the left auricle had been almost completely severed functionally from the rest of the heart by a cut just to the left of the auricular septum it for a time contracted synchronously with the ventricles. A cut made later so as to divide the auriculo-ventricular bundle demonstrated that the ventricles had been driven by tissue located above such cut.

In Experiment 33 the excision of the region of the great veins resulted in prolonged stoppage of the whole heart. After the heart had resumed its beat it was found that the As-Vs interval was short, but no significance can be attached to this finding because of the fact that at the same time the rate of beat was slow. Later, while the right auricle was not influencing the beat of the rest of the heart, the left auricle and ventricles were for a time practically synchronous. At this time a cut separating the coronary sinus region from the anterior part of the auricular septum slowed the beat of the ventricles as well as that of the left auricle. Thus proof was obtained that, despite the fact that the left auricle was beating after the ventricles, both parts were being driven by the same part of the auricle. Other proof of this fact was also obtained, but a discussion of it would necessitate the introduction of unimportant details.

In Experiment 34 the contractions of the auricles and ventricles became synchronous after the region of the great veins had been functionally separated from the heart by means of a clamp. Then, after partial separation of the right auricle from the heart, the ventricles contracted before the left auricle, the right auricle later. At a time when the left auricle was not beating (owing to its functional separation from the rest of the auricular tissue), and when the right

auricle was contracting after the ventricles, the auriculo-ventricular bundle was presumably cut. After a short preliminary acceleration the ventricles became quite slow, but the right auricle, after giving a few irregular beats, continued to beat with unchanged rate. Then it could be seen that the auricular beat originated in the septum and reached the right auricle by traversing a connecting fringe of auricular tissue which remained attached to the auriculo-ventricular junction after the region of the great veins had been excised.

The data of Experiments 35 and 36 are similar in almost every respect to those of the foregoing experiments and need not therefore be reviewed here.

This analysis of the experiments in which long records were made will serve to demonstrate that synchronism of auricles and ventricles is not an infrequent result of operations for the functional removal of the region of the great veins and partial separation of the auricles from the septum. What is the cause of this phenomenon? Three answers to this question suggest themselves :

1. The first thought suggested is that which has been advanced in explanation of the synchronism following stimulation of the vagus nerve and associated with stimulation of the accelerator nerve; namely it is the result of the usurpation of the function of pace-maker to the heart by the auriculo-ventricular bundle. This explanation does not, however, bear criticism. Our experiments have shown (*a*) that the auriculo-ventricular bundle, or at least the parts of the heart therewith connected, possess a relatively low grade of rhythmicity. It, or better the auricular septum, is surpassed in this regard not alone by the region of the great veins, but by the right auricle and coronary sinus region as well. Not alone has the auricular end of the auriculo-ventricular bundle a low grade of rhythmicity as judged by the rhythmicity of the tissue in which it lies, but the ventricular end has even a lower grade if we may be justified in estimating this by the rate of beats of the tissue, the ventricles, with which the bundle is connected. (*b*) In the second place, we have been able to show by direct experiment that neither the ventricles nor the auriculo-ventricular bundle, at least the ventricular portion of it, is a causative factor in the production of synchronous beats. For section of the auriculo-ventricular bundle some distance posterior to the membranous septum had no influence upon the contraction of the auricles when beating synchronously with the ventricles, whereas by it the rate of the ventricles was permanently slowed (Fig. 11).

2. As the result of the operations upon the heart the cardiac impulse travels such a course from its point of origin to the bulged portions of the auricles and to the ventricles that these two regions contract at almost the same time. In some of the experiments we have succeeded in demonstrating that this is the cause of the synchronism. The impulse arising, for instance, in the coronary sinus region, owing to the damage produced by the cut or other operation removing the region of the great veins or reducing the functional connection of the auricles with the septum, finds the ventricles quicker of access than the auricles, and therefore causes the former to contract before, synchronously with or even after the latter. Thus, in Experiment 34, the coronary sinus region could be seen to contract first, the ventricles responding soon after, the right auricle still later. The passage of the impulse, or rather of the contraction wave, from the coronary sinus region into the right auricle could be watched. To reach the right auricle it had to traverse a narrow connecting band of tissue.

3. The synchronism of beats may be the result of a lowering of the irritability of the auricles from the insults to which they have been subjected. They would then have a long latent period, and might therefore respond later than the ventricles to a stimulus arising, say, in the coronary sinus region. Our experiments do not seem to support this suggestion.

We may then summarize the foregoing discussion with the statement that the synchronism of auricles and ventricles which so frequently follows the liberal excision of the great veins is probably the result of the production of a partial block on the path of the impulse from the new rhythmical centre to the bulged parts of the auricles. These may contract synchronously with, or even later than, the ventricles. The auriculo-ventricular bundle under the conditions of our



FIGURE 11.—To show synchronous contractions of the left auricle and ventricles and delayed contractions of the right auricle after crushing and excising the region of the great veins. The auriculo-ventricular bundle was cut where the record is irregular. The ventricles at once slow and become independent of the auricles. The time relation between the contractions of the auricles is unaltered. Rabbit.

experiments possesses a very low grade of rhythmicity, and is not the cause of the synchronism.

Since the auriculo-ventricular bundle possesses such a low grade of rhythmicity, and since synchronous beats of the auricles and ventricles may be obtained without the intervention of the auriculo-ventricular bundle, the question suggests itself, Is it necessary to conclude that because during stimulation of the accelerator nerve or after stimulation of the vagus nerve, the auricles and ventricles beat synchronously, or because these parts beat synchronously for any other reason, therefore the beat of the heart begins in the auriculo-ventricular bundle? We do not deny that the beat may begin there but we question the logic of the argument. Since this whole question is one amenable to settlement by the experimental method, we do not deem it wise to suggest other explanations for synchronism of beats obtained by means of stimulation of nerves.

SUMMARY.

In the foregoing pages the results are reported of experiments testing the effect of functional isolation, both partial and complete, of various parts of the auricles. These experiments were performed for the most part upon the excised perfused heart of the rabbit. The functional isolation was accomplished either by means of actual separation of parts with scissors or by means of lines of crushing or by means of torsion. The results obtained by all methods may be summarized as follows :

1. The region of the right auricle in the vicinity of the mouths of the great veins (sinus reuniens?) is possessed of the highest degree of rhythmicity. In at least a vast majority of instances this region normally sets the pace of the whole heart.

(a) The sudden withdrawal of the influence of the region of the great veins over the rest of the heart frequently results in transitory stoppage of the parts of the heart below it, which after recovery usually beat at a permanently slowed rate.

(b) When completely isolated by means of a circular crush, the region of the great veins has sometimes been seen beating more rapidly than the rest of the heart.

(c) When this region is suddenly but not permanently isolated, for example, by means of gentle torsion, there may be witnessed phenomena undoubtedly similar in every respect to those seen when

a transitory block at the auriculo-ventricular junction is established. These phenomena in the order of their occurrence consist of, 1st, stoppage of the auricles and ventricles; 2d, gradual development of the rhythmicity of these chambers (presumably complete sino-auricular heart block); 3d, such alteration in the duration of cardiac cycles as (even in the absence of visible sinus beats) practically proves the presence of a partial sino-auricular block, with such rhythms as 3 : 1, 2 : 1, alternate 2 : 1 and 1 : 1; and, 4th, restoration of normal beat.

2. The region of the coronary sinus possesses a relatively high grade of rhythmicity; in a few instances at least it seems to be acting as pace-maker to the heart.

3. All parts of the right auricle (excluding here the region of the great veins) possess a grade of rhythmicity which is second only to that of the region of the great veins: all parts of it beat when functionally isolated from the rest of the heart. The right auricle may determine the beat of the whole heart after the region of the great veins has been removed.

4. The left auricle, isolated from the auricular septum and right, is not, or at least is rarely, spontaneously rhythmical.

5. The auricular inclusive of the coronary sinus has practically the same degree of rhythmicity as the right auricle exclusive of the region of the great veins.

6. All parts of the auricular septum proper are spontaneously rhythmical, not, however, to the same degree as are the other parts of the auricles which possess rhythmical properties.

7. It therefore follows that the auriculo-ventricular bundle, or at least the parts of the auricular and ventricular septa in which it is embedded, are the least rhythmical of all the parts which go to make up the walls of the right auricle. A small bit of the septum has been seen driving the ventricles after the rest of the auricles had been functionally separated from it.

8. Partial and complete block may be established between any two parts of the auricles, provided one of the parts is spontaneously rhythmical, by narrowing the functional connection between them. When a transitory complete block is established in this way, the less rhythmical part stops beating, but, in case it possess spontaneous rhythmicity, it will soon begin to beat again, first in complete block and then in partial block, with all the rhythms which have been observed, for example, in transitory auriculo-ventricular block. It is

thus possible to obtain a 2 : 1 rhythm between the right auricle and left auricle plus septum, together with a 2 : 1 rhythm between the latter parts and the ventricles. Any other combinations may be obtained.

9. Synchronism of beats of auricles and ventricles not infrequently follows operations severely injuring the region of the great veins. This synchronism is not the result of the assumption by the auriculo-ventricular bundle of the function of pace-maker to the heart; it is due, we believe, to the establishment of a block to the passage of the impulse from the new pace-maker of the heart to the bulged portions of the auricles, — a block which is relatively greater than that in the way of the impulse into the ventricles.

10. Impulses of auricular origin may succeed in reaching the ventricles, and *vice versa*, when there remains of the auricular septum only a narrow border above the ventricular septum, a border connected with the body of the auricle by a strand alongside of the aorta and reaching posteriorly possibly not further than 2 to 3 mm. from the posterior edge of the membranous septum (small rabbit).

11. Operations upon the auricles not infrequently result in stoppage of the whole heart or of parts thereof not directly handled. So far as we have been able to discover such stoppage ("shock") occurs presumably only under two conditions, namely, (*a*), when a more rhythmical part of the heart is separated functionally from one less rhythmical, the latter then ceasing to beat temporarily or permanently, and (*b*) when the part of the heart which presumably is determining its beat is irritated, when the whole heart may stop. Stoppage never has been caused by other, even severe, operations upon the heart.

12. Since all of the phenomena of heart block can be obtained by narrowing the functional connection between any two spontaneously rhythmical parts of the auricles, there seem to be no good reasons for assigning to the auriculo-ventricular bundle any special physiological properties other than those which result from the fact that it is a narrow bridge of tissue joining two other spontaneously rhythmical masses of heart tissue.

THE OXIDATION OF VARIOUS SUGARS AND THE OXIDIZING POWER OF DIFFERENT TISSUES.

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IT has been known, since the work of Voit,¹ Külz,² and others, that a marked difference exists in the ease with which the body can utilize the different sugars as foods, but the causes of this difference are not known. The work of Fischer,³ which has directed attention mainly to the structure of the sugar molecule, has obscured, or at least caused the neglect of, differences in the dynamic properties of these molecules. Fischer observed that some of the sugars, as Pasteur⁴ had shown for some organic acids, were fermented by a given mould or bacterium, while others were either not fermented at all or only at a very slow rate. He ascribed this difference to differences in the inherent characters of the moulds or the structure of the sugars rather than to the ease with which the sugars could be fermented, and drew the conclusion that there must be a structural relationship between the constituents of the protoplasm of the mould or bacterium and the sugar fermented. If this relationship was wanting, the sugar was not fermented. Illuminating as this idea is, it is nevertheless clear that there are several other possible explanations of the facts. In the first place, his explanation takes no account of the differences in the ease of the decomposition of the sugars; in the second, it makes no sharp distinction between the two different factors concerned in the fermentation or oxidation of sugars, *i. e.*, the potential factor and the rate factor. The lock-and-key fermenta-

¹ VOIT, C.: *Zeitschrift für Biologie*, 1891, xxviii, p. 245.

² KÜLZ, E.: *Archiv für die gesammte Physiologie*, 1881, xxiv, p. 1.

³ FISCHER, E.: *Berichte der deutschen chemischen Gesellschaft*, 1894, xxvii, pp. 2985, 3479; 1895, xxviii, p. 1429; 1896, xxiii, p. 2137; 1898, *Zeitschrift für physiologie Chemie*, 1898, xxvi, p. 60.

⁴ PASTEUR: *Comptes rendus*, 1858, lviii, p. 179; 1859, xlvi, pp. 640, 735; 1860, l, p. 1083.

tion hypothesis carries with it the assumption that the ferment influences the potential factor in the transformation; in other words, the ferment itself brings about the degradation of the sugar molecule. This conception is in sharp contradiction to the theory most generally accepted at present, namely, that ferments are incapable of initiating a reaction or of determining the character of dissociation. Their influence is mainly or altogether on the rate of the reaction or amount of decomposition. According to this latter view of ferment actions, an organism may be unable to utilize a sugar for either of two reasons. First, because it has not sufficient energy to bring about the oxidation of the sugar after dissociation of the latter. In other words, the potential of its oxidizing energy is not high enough to affect one sugar, while it may be high enough to oxidize another. Or, second, the potential may be high enough, but the catalytic agent necessary to cause the oxidation or dissociation to take place with sufficient speed may be lacking. In this case the organism is able to decompose the sugar so far as energy is concerned, but lacks the agent necessary to cause the process to take place with sufficient speed to make the sugar available as a food.

In view of these facts it was suggested to me, by Professor A. P. Mathews, that I should attempt to determine the difference, if any, in the ease of oxidation of sugars outside of the body; second, to determine the differences in the oxidizing power of the various organs of the body; third, to endeavor to find whether there was any relationship between the power of the body to utilize a sugar as food and its ease of oxidation outside the body. These questions derive additional interest from the point of view of protoplasmic respiration or oxidation, since up to the present time all theories of oxidation have made no distinction between the potential and rate factors of the oxidation. This research is primarily an attempt to distinguish between these two factors.

A. THE DIFFERENCES IN THE EASE OF OXIDATION OF THE VARIOUS SUGARS.

The first point to be determined was the difference that exists in the ease of oxidation of various sugars by ordinary chemical reagents. If it could be shown that the monosaccharides differ in their ease of oxidation by some inorganic oxidizing agent, it would no longer be necessary to assume a structural relationship between them and the

oxidizing substance of the body in order to explain their different ease of oxidation in the organism. The method followed in answering this question was that suggested by Mathews.¹ It depends on the fact that if a standard solution of cupric acetate be taken and progressive amounts of acetic acid added to it, the oxidizing power² of the solution is steadily diminished. By the addition of the acid the solution gradually loses its power to oxidize the various sugars. The sugar first thrown out is lactose; the one that requires the largest amount of acid to prevent its oxidation is levulose.

The first problem, then, was to determine for each sugar the exact amount of acid necessary to be added to varying concentrations of cupric acetate to just prevent a visible reduction to cuprous oxide within a certain time limit. A neutral solution of cupric acetate will oxidize levulose, galactose, glucose, maltose, or lactose, when heated to boiling for half a minute. If a sufficient concentration of acetic acid is added to the neutral cupric acetate solution, it becomes incapable of oxidizing any of the sugars named in the time specified. A very small amount of acid prevents the oxidation of lactose; a little more will prevent the oxidation of maltose; glucose requires more than maltose; then follows galactose and levulose in the order named. By a proper mixture of acetic acid and copper acetate it is possible to oxidize levulose very completely and to leave glucose almost unoxidized. We have thus a simple means of separating or distinguishing these sugars. Sugars so widely separated as lactose and glucose can be very easily distinguished in this way. This is the principle of Barfoed's solution which is used to distinguish the monosaccharides from the disaccharides.

I. **Determination of the amount of acid necessary to stop the oxidizing action of cupric acetate.** — A solution of cupric acetate was prepared from a saturated solution of recrystallized cupric acetate. The supernatant liquid was filtered and the copper in it determined by electrolysis. The solution was then diluted to make a $m/3$ solution. The amount of copper in this last solution determined by electrolysis was 21.0 gm. per litre. The acetic acid was determined by distillation from phosphoric acid, and yielded approximately the theoretical amount per litre.

A 2 per cent solution of sugar was used in each case. The final

¹ MATHEWS: American journal of physiology, 1904, xi, p. 224.

² By "oxidizing power" in this paper is meant the product of speed of oxidation into the potential of oxidation.

concentration of the sugar was approximately the same in each case, although I decided that this could be varied considerably without material error.

The smallest amount of acid necessary to stop the oxidative action of different concentrations of cupric acetate was determined as follows: 1 c.c. of the cupric acetate solution and 1 c.c. of the sugar solution were mixed and heated to boiling to determine whether the neutral salt would oxidize the sugar. If a reduction was obtained, another mixture of the same proportions was taken and a definite amount of acid added, and the result on heating noted. The exact amount of acid required was found by a number of trials. An example will illustrate the method.

In the case of levulose,

- I. 1 c.c. Cu acetate $m/3$,
 1 c.c. 2 per cent levulose,
 1.2 c.c. $n/1$ acetic acid,

were mixed in a test tube. The mixture, when boiled for approximately one-half minute and cooled, showed a reduction and the formation of cuprous oxide.

- II. 1 c.c. Cu acetate $m/3$,
 1 c.c. 2 per cent levulose,
 1.5 c.c. $n/1$ acetic acid,

heated as above, gave no reduction.

The strength of acid that will just prevent the action of cupric acetate in this case is somewhere between 1.2 c.c. and 1.5 c.c. A number of trials proved that

- III. 1 c.c. Cu acetate $m/3$,
 1 c.c. 2 per cent levulose,
 1.4 c.c. $n/1$ acetic acid,

was the proper concentration. More acid than this prevented oxidation for a longer period; less did not prevent it, since a precipitate of cuprous oxide was formed. The final dilutions from these figures are Cu acetate $n/5$ and acetic acid $n/2.42$. The cupric acetate was then diluted to a known concentration and the amount of acid required to prevent its oxidizing action under the same conditions as above, determined. Successive dilutions were made, and the amount of acid required to prevent the oxidative action of each dilution determined,

until the cupric acetate was so dilute that in neutral solution it would not oxidize levulose to the formation of cuprous oxide in the time limit. The amount of acid necessary to prevent the oxidation of other sugars was determined under the same conditions, and the results are given in Tables I and II.

TABLE I.

LEVULOSE.

Cu acetate final dilution ¹ (equivalent solutions).	Dilution of acid necessary to stop oxidation.
5.0	2.42
6.0	3.0
8.0	4.0
15.6	8.5
19.11	11.0
25.0	20.0
41.0	36.0
60.0	50.0
72.0	60.0

The figures are the final dilutions of cupric acetate expressed in normal or equivalent solutions, and opposite them the dilution of acetic acid just sufficient to prevent the oxidative action of the given concentration of copper for one minute.²

TABLE II.

GALACTOSE.

Cu acetate dilution.	Acetic acid dilution.
5.04	4.90
6.0	6.0
10.0	10.0
19.0	24.0
21.6	30.6
36.0	72.0
40.0	80.0
75.0	200.0?

GLUCOSE.

3.68	5.52
7.8	8.6
14.4	24.0
28.8	54.0
57.8	150.0

¹ By dilution is meant the number of litres of solution containing 1 gm. equivalent.

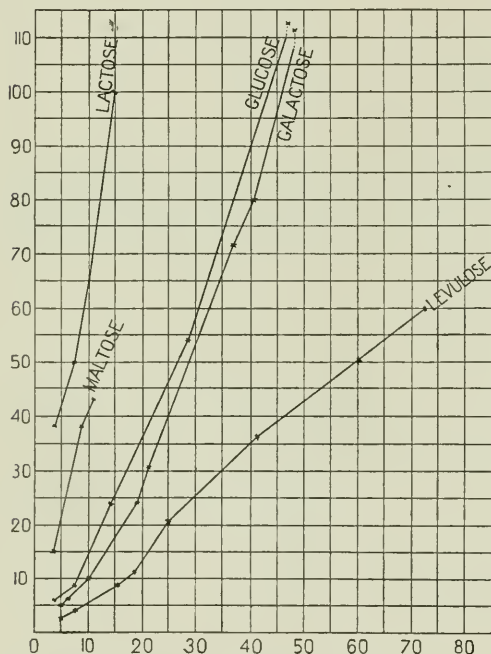
² By utilizing these figures and the interpolations from the chart, solutions may be made capable of oxidizing certain sugars and leaving others largely unoxidized. See Part IV.

TABLE II (continued).

MALTOSE.	
Cu acetate dilution.	Acetic acid dilution.
3.5	15.0
8.4	38.0
11.0	43.0
LACTOSE.	
4.0	38.0
7.5	50.0
15.0	100.0
30.0	200.0

RESULTS.

It is apparent from these figures and the accompanying chart that there is a wide difference in the ease of oxidation of the various sugars,



The curves show the relationship between the concentrations of $\frac{1}{2}$ cupric acetate and of acetic acid necessary to prevent oxidation of the different sugars. Abscissæ represent dilutions of $\frac{1}{2}$ $\text{CuC}_2\text{H}_3\text{O}_2$; ordinates represent dilutions of acetic acid.

In galactose the ratio is approximately 1 : 1 in the $n/5$ solution and 1 : 2 in the $n/40$ th solution. This suggests naturally that we are dealing with an action of the hydrogen ion, the ionization of the acetic acid being more reduced in the strong solution. This matter is discussed more fully in Part IV.

and that they follow the order from the most easily to the least easily oxidized sugar of levulose, galactose, glucose, maltose, and lactose. The figures do not show any constant ratio between the amount of acetic acid necessary to prevent oxidation and the concentration of the acetate in the case of any one sugar, for in concentrated solutions the ratio of acetic acid to acetate is higher than in the more dilute. Thus in the case of levulose the ratio is approximately 2 molecules of acetic acid to one equivalent of the acetate in the $\frac{1}{5}$ normal solution and nearly one molecule to one in the $\frac{1}{72}$ normal. In galactose the ratio is approximately 1 : 1 in the

These facts establish the main point, i. e., that there is a marked difference in the ease with which different sugars are oxidized by cupric acetate solution, and they at once suggest an explanation of why levulose is more easily burned by the body than glucose, and why lactose must be inverted before it can be burned with any speed.

These figures do not show, however, whether the different ease of oxidation of the various sugars is due to the fact that the reducing potentials of the different sugars are different, or to the fact that they dissociate to different degrees so that the reaction takes place much faster in some than in others. This point is taken up in Part IV.¹ Nor do they show whether the addition of acid to cupric acetate reduces its oxidizing power by a reduction of the speed or potential factor.

If we could determine the absolute voltage necessary to oxidize any of these common sugars, and if it were found that the potential was different with the different sugars, we would then be able to state in volts just how much the oxidative energy of the body is reduced

¹ In the beginning of this investigation, I attempted to measure the amount of undissociated acetic acid in the cupric acetate solutions and of undissociated cupric hydrate by measuring the amount of acetic acid given off from the solution at 99.5° and comparing it with the amount given off by various concentrations of acetic acid. The method proved to be wrong and the determination useless, but the interesting fact came out that dilute solutions of cupric acetate will give off acetic acid to a current of air passing through the solution with a speed practically equivalent to that of a pure acetic acid solution of the same strength. This shows the extreme rapidity with which hydrolytic decomposition occurs to make good the equilibrium disturbed by the loss of a molecule of acetic acid to the air. In strong solutions ($m/3 - m/7$), however, the acid came off only about one half as rapidly as it did from an acetic acid solution of the same strength, owing to the amount of hydrolysis in the strong solution being less.

In the preliminary note I called attention to the empirical formula suggested by Professor A. P. Mathews:

$$\frac{V. \text{ Cu acetate} \times Y}{V. \text{ acetic acid} \times X} = K.$$

v. dilution

x. per cent $\text{Cu}(\text{OH})_2$

y. per cent ionized acetic acid.

By applying the formula to the results given in the reference, we obtained an approximate constant. However, it has since been decided that the data used in the calculation of the concentration of the cupric hydrate were obtained by incorrect methods, consequently the constant given is erroneous; yet the order of the constants is correct.

The error in the calculation of the hydrate, whatever its magnitude, was included in each calculation so that, while the size of the constant given is wrong,

when it is unable to oxidize dextrose but still retains the power to utilize levulose. In Part IV it is shown that the potential necessary to oxidize these sugars is not more than 0.668 volt, but the minimum voltage capable of oxidizing them is not known, nor could we determine differences between the potentials in the various sugars. I was, therefore, unable to answer the first question investigated, *i. e.*, whether the sugars had different reducing potentials.

PART II. AN ATTEMPT TO DETERMINE THE RELATIVE OXIDIZING POTENTIAL OF THE DIFFERENT TISSUES.

It is greatly to be desired that we should be able to express in volts the absolute oxidizing potential of the various tissues of the body. If we knew this factor, and knew also how great a potential was necessary to oxidize any food, we could at once state whether that food could be oxidized by the tissue or not. We originally thought that the addition of acid to the cupric acetate diminished the oxidizing potential of the solution, and it was suggested that in this way, by adding acid to the various organs, their oxidizing potential could be determined, at least relatively. Further investigation showed this hypothesis to be incorrect, and that the acid dimin-

the relative order remains the same. The average constants as found, then, are as follows:

Levulose	0.0262
Galactose	0.0182
Glucose	0.0153
Barfoed's reagent	0.0130
Maltose	0.0093
Lactose	0.0078

The correct value for the constants is given in Part IV.

Barfoed's solution which is used to distinguish the monosaccharides from the disaccharides is approximately cupric acetate containing $n/6$ acetic acid. In the use of the solution, if somewhere near the same amount of the reagent as the solution is used and the final dilution calculated, as we have done in the above cases, we get a value which agrees well with the theory, and places the constant between those of the mono- and the di-saccharides, giving it a wide variation for dilution and still remaining useful in the identification of the two groups. It will oxidize the monosaccharides, but will not within the time limit of an ordinary test oxidize maltose or lactose. The limit of the amount of acid which may be used in Barfoed's reagent varies considerably, and yet the solution remains sensitive enough to distinguish between the mono- and di-saccharides. If we wish to distinguish between sugars standing so closely together as glucose and galactose, or glucose and levulose, the variation is much smaller, and more delicate work is necessary.

ished only the *rate* of oxidation in the cupric acetate without affecting its potential. Although the original hope of being able thus to get some idea of the relative oxidizing potentials of the various tissues was not fulfilled, the results obtained are of considerable interest in connection with the speed of oxidation by various tissues, and are given here.

Many determinations have already been made of the so-called "oxidizing power" of the various tissues, but these determinations have always been made by determining the relative amounts of some substance, chosen as a standard, oxidized in a given time (salicylic aldehyde, sugar, uric acid, etc., and without any clear recognition of the two fundamentally different factors of speed and potential involved in these oxidations). Such measurements throw but little light on the question of oxidizing potential, but principally on the "rate" of oxidation by that organ for the particular substance investigated. No conclusions can be drawn concerning absolute oxidizing potential from such experiments. It is clear that if any organ has a catalyser to hasten the oxidation of any substance used as a test, that organ might oxidize great quantities of it, while the actual potential might be lower than that of an organ which oxidized very small amounts, owing to the absence of a catalyser; and, on the other hand, the catalyser might be present in an organ oxidizing not at all owing to its low potential of oxidation. We hoped that, having obtained definite information concerning the oxidizing potential of the various organs, it would be possible in any disease characterized by a loss of power of oxidation of any substance to say whether this loss was due to the lowering of potential or to a loss of catalyser. The method we adopted, however, could only throw light on the rate factor.

Hydrogen peroxide was chosen as the substance oxidized on account of the ease with which the fact of its oxidation can be determined. It is open to the objection that catalysers which hasten its oxidation exist in many tissues. Hydrogen peroxide occupies a position somewhere between cupric and hydrogen ions as an oxidizing agent; that is, it is oxidized by cupric salts, by ferric chloride and silver salts, and oxidizes metals lying above hydrogen in the table of solution tension. We thus know approximately where it stands as regards its oxidizing potential. The speed of the oxidation is greatly retarded by an acid reaction, and if sufficient acid is added the oxidation is almost entirely suppressed for a considerable period.

To test the action of acid on the rate of this oxidation, a series of

experiments were carried out, using ferric chloride as an oxidizing agent. The following mixtures were placed in small bottles and the gas collected over water in graduated tubes.

TABLE III.

		Fe_2Cl_6 <i>n</i> /10	H_2O	H_2SO_4 <i>n</i> /10	H_2O_2
I.	Contained	5 c.c.	25 c.c.	10 c.c.	5 c.c.
II.	"	5 "	30 "	5 "	5 "
III.	"	5 "	40 "	5 "	5 "
IV.	"	5 "	40 "	0 "	5 "

Time in minutes.	Temp. 24° C.			
	I. Gas.	II. Gas.	III. Gas.	IV. Gas.
5	0	0	0	tr
10	0	0	0	8.0
15	0	0	0 ¹	13.5
20	0	0 ¹	0,	22.5
25	0	0	0	27.5
30	0	0	0.5	32.0
35	0	0.5	1.2	35.5
40	0	3.0	3.0	39.0
45	0	7.5	8.5	41.5
50	0 ¹	10.5	11.0	42.5
55	0	14.0	12.0	44.0
60	0	17.0	13.5	44.5

TABLE IV.

		Fe_2Cl_6 <i>n</i> /10	H_2O	H_2SO_4 <i>n</i> /10	H_2O_2
I.	Contained	15 c.c.	5 c.c.	25 c.c.	5 c.c.
II.	"	15 "	10 "	20 "	5 "
III.	"	15 "	15 "	10 "	5 "
IV.	"	15 "	30 "	0 "	5 "

Time in minutes.	Temp. at 24° C.			
	I. Gas.	II. Gas.	III. Gas.	IV. Gas.
11.00 A.M.	0	0	0	0
110.5	0	0	0	2.5
11.10	0	0	0 ¹	17.0
11.15	0	0 ¹	0.5	29.0
11.20	0 ¹	0	2.0	34.5
11.25	0	0	4.5	40.0
11.30	0	0	7.0	42.5
11.35	0	1.0	10.5	44.5
1.35 P.M.	17	34.5	50.0	47.0

¹ Indicates a fermenting appearance, but no gas yet given off.

The effect of dilution on catalysis is shown by the following experiment:

TABLE V.

		Fe ₂ Cl ₆ <i>n</i> /10	H ₂ O	H ₂ O ₂
I.	Contained	5 c.c.	10 c.c.	5 c.c.
II.	"	5 "	25 "	5 "
III.	"	5 "	50 "	5 "
IV.	"	5 "	100 "	5 "

Time in minutes.	I. Gas.	II. Gas.	III. Gas.	IV. Gas.
1.45	0	0	0	0
1.50	0.5	0	0	0
1.55	11.0	4.5	1.5	0
2.00	19.5	12.0	4.5	1.5
2.10	32.0	28.0	15.5	7.0
2.25	37.5	38.0	26.5	15.0
2.50	38.5	45.0	35.5	23.0

From this it is seen that the action is retarded by dilution.

From experiments of this kind I. determined the amount of ferric chloride solution (*n*/10) that a given concentration of acid would stop for thirty minutes. The final volume of the solutions was 50 c.c.; normal acid was used and the concentration calculated from the final dilution. The result is given in Table VI.

TABLE VI.

Vol. <i>n</i> /10 Fe ₂ Cl ₆	Concentration of sulphuric acid necessary to prevent gas evolution for thirty minutes.
5 c.c.	<i>n</i> /100
8 "	<i>n</i> /80
10 "	<i>n</i> /70
13 "	<i>n</i> /60
15 "	<i>n</i> /50
15 "	<i>n</i> /40 (?)
20 "	<i>n</i> /30
28 "	<i>n</i> /20

The influence of time is shown when the amount of acid necessary to prevent gas evolution for twenty-four hours are given:

<i>n</i> /10 Fe ₂ Cl ₆	Sulphuric acid to prevent evolution of gas.
4 c.c.	<i>n</i> /30
2 "	<i>n</i> /70

These experiments show that the addition of acid to ferric chloride does not prevent the oxidation of hydrogen peroxide by ferric chlo-

ride, but only reduces the rate at which the reaction goes on. This reduction of rate is to be interpreted in the light of the work done in Part IV. The addition of the acid does not greatly alter the number of ferric ions; its main action is to reduce hydrolytic dissociation, to reduce the number of oxygen and hydroxyl ions, and possibly to reduce the dissociation of the hydrogen peroxide. Its action, therefore, is not that of changing the oxidizing potential of the solution, which is determined by the ferric ions present, but only that of reducing in number some or all of the active particles, ferric ions, oxygen ions, or the products of dissociation of hydrogen peroxide, taking part in the reaction, and thus reducing the speed. I think it probable, in the light of Part IV, that the main action of the acid is the reduction of the concentration of the oxygen ions and the reduction of the dissociation of the hydrogen peroxide.

If the concentration of the peroxide is kept constant, and if the time is kept constant, the addition of acid to the different tissues should enable us to form some estimate of the *number of active oxidizing particles* capable of oxidizing hydrogen peroxide in the different tissues.

In order to prepare a uniform sample of the tissues, the animals were bled to death under ether anæsthesia. The blood was drained out as completely as possible without washing. The organs were then quickly removed and ground to a pulp in a grinder, and placed in 95 per cent alcohol for twenty-four hours. After this time the alcohol was filtered off and the residue washed with alcohol and ether. It was then spread on a porcelain plate and dried in the air. When completely dry, the residue was ground to a fine powder and any connective tissue was removed by sifting. In this form the tissue was used in the experiments. The method of preparation is open to criticism, as the alcohol unquestionably affects the oxidizing power, and the various tissues may be differently affected, but for this preliminary investigation it was decided to use it. Two tenths of a gram of the dried powder was used in each case. It was weighed and placed in a medium-sized test tube, and 15 c.c. of HCl of known strength added. A series was prepared in the same way with varying concentrations of acid. The acid was allowed to macerate the samples for thirty minutes. After this time, 5 c.c. of neutral hydrogen peroxide (Aqua Hydrogenii Dioxidi U. S. P.) was added, and the gas evolved collected in a eudiometer tube over water. Where the tissue showed a tendency to rise out of the liquid, owing to gas bubbles ad-

hering to the powder, a little glass wool was inserted in the tube before the addition of the acid.

The strength of the acid just sufficient to prevent for a certain time the action of the powdered tissue on the peroxide, was determined from a number of trials. If, for example, it was found that acid of the strength $n/20$ prevented the evolution of gas while $n/50$ gave a considerable amount, the strength just sufficient to prevent the action of the powder lies somewhere between $n/20$ and $n/50$. By comparing a number of tubes between these concentrations the correct point can be approximately obtained. It is necessary that the acid should act on the tissue before the addition of the neutral peroxide, otherwise the ferment may act on the peroxide before the acid has had time to penetrate the tissue powder. Fairly constant results can be obtained by this method.

Is the oxidative power of homologous organs the same or nearly the same in animals of the same species? To determine this the tissues of a number of animals prepared as above were used. The results show that there are slight individual differences, but the order of the different organs of one animal holds for all of the species, and varies within a comparatively narrow range. The variation in the muscles of different animals is much less than the variation between the muscles and any other organ. This statement holds for the other tissues.

In this preliminary work I used the tissues of four rabbits, four dogs, one cat, and one hog. Clearly, not enough to make a sweeping statement from the results, yet the uniformity is striking.

The figures in Table VII give the variations in the results obtained:

TABLE VII.

Tissue.	Acid necessary to prevent gas formation for one hour.	After one hour gas in control, no acid.	Temperature. C.
Muscles — dog	1 $n/100$	3.5 c.c.	21°
“ “	2 $n/100$	3.6 “	“
“ “	3 $n/120$	6.5 “	“
“ “	4 $n/120$?	“
Pancreas — dog	1 $n/90$	5.8 “	“
“ “	2 $n/70$	7.5 “	“
“ “	3 $n/80$	35.0 “	“
“ “	4 $n/60$	20.0 “	“
Lung — dog	1 $n/80$	10.4 “	21°-23°
“ “	2 $n/60$	8.0 “	“
“ “	3 $n/60$	9.5 “	“
“ “	4 ?	?	“

TABLE VII (continued).

Tissue.	Acid necessary to prevent gas formation for one hour.	After one hour gas in control, no acid.	Temperature. C.
Spleen — dog	1 <i>n</i> /60	27.0 c.c.	21°
“ “	2 <i>n</i> /50	6.0 “	“
“ “	3 <i>n</i> /50	35.0 “	“
“ “	4 <i>n</i> /50	10.0 “	“
Liver — dog	1 <i>n</i> /50	25.0 “	“
“ “	2 <i>n</i> /50	20.0 “	“
“ “	3 <i>n</i> 30	24.7 “	“
“ “	4 <i>n</i> /25	10.4 “	“
Kidney — dog	1 <i>n</i> /30	20.6 “	21°-23°
“ “	2 <i>n</i> /25	19.5 “	“
“ “	3 <i>n</i> /20	15.0 “	“
“ “	4 <i>n</i> /25	16.0 “	“
Muscle — rabbit	1 <i>n</i> /85	8.0 “	23°
“ “	2 <i>n</i> /85	7.5 “	“
“ “	3 <i>n</i> /90	10.0 “	“
“ “	4 <i>n</i> /100	10.0 “	“

PANCREAS NOT TAKEN.

Liver — rabbit	1 <i>n</i> /60	45.0 “	“
“ “	2 <i>n</i> 60	40.0 “	“
“ “	3 <i>n</i> /65	45.0 “	“
“ “	4 <i>n</i> /60	“
Kidney — rabbit	1 <i>n</i> /20	33.0 “	“
“ “	2 <i>n</i> /35?	34.5 “	“
“ “	3 <i>n</i> /20	36.0 “	“
“ “	4 <i>n</i> /15	35.0 “	“
Spleen — rabbit	1 <i>n</i> /80	29.5 “	“
“ “	2 <i>n</i> /80	24.3 “	“
“ “	3 <i>n</i> 90	29.5 “	“
“ “	4 <i>n</i> /90?	“
Lung — rabbit	1 <i>n</i> /80	15.0 “	“
“ “	2 <i>n</i> 80	26.0 “	“
“ “	3 <i>n</i> /70	48.0 “	“
“ “	4

The average results for the tissues for four rabbits are given in Table VIII.

TABLE VIII.

	Strength of acid to prevent action.	Average gas in control tube in one hour.	Temp. of room and solution C.
Muscle	<i>n</i> /90	8.8 c.c.	21°
Pancreas
Spleen	<i>n</i> /85	26.9 “	21°
Lung	<i>n</i> /80	22.0 “	23°
Liver	<i>n</i> /60	42.5 “	21°
Kidney	<i>n</i> /25	34.0 “	“

Average result from the tissue of four dogs.

TABLE IX.

	Strength of acid to prevent action.	Average gas in control tube in one hour.	Temp. of room and solution C.
Muscle	<i>n</i> /110	4.6 c.c.	21°
Pancreas	<i>n</i> /75	18.0 "	"
Spleen	<i>n</i> /60	19.5 "	"
Lung	<i>n</i> /60	9.3 "	"
Liver	<i>n</i> /40	23.2 "	24°
Kidney	<i>n</i> /25	18.0 "	21°

Average result of one cat's tissues.

TABLE X.

	Strength of acid to prevent action.	Average gas in control tube in one hour.	Temp. of room and solution C.
Muscle	<i>n</i> /100- <i>n</i> /90	6.5 c.c.	23°
Pancreas
Spleen	<i>n</i> /90	19.5 "	23°
Lung	<i>n</i> /60	35.0 "	"
Liver	<i>n</i> /50	27.5 "	"
Kidney	<i>n</i> /45	20.5 "	"

Result of tissues of hog — not taken when fresh, but within twenty-four hours.

TABLE XI.

	Strength of acid to prevent action.	Average gas in control tube in one hour.	Temp. of room and solution C.
Muscle
Pancreas	<i>n</i> /80	2.5 c.c.	21°
Spleen	<i>n</i> /80	11.5 "	23°
Lung	<i>n</i> /40	7.0 "	21°
Liver	<i>n</i> /40	30.0 "	23°
Kidney	<i>n</i> /30- <i>n</i> /40	20.0 "	22°

DISCUSSION OF RESULTS.

These results, I think, are as nearly correct as the methods will yield. They show that the catalysis of hydrogen peroxide is slowed to the point where no visible evolution of gas occurs in one hour, with different ease in the different organs. It takes, in rabbits, cats, dogs, and hogs, most acid to check the kidney; the liver comes next, requiring two thirds to one half as much acid as the kidney; the lung curiously comes next, the spleen and pancreas come close together, and the muscles require the least. It requires roughly three times as much acid to slow down the kidney to a certain point as is required for muscles. It might be supposed that the acid-combining powers of

the tissues come into action here, but were that the case the muscle should require more acid than the kidney.

Some idea of the meaning of these results appears clear from a study of the following paper (p. 199). It is shown there that the speed of oxidation of sugar by cupric acetate is proportional to three things, the concentration of oxygen ions, of cupric ions, and of dissociated sugar particles in the solution. Similar factors are probably found here, although other factors not found in the simpler case have also to be considered. The active particles in this catalysis are, first, the oxidizing positively charged particles of unknown nature in the tissues; second, probably the oxygen ions; third, the dissociated hydrogen peroxide; and fourth, the catalase, which may act by increasing the dissociation of the hydrogen peroxide. If the speed is constant, and the dissociation of the hydrogen peroxide is constant, the variation must be in the other two factors. It must be, therefore, either that those tissues which require most acid contain substances which increase the dissociation of the hydrogen peroxide, or that the concentration of the positive oxidizing particles in such tissues is greater than in tissues requiring less acid. They must be greater in the kidney than in the muscle. The latter view appears to the writer the more probable, for the reason that no clear relationship exists between the amount of acid necessary to add and the amount of catalase in the tissue. This becomes apparent by an inspection of Tables VIII, IX, X, and XI. The kidney, yielding less gas in an hour than the liver, nevertheless requires more acid to check it. On the other hand, the muscle containing least catalase or fewest active particles also requires the lowest concentration of acid to check it. We may assume, from analogy with the sugar oxidation, that the action is, at least in part, on the concentration of oxygen ions. If this is the case, then, as the concentration of the oxygen ions varies inversely as the square of the concentration of the hydrogen ions, the various concentrations of the positive particles (not the catalase) in these organs will be proportional to the square of the concentrations of the hydrogen ions necessary to check their action. For dogs we have, then, assuming for the sake of simplicity that the acids are entirely dissociated and uncombined:

	V_H	V^2_H		V_H	V^2_H
Muscle . . .	100	10,000	Spleen . . .	60	3,600
Pancreas . . .	90	8,100	Liver . . .	50	2,500
Lung . . .	80	6,400	Kidney . . .	30	900

$$\frac{C \text{ Kidney}}{C \text{ Liver}} = \frac{2,500}{900} = 2.77.$$

$$\frac{C \text{ Kidney}}{C \text{ Spleen}} = \frac{3,600}{900} = 4.$$

$$\frac{C \text{ Kidney}}{C \text{ Lung}} = \frac{6,400}{900} = 7.1.$$

$$\frac{C \text{ Kidney}}{C \text{ Pancreas}} = \frac{8,100}{900} = 9.$$

$$\frac{C \text{ Kidney}}{C \text{ Muscle}} = \frac{10,000}{900} = 11.1.$$

In other words, the concentration of positive oxidizing particles capable of oxidizing hydrogen peroxide in the kidney powder of the dog is four times that of the spleen; two and seventy-seven hundredths times the liver; seven and one-tenth times the lung; nine times that of the pancreas; and eleven and one-tenth times that of the muscle.

These figures can be regarded only as rough approximations possibly wide of the mark, and it is of course doubtful how far they may be carried over to the living tissue, but such wide variations are certainly of very great interest. As regards the potential of these positive particles, it cannot be less than that of hydrogen ions, that is, 0.16 volt since they then oxidize the peroxide. How much greater potential they have than this cannot at present be said. We are, therefore, certain, from the results of this investigation and that of the oxidation of sugars, that these tissues contain positive particles having a tension of at least 0.16 volt, and probably somewhat higher than this. And we may infer, also, that in the powder of the dried organs these particles are present in greatest numbers in the kidney and in the other organs in the order named.

It is worthy of notice that these tissues behave thus towards acids in the direct ratio of their erepsin content. Vernon¹ has shown that erepsin is more abundant in the kidney than in any other organ, while the muscle and brain tissue contain the least. From the result obtained of the action of acid on the tissues we might conclude that the muscles are the most easily injured in their oxidative powers by an increase of acidity of the blood, while the kidneys are comparatively hard to injure by the acid. This may be due to a tolerance created by the acid urine. The smallest amount of acid lowers the oxidative power somewhat, a larger amount injures it still more.

¹ VERNON: *Journal of physiology*, 1904, xxxii, p. 33.

An example will show how an increase in the amount of acid acts on the catalysis. — Dog's liver, 0.74 gm., treated as above :

TABLE XII.

Time.	<i>n</i> /25 acid. Gas.	<i>n</i> /30 acid. Gas.	<i>n</i> /40 acid. Gas.	<i>n</i> /50 acid. Gas.	No acid. Control. Gas.
9.30	0	0	0	0	0
9.35	0	0.5	0.7	1.3	3.2
9.45	tr.	1.0	1.3	1.8	4.0
10.05	"	1.3	1.8	2.4	5.8
10.15	"	1.3	2.10	2.4	8.5
10.30	"	1.5	2.3	3.3	10.4

The strength of acid to prevent action here was *n*/25. *n*/30 was too weak to entirely stop the action for one hour, but it markedly inhibited it. Weaker solutions have less action.

Some work was done on the brain, but the method of preparation of the tissues did not yield a powder with organs of a lipoid nature; so the results are not included. It can be stated, however, that the brain tissue requires less acid than does the muscle to prevent its action on hydrogen peroxide.

A comparison of my results with those of other investigators is of interest. Medwedew,¹ in a review of the history of oxidation in the animal body, gives the order obtained by Salkowski² and Abelous and Biarnès.³ Spitzer⁴ has also determined the oxidative energy of fresh tissues, and we give his results and those of the other authors mentioned along with those found by myself.

I.	II.	III.	IV.
Spitzer.	Salkowski.	Abelous and Biarnès.	McGuigan.
Blood.	Liver.	Spleen.	Kidney.
Spleen.	Spleen.	Liver.	Liver.
Liver.	Kidney.	Lungs.	Lungs.
Pancreas.	Pancreas.	Thyroid.	Spleen.
Thymus.	Muscle.	Kidney.	Pancreas.
Brain.		Thymus.	Muscle.
Muscle.		Muscle.	Brain.
Ovary.		Brain.	
Fallopian tube.		Pancreas.	

¹ MEDWEDEW: *Archiv für die gesammte Physiologie*, 1896, lxxv, p. 249.

² SALKOWSKI: *Centralblätter für die medicinischen Wissenschaften*, 1894, xxxii, p. 913; also SALKOWSKI (mit Jamagiwa): *Virchow's Archiv für pathologische Anatomie*, 1897, cxlvii, p. 1.

³ ABELOUS and BIARNÈS: *Archives de physiologie*, 1895, vii, pp. 95, 239; *Ibid.* 1896, viii, p. 311.

⁴ SPITZER: *Archiv für die gesammte Physiologie*, 1897, lxxvii, p. 621.

It is important to bear in mind that both rate and potential factors are involved in this order, since the oxidative power over different substances has been compared to be oxidized.

Ralph S. Lillie¹ has shown that there exists a general similarity between the distribution of the nuclear matter and the oxidative action in the cell. This would lead to the belief that those organs containing the greatest amount of nucleins probably possess greater oxidative powers than those poorer in nucleins. Spitzer² has shown that the nucleo-proteids of the liver, kidney, thymus, and blood corpuscles possess as great an oxidative energy as the original tissue. Iron is generally found in nucleo-proteids, but whether it has anything to do with their oxidative action is very doubtful.

PART III. THE EASE OF OXIDATION OF THE SUGARS IN THE BODY.

Having determined the order in which the sugars are oxidized *in vitro*, the next question to decide is, whether there is any evidence that the same order holds for the oxidation within the animal body. Different individuals show wide variations in the amount of sugars which they can oxidize, so that, if the order as given *in vitro* cannot be obtained from a few experiments, it would not be a surprise. However, we ought to find approximately the same order as that found *in vitro* if a series of animals were taken, although in all these experiments we are dealing with a rate or catalytic factor as well as the potential factor.

Pavy³ in 1899 made an extensive and careful study of the intravenous injection of carbohydrates, and in his article gives the extensive literature of the subject. He determined the amount of sugar remaining in the blood, and the amount in the urine after the intravenous injection of a known amount. In this way he arrived at the amount utilized or oxidized by the body. He employed the rabbit throughout, and considered it especially suited for the investigation, both from the extent to which carbohydrate naturally enters into the food and also from the facility with which intravenous injection can be made. There are, however, some objections to the use of the rabbit. It is known that sugar will appear in the urine at times after the most

¹ LILLIE: American journal of physiology, 1902, vii, p. 412.

² SPITZER: Archiv für die gesammte Physiologie, 1897, lxxvii, p. 615.

³ PAVY: Journal of physiology, 1899, xxiv, p. 479.

trivial operation, but no method and no animal can be used which will be entirely free from objection.

Pavy found that the disaccharides, saccharose, and lactose were cast out with the urine and comported themselves within the general system as so much unserviceable foreign matter. His results for maltose are ambiguous. After intravenous injection it was eliminated to an equal or greater extent than lactose, while, when it was introduced subcutaneously, it coincided instead with the monosaccharides. He found that the monosaccharides were utilized to a great extent, and dextrose was utilized to a greater extent than levulose, although there was very little difference between them, while galactose was utilized to a less extent than either of them. There is a very wide variation in his individual results with each of these sugars. In twelve trials for dextrose where he injected intravenously 1.0 gm. of sugar per kilo of body weight, the amount of sugar eliminated in the urine at the end of an hour varied from 2.8 per cent to 31.5 per cent of the amount injected. The amount obtained from the blood in the same length of time varied from 0.70 to 1.55 parts per 1000 for the same sugar (dextrose).

Obviously, from his results, it would be very difficult to state definitely just what sugar is utilized to the greatest extent or with the greatest ease. The variation of the animals renders it difficult, if not impossible, to draw conclusions that can be considered absolutely accurate. From both intravenous and subcutaneous injection Pavy places the sugars in the following order:

1, dextrose; 2, levulose; 3, galactose; 4, maltose; 5, lactose, the last named being the least utilized by the body.

The order of oxidation which I find *in vitro* is: 1, levulose; 2, galactose; 3, dextrose; 4, maltose; 5, lactose.

The results obtained for the disaccharides agree with the work of Pavy and others. The order of the monosaccharides is slightly different.

To test the matter by a little different method than that used by Pavy, Dr. S. A. Mathews and the writer injected the various sugars intravenously, and determined the amount of sugar necessary to inject before the appearance of sugar in the urine. It is well known that when the sugar content of the blood is increased, or, rather, when free sugar is found to any extent in the blood, the tendency of the organism is to excrete that part which cannot be utilized by the body. That sugar which is hardest to oxidize or to fix in the tissues would be the first thrown out, and can be demonstrated in the urine.

METHOD.

Our method was to inject sugar solutions sufficiently dilute that small variations in the amount of the sugar could be easily read off in cubic centimetres. One-half per cent solution in water of the pure sugar was used in each case and injected at body temperature (approximately 40° C.) into the jugular vein of rabbits. We did not dissolve the solution in normal salt, because of the great tendency of salt to cause glycosuria in these animals. The solution was run in at the rate of 5 c.c. every five minutes, and the urine tested frequently for the first appearance of the sugar. When the sugar appeared — as proven by the usual test, reduction of Fehling's and fermentation with yeast (in the case of the disaccharides, the sugars were inverted before testing) — the amount of the sugar solution run in was observed. The urine was taken from a bladder cannula so fixed that only a very few drops could be retained in the bladder, so that the result was much the same as if the cannulas had been inserted directly into the ureters. The amount of urine secreted was small.

Results: Rabbit, 2530 gm. Urethane anæsthetic.

I. **Levulose.** — 80 c.c. had run in before the first trace of sugar appeared.

Time.	Sol. run in.	Urine.	Remarks.
1.45	5.0 c.c.
1.50	5.0 "	Commencing to run.	No sugar.
2.45	60.0 "	9.0 c.c.	" "
2.55	10.0 "	1.0 "	Sugar.

II. **Dextrose.** — Rabbit, 1800 gm. — anæsthetic, urethane. 40 c.c. run in before sugar appeared.

Time.	Sol. run in.	Urine.	Remarks.
1.20	5.0 c.c.
1.25	5.0 "	Commencing to run.	No sugar.
2.00	30.0 "	5.0 c.c.	" "
2.05	5.0 "	2.0 "	Sugar?
2.10	5.0 "	1.0 "	Sugar!

III. **Galactose.** — Rabbit, 1550 gm. — methane. 60 c.c. run in before sugar appeared.

Time.	Sol. run in.	Urine.	Remarks.
3.05	5.0 c.c.
3.50	45.0 "	5.0 c.c.	No Sugar.
4.00	10.0 "	1.5 "	Trace "
4.05	5.0 "	1.0 "	Sugar.

IV. **Maltose.** — Rabbit, 2800 gm. — 45 c.c. run in before sugar appeared.

Time.	Sol. run in.	Urine.	Remarks.
7.50	5.0 c.c.
8.15	25.0 "	0.5 c.c.	No sugar.
8.30	15.0 "	2.0 "	Trace.
8.55	25.0 "	2.0 " (?)	Large reduction. Urine very thick.

V. **Lactose.** — Rabbit, 2400 gm. — methane. 25 c.c. run in before sugar appeared.

Time.	Sol. run in.	Urine.	Remarks.
9.40	5.0 c.c.
10.00	20.0 "	3.0 c.c. (?)	Sugar in first urine excreted.

From these results, taken without consideration of body weight, maltose seems easier oxidized in the body than dextrose; otherwise the order is the same as I found *in vitro*. However, when we correct for body weight this order is slightly changed. Maltose occupies the position assigned to it by the determination *in vitro*, and the only change from that order is that galactose appears more easily oxidized than levulose. Arranged in the order in which they appeared in the urine, calculated to a body weight of 2000 gm., they appear as follows:

1. Lactose	20 c.c.	4. Levulose	63 c.c.
2. Maltose	32 "	5. Galactose	77 "
3. Dextrose	44 "		

The order in which they are placed by the determination *in vitro* is:

1. Lactose.	4. Galactose.
2. Maltose.	5. Levulose.
3. Dextrose.	

While these few results by themselves are of little value, yet taken in connection with the work of Pavy, Külz, Voit, and others and the results obtained by me *in vitro*, they show that lactose and maltose occupy the position assigned in these tables, and that dextrose is slightly more difficult to oxidize than levulose. Whether galactose is easier to oxidize than levulose is not shown definitely by animal experiments. The work of Minkowski¹ and Voit² shows that galac-

¹ MINKOWSKI: Archiv für experimentelle Pathologie und Pharmacologie, xxxi, p. 85.

² VOIT: Zeitschrift für Biologie, 1892, xxix, p. 147.

tose, like levulose, can sometimes be used when the organism cannot utilize glucose. The order given by experiments *in vitro* is, on the whole, more satisfactory than any of the others. Until definite proofs to the contrary are furnished by animal experimentation, we may assume that the order in the body is the same as the order *in vitro*, although the rate at which they are oxidizing may differ.

SUMMARY OF OBSERVATIONS.

1. If acetic acid is added to cupric acetate solutions, the speed of oxidation of sugars by the solution is checked.

2. If the amount of acid necessary to add to different concentrations of the acetate to check the oxidation of any sugar to any given amount is determined, it is found to be greater the more concentrated the cupric acetate solution.

3. The amount of acid necessary to check oxidation to a certain point when different concentrations of acetate are used, was determined accurately.

4. If the same concentration of different sugars is oxidized by the same concentration of cupric acetate, the amount of acid necessary to check the oxidation to a certain point varies with the different sugars, being the most for levulose; then galactose, glucose, maltose, and lactose follow in the order named.

5. The sugars are oxidized *in vitro* with varying speeds, corresponding in general with their ease of oxidation in the body.

6. Equal quantities of the various tissues of the body, dried and powdered, require different quantities of acid to slow the catalysis of hydrogen peroxide to a certain point. The kidney requires most acid, then the liver, the spleen, pancreas, and muscle, following in the order named, requiring progressively less acid.

7. In the powders of the various organs, particles with a pressure of at least 0.125 volt of positive electricity exist in greatest numbers in the kidney and in the other organs in the order named.

8. It is very probable that a body may be able to utilize levulose or galactose and yet be unable to use glucose. This change may be due either to diminished potential of oxidation or to diminished speed of oxidation, but is probably due to diminished speed of oxidation.

9. The mechanism of oxidation in the cell is in all probability the same as that of cupric acetate, protoplasm hydrate and oxide taking the place of the copper.

10. The oxidation of H_2O_2 by iron chloride is checked by the addition of acid in a manner similar to its action on cupric acetate or powdered tissue.

11. The results indicate that rate of oxidation and potential of oxidation are distinct, and not necessarily parallel factors in animal oxidations.

A STUDY OF THE OXIDIZING POWER OF CUPRIC ACETATE SOLUTIONS.

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IN the preceding paper it was shown that the addition of acetic acid to any cupric acetate solution reduced its oxidizing power,¹ and that it was possible practically to prevent the oxidation of any sugar, for a certain time at least, by adding acetic acid to the acetate-sugar mixture. The quantity of acetic acid necessary markedly to inhibit the oxidation by any acetate solution varied with the concentration of the cupric acetate, being greater where the acetate was more concentrated. By taking various concentrations of the acetate and determining the various concentrations of acetic acid just necessary to prevent the oxidation of any particular sugar, a series of solutions were prepared which had approximately equal powers of oxidation. Thus a 0.272 N solution of cupric acetate containing 0.181 N acetic acid would just fail of oxidizing glucose; and also a solution of 0.0173 N cupric acetate containing 0.00666 N acetic acid. These two solutions were of equal oxidizing power. The question we sought to answer was: How do these solutions oxidize? We began by an attempt to determine what these solutions have in common which give them equal oxidizing powers.

From the electrical theory of oxidation developed by Ostwald it was inferred that the oxidation must be due to the transfer of a positive charge of electricity from the oxidizing to the oxidized body; in other words, from some constituent of the cupric acetate solution to the sugar molecule. The positive charge was accompanied by an oxygen atom, since this was added to the sugar molecule. It is clear at the outset that any such transfer of electrical charges must involve two factors, namely, the oxidizing power or potential of the cupric

¹ The words "oxidizing power" are used in this paper to signify the product of the speed of oxidation into the oxidizing potential.

salt, or the oxidizing agent, and the oxidizing power or potential of the sugar molecule, or the oxidized substance. The actual transfer of energy from the acetate to the sugar must be dependent upon the difference of potential of the cupric acetate solution and the sugar molecule respectively. This being the case, it is clear that, by the addition of acetic acid to the cupric acetate so that no interchange of energy between the acetate and sugar takes place within the time of the experiment, the acid must either in some way have equalized the potential between these two bodies or enormously reduced the rate of reaction. It might equalize the potential difference by acting on the sugar and thus raise its oxidizing power, or by acting on the cupric acetate and thus reduce its oxidizing potential to the level of the sugar; or it might act both on the sugar and the acetate, raising the oxidizing potential of one and lowering that of the other until they are finally equal; or it might in some other way reduce the rate of the reaction, leaving the potential unaltered.

As it was found by experiment that variations of the concentration of the sugar within rather wide limits made no very great difference in the result, whereas every variation in concentration of the cupric acetate required a corresponding change in the concentration of the acetic acid needed, it was at first concluded that the main action of the acid consisted in lowering the oxidizing potential of the cupric acetate rather than in raising that of the sugar or altering the speed of the reaction primarily. Subsequent observation, however, showed this conclusion was wrong.

As we were dealing with a transfer of positive electricity, our attention was naturally directed in the first instance to the cupric ion as the essential oxidizing agent, since it carries positive electricity at a high potential¹ (0.668 volt) and loses one of its charges during the oxidation. It was clear, however, that it could not be the cupric ion alone which determines the oxidizing power (speed \times potential) of the solution, since a cupric sulphate solution containing the same number of cupric ions as the acetate had a much lower oxidizing power, and since the addition of the acetic acid to the acetate, which so remarkably changes its oxidizing power, does not materially alter the concentration of the cupric ions present.

It seemed probable that the other element involved must be the oxygen ion, for there is removed from the solution with every mole-

¹ MATHEWS: Biological studies by the pupils of Wm. Thompson Sedgwick, Boston, 1906, p. 92.

cule of sugar oxidized an atom of oxygen united with the cuprous ion and an atom of oxygen united with the sugar. As a working hypothesis, it seemed likely that the oxidizing power of the solution must involve both the cupric and the oxygen ions in the solution.

As we had no good hypothesis at the outset, the matter clearing up only after we had got some way into it, our actual procedure was to measure as well as we could the concentration of the various ions in the solution, to find out what solutions of equal oxidizing powers had in common.

The first question which arose was whether we were dealing with a potential or a rate factor in these oxidations. When a given mixture of acetate and acetic acid fails to oxidize under the conditions of the experiment, that is, after boiling one minute, may we conclude from this that the oxidizing potential of the solution has been so reduced that it can no longer oxidize, however long the sugar molecule and the cupric acetate solution be in contact; or are we dealing only with a *rate* phenomenon, in that we have by the acid so reduced the number of active particles that the rate is so reduced that oxidation does not occur to an appreciable amount within the limits of the time of the experiment, the actual oxidizing potential remaining unaltered?

This question was settled by direct experiment. Cupric sulphate solutions are quite acid owing to the hydrolytic decomposition in the solutions. As is well known, no visible reduction occurs in such solutions if levulose or glucose is added and the solution boiled for a minute or so. By boiling, however, for one hour or longer a considerable reduction to metallic copper is produced. Similarly it was found impossible to prevent for twenty minutes or more the oxidation of levulose by cupric acetate-acetic mixtures by the addition of even very much greater quantities of acetic acid than were found to check oxidation for a short time. Thus a 1 per cent solution of glucose is oxidized in a few moments at 100° by a $\frac{1}{16}$ N solution of cupric acetate; whereas, if the glucose is heated in a sealed tube to 99.5° C. with a mixture containing $\frac{1}{16}$ N cupric acetate and $\frac{1}{8}$ N acetic acid, the reduction becomes visible only after twenty minutes of heating, and thereafter proceeds at a slow rate, but ultimately the solution is completely reduced. Similar experiments with other sugars showed this same result, that we could not permanently prevent the oxidation of the sugar and the reduction of the copper in acid solutions, but could only reduce the rate at which the reaction was taking place.

Experiments to determine the effect of the addition of acid on the potential of oxidations in such solutions were no less decisive. It was found impossible to prevent the reduction of the cupric ions even by enormous dilutions, — a process which greatly raises the decomposition tension of the ion, and might accordingly be supposed to reduce its oxidizing potential. This, however, was not found to be the case. In fact, all solutions containing cupric ions appeared to have the same oxidizing potential regardless of the concentration of these ions. For example, it was found that the addition of cyanide to an alkaline cupric sulphate solution containing glycerine (Haines' solution), a procedure which reduces the number of cupric ions enormously, could not prevent the reduction by levulose and glucose. Furthermore, some experiments were tried with cupric sulphate and potassium iodide. In this case the cupric ion oxidizes the iodine ion by transferring to it a positive electric charge. The rate of this transformation, contrary to that of the sugar, appeared to be independent of the concentration of the oxygen ions, the transfer going on rapidly even in very acid solutions, and the copper oxidizing the iodine even when present in very small quantities. These facts showed clearly that the diminution in the oxidizing power of a cupric acetate solution by the addition of acid is due to the slowing of the rate of the reaction rather than to a reduction of the potential of the oxidizing substance.

The most probable explanation of the slowing of the rate of the reaction was that the acid had reduced the number of some or all of the "active" constituents taking part in the reaction. There are three such constituents: the cupric ions, the oxygen ions, and the "active"¹ sugar molecules. It is evident that the cupric ions are but slightly affected by the addition of the acid. There remain the concentration of the oxygen ions and the concentration of the "active" (dissociated?) sugar molecules. Of these latter the concentration of the oxygen ions is of course reduced with every addition of acid. We could not measure the concentration of the dissociated sugar molecules, so we tried to keep this constant by using the same concentration of sugar each time. It is, however, possible that the dissociation or activity of the sugar may be reduced by every addition of acid. We took, therefore, those various solutions which would just

¹ The term "active" is used here to designate the particles which are in such a condition that they can enter into a reaction. It is used in the sense of dissociated particles, whether ionic or non-ionic.

fail to give a visible reduction with a given quantity of levulose when boiled one-half minute, and determined the concentration of cupric and hydrogen ions in them, and from these figures computed the decomposition tension of the $\overset{++}{\text{Cu}}$ ions and $\overset{-}{\text{O}}$ ions. If the speed of the reaction is proportional to the concentration of the cupric ions and the oxygen ions, then it is proportional to the product of their concentrations, and the decomposition tension of the dissociated cupric oxide in those solutions which oxidize sugar solutions of the same concentration at the same rate should be constant. We did not come to this conclusion in the beginning, however, on the basis of this reasoning, but from an hypothesis which proved to be erroneous. The results showed, however, that the decomposition tension was constant in the circumstances cited.

We did not attempt to measure the decomposition tension of the dissociated cupric oxide directly by the polarization method, owing to the well-known difficulty of determining the exact point within a tenth of a volt, within which the deposition of copper begins. Instead, we measured the concentration of the cupric ions and of the hydrogen ions, and from the concentration calculated the decomposition tension.

A. DETERMINATION OF THE CONCENTRATION OF CUPRIC IONS.

This involved some difficulty. The conductivity method could not be relied on owing to the possibility that most of the dissociation was in the form $\overset{+}{\text{Cu}}$ acetate $\overset{-}{\text{acetic}}$. The only way we could determine the $\overset{++}{\text{Cu}}$ ions was to measure the potential of a copper plate immersed in the cupric acetate-acetic mixture. This depends, as is well known, on the concentration of the cupric ions in the solution. While this method is not very accurate, since a comparatively large change in concentration involves only a small change in potential of the plate, yet this enables us to determine whether the dissociation is chiefly into $\overset{++}{\text{Cu}}$ $\overset{-}{\text{acetic}}$ ions or not. We accordingly made up a cell as follows:

Copper — Cupric acetate + acetic acid — Cupric sulphate — Copper.

The acetate and sulphate were chosen of the same concentration; the acetic acid in the acetate-acetic mixture was always twice as concentrated (equivalent solutions) as the acetate. In such a cell the electromotive force is due chiefly to differences in concentration of

the cupric ions in the two solutions; since that developed at the point of contact of equivalent solutions of cupric sulphate and cupric acetate is certainly small and was neglected. The electromotive force, neglecting the differences in potential due to the contact of the two solutions, is given by the formula for $T = 18^{\circ} \text{C.}$:

$$E = 0.0288 \text{ volt } \log C_1/C_2.^1$$

Before proceeding to these measurements, one of us (A. P. M.) made a series of measurements of the E. M. F. of copper electrodes in different concentrations of cupric sulphate as a control. The sulphate was carefully recrystallized, the first time after the addition of sulphuric acid, then twice from water. The electrodes were prepared by heavily electroplating sheet copper in a concentrated cupric sulphate solution to which sulphuric acid had been added, using a copper anode. The cathode was then washed carefully, dried, rubbed with a clean dry linen cloth, and cut into halves, which served as the electrodes, each electrode being about 6 sq. cm. By this means electrodes may be obtained which, immersed in a cupric sulphate solution, are isoelectric within 0.0002 volt or less.

The solutions to be measured were placed in a concentration cell which contained about 80 c.c. of each solution. The electrodes were entirely immersed in the solutions, the wires (copper) being paraffined up to the electrode, to diminish the action of oxygen. The cell was placed in a thermostat (water) and the measurements made at a temperature of 18° .

It was found that a slight difference in temperature between the two electrodes produced a very much greater divergence in the E. M. F. than was anticipated from the formula. If the electrode in the weaker solution was warmed, the electromotive force was greatly reduced; if the strong solution was warmed, the E. M. F. was greatly increased. For example, in measuring the E. M. F. between a molecular and an $\frac{N}{100}$ solution of CuSO_4 , it was found that warming the weak solution from 17° to 34°C. , the strong solution being at 17°C. , caused a fall of the E. M. F. from 0.041 volt to 0.027 volt; when the weak solution was at 25° , the strong was warmed to 34°C. , the E. M. F. rose to 0.053 volt.

The cause of this great variation is not clear, but we avoided the error by keeping both solutions at the same temperature of 18° . The E. M. F. was measured in the usual way by balancing it against a

¹ See NERNST: *Theoretische Chemie*, 1904, iv.

Weston standard cell of 1.0186 volts. In some experiments a capillary electrometer, in others a dead-beat galvanometer of the D'Arsonval type, of the convenient form made by Gärtner, was employed.

The results obtained are given in Table I.

TABLE I.

C ₁ Molecular concentration.	C ₂ Molecular concentration.	E ₁ Calculated from disso- ciation of CuSO ₄ .	E ₂ Observed.
1.	0.1	0.0238	0.0187
0.1	0.01	0.0266	0.0215
0.01	0.001	0.0278	0.0258
0.001	0.0001	0.0307	0.0325
1.	0.01	0.0504	0.0386
1.	0.001	0.0782	0.0642
1.	0.0001	0.1089	0.0939
0.1	0.001	0.0544	0.0479
0.1	0.0001	0.0851	0.0711
0.01	0.0001	0.0585	0.0611
1.	0.5	0.0064	0.0048
0.5	0.01	0.0440	0.0366
0.5	0.1	0.0174	0.0145
0.5	0.001	...	0.0598
0.1	0.002	0.0456	0.0359

Cell: Cu. CuSO₄(C₁) CuSO₄(C₂) Cu.

$$E = \frac{2V}{U+V} 0.0288 \log C_1/C_2 \text{ volts.}$$

The figures in the last column are the means of several determinations. The deviations from the mean are indicated in the following examples of the measurements from *M* to 0.1 *M*, and from 0.1 to 0.01 *M* solutions.

M to 0.1 M.	0.1 to 0.01 M.
0.0182	0.0214
0.0192	0.0203
0.0172	0.0216
0.0189	0.0208
0.0200	0.0214
0.0185	0.0227
0.0178	<u>0.0226</u>
0.0194	Mean = 0.0215
<u>0.0188</u>	
Mean = 0.0187	

Considerable difficulty was experienced in avoiding polarization, particularly in the weak solutions. Also a deposit of an oxide occurs on the positive electrode if the difference in concentration of the two solutions is great. Only those measurements were used which were obtained with newly electroplated and bright electrodes. These give the most constant and highest figures; the E. M. F. diminishing if the electrodes are left in the solution.

From Table I it may be seen that while a general agreement exists between the observed E. M. F. and that calculated from the formula, using the figures computed for the dissociation from the conductivity of the sulphate, yet in practically all cases the observed value was less than the calculated and in some cases very much less. The causes of this discrepancy are not clear to the writers. It may be stated, however, that if the dissociation of the stronger solutions of the sulphate is less than that calculated from the conductivity a much better agreement with the observed results is obtained. It was assumed, however, that the conductivity figures are correct, and the measurement of the E. M. F. between the acetate and the sulphate was proceeded with.

For this purpose acetic acid was added to the acetate mixtures until the concentration of the acetic was double that of the acetate. The acid was added for several reasons. In the first place, it reduces hydrolytic dissociation, and thus reduces or prevents the deposition of a coating of oxide on the acetate electrode; in the second place, it equalizes to some extent the number of hydrogen ions in the two solutions, the sulphate being more acid normally than the acetate; and in the third place, our other experiments were tried with acetate-acetic mixtures. A certain source of error exists in the different concentration of the acid in the two solutions, since experiment showed us that the addi-

tion of acid to an electrode in sulphate solution altered its potential somewhat. The amount of acid in the solution is certainly small, and we do not believe that this error is of very great importance in view of the close agreement between the results obtained in this way in the dilute solutions and that obtained by measuring the hydrogen ions.

The actual measurements are given in Table II.

TABLE II.

V CuSO ₄ (Molecular).	V' Cu Acetate (Molecular).	V'' Acetic Acid.	E Volts.	C Cu Ions CuSO ₄ .	C' Cu Ions Cu Acetate.	$\alpha =$ % dissociation $\frac{1}{2}$ Cu Acetate
8	8	2	0.0068	0.03724	0.02150	18.
16	16	4	0.0055	0.02311	0.01482	23.7
32	32	8	0.0055	0.01271	0.00815	26.08
64	64	16	0.0052	0.00755	0.00495	31.69
128	128	32	0.0050	0.00426	0.00284	36.42
256	256	64	0.0044
512	512	128	0.0044
1024	1024	256	0.0042
2048	2048	512	0.0022

In Table II it may be seen that the E. M. F. between copper plates immersed respectively in equivalent solutions of cupric acetate and cupric sulphate is very small, about 0.005 of a volt for those concentrations which we are measuring. The E. M. F. is somewhat greater in concentrated than in the more dilute solutions. In Column 5 are given the concentrations of cupric ions in cupric sulphate as computed from the conductivity at 18° C.; and in Column 6 the concentration of cupric ions in the corresponding cupric acetate-acetic solutions. We have not computed the numbers in concentrations less than $\frac{M}{128}$. The change in the E. M. F. with the dilution indicates that in the stronger solutions the cupric acetate dissociates in part as Cu⁺ acetate, as well as into Cu⁺ ions.

These values correspond in the more dilute solutions with the values deduced from the determination of H ions, but in the stronger

solutions the values are different from those obtained by that method. As is pointed out on page 209, the determination of the hydrogen ions in the strong solutions was unreliable.

B. DETERMINATION OF THE CONCENTRATION OF THE HYDROGEN IONS.

The concentration of the hydrogen ions may be calculated from the figures derived from the study of the conductivity, or from those obtained in the method just described, by applying the law of mass action and determining the extent to which the ionization of the acetic acid will be repressed by the acetions present. It was, however, desirable to determine the hydrogen ions directly. For this purpose the inversion method was used. It is unfortunately impossible entirely to suppress the oxidation of the levulose by the addition of acetic acid, in the case of strong solutions, although by the addition to the acetate of sufficient acetic the oxidation was much retarded and that of the glucose formed almost altogether prevented. In the weaker solutions the levulose was not noticeably oxidized.

We proceeded as follows: Equal volumes of the cupric acetate-acetic acid solution and a 10 per cent cane sugar solution were mixed, and a portion was polarized at once. Owing to the fact that the ionization of the acid is greatly reduced in the presence of the acetate, the inversion goes on very slowly at room temperature. In strong solutions, *i. e.*, $\frac{x}{8}$ or over, it was necessary to precipitate the copper from the solution before polarization, since the blue solution absorbs too much of the sodium light used. For this purpose a strong solution of Na_3PO_4 was run from a burette into a known quantity of the sugar-copper acetate mixture until precipitation was complete, but the solution still acid. The precipitate of cupric phosphate was filtered off and the clear solution then polarized, a correction being made for the dilution by the phosphate. A three-shadow Schmidt and Haensch Landolt-Lippich polariscope reading to 0.01 of a degree was used.

The remainder of the cupric acetate-acetic mixture was placed at once, after mixing, into ignition tubes holding about 30 c.c. each. The solution almost filled the tubes, leaving but a small air space at one end of each. The tubes were sealed and placed in a steam bath at a temperature of 99.2° – 99.5° , where they remained for a varying time, twenty minutes to two hours, but generally for sixty minutes.

They were then removed, cooled under the tap, and their contents polarized at 22°–23° C., either directly or after precipitation of the copper. Several determinations were made for each concentration of acetate and acetic. From the readings K , the inversion constant was determined from the formula, $\frac{1}{t} \log \frac{A}{A-a} = K$. The inversion constant for acetic acid of known strength was then determined for the same conditions, and the hydrogen ions calculated by the proportion $K_1 : K_2 :: C_1 : C_2$. In this C_2 and C_1 are the concentrations of hydrogen ions in the acetic acid and the acetate-acetic mixture respectively, K_2 being the inversion constant of acetic acid. A few experiments were tried with the solutions in flasks with paraffine over the solution.

In all the concentrations of the acetate up to $\frac{N}{16}$ considerable reduction occurred if the tubes were left in the water bath for over twenty minutes. This reduction appeared to take place first on the walls of the tubes, and may perhaps appear here first, owing to the partial solution of the glass and an increase in the concentration of hydroxyl ions in this region. A noticeable precipitate of cuprous oxide occurred in many tubes, particularly $\frac{1}{4}$ and $\frac{1}{8}$ N Cu acetate. We did not find it possible to prevent the oxidation of the levulose during long heating, even by the addition of a large amount of acid. This oxidation of the levulose introduces two errors into the results. By it an acid is produced, and the inversion proceeds at a constantly accelerating pace in consequence. This may easily be seen by contrasting the inversion constants at twenty, forty, sixty, and eighty minutes in Table IV. In such cases we have taken the mean constant, although this is too high and the number of hydrogen ions is greater in consequence than was originally present in the solution. The error from this source is, however, lessened somewhat by the second error. By the oxidation of the levulose its levulo-rotary power is diminished. Consequently less sugar will appear to be inverted than is actually inverted, provided of course that the glucose is not oxidized at the same rate.

We tried the following experiments to show the influence of oxidation on the rotation of levulose: 20 c.c. of levulose of approximately 5.5 per cent and 20 c.c. of a mixture containing $\frac{1}{2}$ N cupric acetate and N acetic acid were sealed in tubes and placed in boiling water for twenty, forty, and sixty minutes respectively, with the following result:

TABLE III.

Time.	Angle of rotation before.	Angle of rotation after.	Degrees lost by oxidation.
20 minutes	- 5.57°	- 4.44°	1.14°
40 "	"	- 4.37	1.20
60 "	"	- 4.32	1.25

That the glucose is oxidized in such mixtures at a much slower rate than the levulose is indicated by the following experiments. Two tubes of cupric acetate $\frac{N}{16}$ and acetic acid $\frac{N}{8}$; one containing levulose 0.5 per cent and the other 0.5 per cent glucose were sealed and left one hour at 99°. In the levulose tube there was a strong reduction of the cupric salt; the glucose tube showed but a slight appearance of reduction.

The two errors just cited are in opposite directions and partially neutralize each other, but that due to the production of acid from the levulose by oxidation undoubtedly overbalances the other, as is shown by the great increase in the inversion constant with the time. In all the concentrations greater than $\frac{N}{32}$ the method undoubtedly, therefore, yields too high a result for the hydrogen ions. Table IV gives the results obtained.

A solution of cane sugar of approximately 5 per cent containing 0.00943 N acetic in sealed tubes and under the same conditions as the preceding experiments, gave a value of 0.004132 as the inversion constant. Such a solution contains hydrogen ions of a concentration 3.836×10^{-4} N.

The concentrations of hydrogen ions in the dilution of acetate $V = 4$ to $V = 32$, given in Table V, are undoubtedly too large owing to the partial oxidation of the levulose and the formation of acid from it. In the concentrations $V = 512$ and $V = 1024$ hydrolytic dissociation probably takes place also to some extent, so that the constant is probably too high here also. The dilutions 16 to 128 inclusive are the most nearly correct.

From the concentrations of hydrogen ions given in Table V the number of acetions in the cupric acetate solution may be calculated. This is done by the formula: $KC_{\text{acetic}} = \overset{+}{C}_{\text{H}} + \bar{C}_{\text{acetions}}$.

In this formula K is 0.00018 the dissociation constant of the acetic acid, C_H is the concentration of the hydrogen ions, and the C_{acetic} is the concentration of the undissociated acetic acid, that is, of the added acetic acid minus the concentration of the hydrogen ions. Since the latter factor is very small compared to the former, it may be neglected and for C_{acetic} the concentration of the acetic acid may be used. By this formula the results were obtained given in Table VI. Column 4 of this table has the values of the per cent of dissociation of $\frac{1}{2}$ Cu acetate calculated from the value of the acetions found.

From the values of α given in Table VI, a curve was plotted and the value of α for any required dilution between 4 and 72 was obtained from it. From α so obtained the number of acetions in any mixture of cupric acetate and acetic acid could be computed, and by substituting the values from this table in the formula on page 210 the number of hydrogen ions in any mixture of cupric acetate and acetic acid within the dilutions measured could be calculated. The figures given in Table VII, Column 5, on page 215, were computed in this way. As these figures, for the reasons already stated, are too high in concentrations of the acetate above $\frac{1}{16}$ normal, they were not used in subsequent computations, but instead of them the values given in Column 4, which were obtained by assuming the concentration of acetions to be double that of the cupric ions found by measurement to be present. This value is a minimum value, the actual number of acetions present being somewhat higher than this. It will be seen that there is a fair agreement between the number of hydrogen ions computed to be present in this way and the number found by the inversion method for all dilutions greater than 15; but it was not good for the more concentrated solutions owing to the oxidation of the levulose.¹

C. THE CALCULATION OF THE DECOMPOSITION TENSION OF THE CuO^{++-} .

For the sake of convenience in computation we have computed the decomposition tension as it is at 18° C. The value of the decomposition tension of the cupric ions is obtained from the formula :

¹ I tried to determine the rate of inversion in the strong solutions by using very concentrated acetic acid and reading the polariscope in the blue light of the blue line of mercury vapor, but in all cases for some reason the cupric acetate retarded the inversion far more than could be accounted for by a diminution of ionization of the acetic acid, even had the cupric acetate been entirely dissociated. After a few trials the method was given up. (A. P. M.)

TABLE IV.

1 Time.	2 V $\frac{1}{2}$ Cu Acetate.	3 V/ Acetic Acid.	4 Polariscope.		6 a Degrees lost.	7 A Total rota- tion degrees.	8 K $\frac{1}{t} \log \frac{A}{A-a}$.	9 Remarks.
			Before.	After.				
min.	4	2	6.62	5.55	1.07	8.80	0.00282	Reduction.
20	"	"	"	4.48	2.14	"	0.00303	Sealed tube.
40	"	"	"	5.62	1.00	"	0.00262	Flasks. Paraffine.
20	"	"	"	4.02	2.60	"	0.00380	"
40	"	"	6.66	5.56	1.10	8.86	0.00288	Sealed tube.
20	"	"	"	4.15	2.50	"	0.00361	"
40	"	"	"	2.81	3.85	"	0.00414	"
60	"	"	6.32	4.84	1.48	8.40	0.00280	"
30	8	4	"	3.87	2.45	"	0.00249	"
60	"	"	"	3.53	2.78	"	0.00291	"
60	"	"	6.72	3.67	3.05	8.94	0.00300	"
20	16	8	6.73	6.11	0.62	"	0.00157	Flasks.
40	"	"	"	5.49	1.24	"	0.00162	Flasks. Reduction.
60	"	"	"	4.76	1.97	"	0.00181	"
80	"	"	"	4.09	2.64	"	0.00190	"
20	"	"	"	6.10	0.63	"	0.00160	"
40	"	"	"	5.57	1.16	"	0.00151	"
60	"	"	"	4.71	2.02	"	0.00186	"

32	32	6.68	5.50	1.18	S.88	0.00183	Scaled tube.	Reduction.
62	"	"	4.52	2.16	"	0.00189	"	"
82	"	"	3.48	3.20	"	0.00232	"	"
60	"	6.32	4.41	1.91	S.40	0.00186	"	"
60	"	"	4.47	1.85	"	0.00180	"	"
40	16	6.73	5.80	0.93	S.94	0.00120	Flask. No reduction.	
60	"	"	5.31	1.43	"	0.00126	"	Slight "
80	"	"	4.61	2.12	"	0.00147	Scaled tube.	"
105	"	6.68	3.96	2.72	S.88	0.00148	"	"
60	"	"	5.23	1.45	"	0.00123	"	"
60	"	6.32	4.85	1.46	S.40	0.00138	"	"
60	"	"	4.88	1.44	"	0.00136	"	"
105	32	6.68	4.66	2.02	S.88	0.00103	"	"
63	"	6.32	5.21	1.11	S.40	0.00097	"	"
61	64	"	5.36	0.96	"	0.00086	"	"
50	"	"	5.65	0.67	"	0.00072	"	"
62	"	"	5.37	0.95	"	0.00084	"	"
61	128	"	5.54	0.78	"	0.00068	"	"
35	"	6.59	6.13	0.46	S.76	0.00067	"	"
60	256	6.32	5.71	0.61	S.40	0.00054	"	"
120	512	6.75	5.57	1.18	S.98	0.00051	"	"
122	"	"	5.33	1.42	"	0.00061 (?)	One determination.	
122	"	"	5.42	1.33	"	0.00057	"	"
60	"	6.65	6.04	0.61	S.85	0.00052	"	"

TABLE V.

V $\frac{1}{2}$ Cu Acetate.	V' Acetic.	Mean values of K.	C Hydrogen ions.
4	2	0.00327	3.036×10^{-4}
8	4	0.00280	2.600×10^{-4}
16	8	0.00169	1.569×10^{-4}
32	16	0.00134	1.244×10^{-4}
64	32	0.00100	9.284×10^{-5}
128	64	0.000806	7.47×10^{-5}
256	128	0.000677	6.28×10^{-5}
512	256	0.000539(?)	5.00×10^{-5}
1024	512	0.000553(?)	4.91×10^{-5}

TABLE VI.

V $\frac{1}{2}$ Cu acetate.	V ₁ Acetic acid.	C Acetions.	$\alpha =$ per cent dissociation $\frac{1}{2}$ Cu acetate.
4	2.0	0.02965	11.86
8	4.0	0.01730	13.85
16	8.0	0.01433	22.94
32	16.0	0.009043	28.94
64	32.0	0.006058	38.77
128	64.0	0.003765	48.20
8	1.8	0.02429	19.42*
16	1.67	0.0162	26.00*

* These results obtained with stronger acetic acid are the more correct.

$$E_{Cu}^{\dagger\dagger} = -0.606 + 0.0288 \log \frac{C_1}{C_2} \text{ volts.} \quad (2)$$

In this formula — 0.606 is the decomposition tension of the cupric ions in a normal ionic solution; C_1 is 1; and C_2 the concentration

TABLE VII.

	1	2	3	4	5	6	7	8.
	C One-half Cu ace- tate.	C Acetic acid.	C Cu ions 18°.	C × 10 ⁴ H ions by com- puta- tion 18°.	C × 10 ⁴ H ions by in- version 99.5°.	Decom- position tension Cu ions in volts.	Decom- position tension O ions (from Col. 4).	Decom- position tension of CuO in volts (6 + 7).
Levulose	0.2	0.4166	0.01993	1.882	2.917	-0.557	1.264	0.707
"	0.1667	0.3333	0.01667	1.807	2.653	-0.555	1.264	0.709
"	0.125	0.2489	0.01482	1.512	2.325	-0.553	1.259	0.706
"	0.0641	0.1176	0.00836	1.267	1.578	-0.546	1.254	0.708
"	0.0523	0.0909	0.00707	1.158	1.367	-0.544	1.252	0.708
"	0.0400	0.04988	0.00600	0.748	0.844	-0.542	1.242	0.700
"	0.02439	0.02778	0.00415	0.603	0.650	-0.537	1.236	0.699
"	0.01667	0.02000	0.00300	0.600	0.588	-0.533	1.236	0.703
"	0.01389	0.01667	0.00264	0.568	0.549	-0.532	1.235	0.703
							Mean =	0.705
Galactose	0.1984	0.2041	0.01975	0.930	1.485	-0.557	1.247	0.690
"	0.1666	0.1666	0.01750	0.859	1.327	-0.555	1.245	0.690
"	0.1	0.1	0.01200	0.750	1.050	-0.551	1.241	0.690
"	0.05235	0.04167	0.00707	0.530	0.630	-0.544	1.233	0.689
"	0.04630	0.03268	0.00672	0.438	0.527	-0.543	1.228	0.685
"	0.02778	0.01389	0.00458	0.273	0.301	-0.539	1.216	0.677
"	0.02500	0.01250	0.00425	0.265	0.290	-0.538	1.215	0.677
"	0.01333	0.00500	0.00260	0.173	0.169	-0.532	1.205	0.673
							Mean =	0.684
Glucose	0.2721	0.1812	0.02449	0.666	1.108	-0.560	1.238	0.678
"	0.1282	0.1163	0.01520	0.689	1.066	-0.554	1.239	0.685
"	0.06944	0.04167	0.00868	0.432	0.535	-0.547	1.228	0.681
"	0.03472	0.01852	0.00504	0.331	0.353	-0.540	1.211	0.681
"	0.01730	0.00666	0.00294	0.204	0.194	-0.533	1.209	0.676
							Mean =	0.680
Maltose	0.2857	0.06667	0.02574	0.233	0.389	-0.560	1.212	0.652
"	0.09091	0.02325	0.01093	0.192	0.257	-0.530	1.207	0.657
"	0.11906	0.02631	0.013690	0.173	0.251	-0.552	1.205	0.653
							Mean =	0.654
Lactose	0.25	0.02631	0.02250	0.105	0.169	-0.539	1.192	0.633
"	0.1332	0.02000	0.015805	0.114	0.179	-0.534	1.194	0.640
"	0.0666	0.01000	0.008665	0.104	0.131	-0.547	1.192	0.645
"	0.0333	0.00500	0.005335	0.084	0.097	-0.541	1.187	0.646
							Mean =	0.641

of the cupric ions in the solution as given in Table VII, Column 3. The values obtained by this computation are given in Column 6, Table VII.

The computation of the decomposition tension of the oxygen ions is made in the same manner. The decomposition of the oxygen in a solution normal as regards the hydrogen ions is 1.479 volts.¹ The concentration of the oxygen ions varies inversely as the square of the concentration of the hydrogen ions, as is shown by the equation $K_{C_{H_2O}} = C_O \times C_H^2$. The left-hand member of the equation is a constant.

We have, therefore, for the decomposition tension of oxygen in any solution of which we know the concentration of hydrogen ions,

$$E_{\bar{O}} = 1.479 - 0.0288 \log (1/C_2)^2 \text{ volts.} \quad (3)$$

In this C_2 is the concentration of the hydrogen ions in the solution. 1.479 is the decomposition tension of \bar{O} in normal \bar{H} solutions.

Using the values of C_H given in Column 4 of Table VII, the values of $E_{\bar{O}}$ of Column 7 were computed by Formula 3. The sum of the decomposition tensions of the cupric ions and the oxygen ions gives the decomposition tension of the dissociated $Cu\bar{O}^{++}$ in the solution (Column 8).

D. RESULTS OBTAINED.

An inspection of Column 8, Table VII, p. 215, shows that in solutions of cupric acetate-acetic acid of equal oxidizing power the decomposition tension of the dissociated cupric oxide in the solutions is constant. The concentration of the cupric ions may vary within wide limits, — in the case of levulose, for instance, from the concentration of 0.0124 to a concentration of 0.00273. Similarly, in these same solutions the concentration of the hydrogen ions varies from 1.882×10^{-4} to 0.568×10^{-4} . The variation, however, is of such a character that the total decomposition tension of the cupric oxide in these solutions remains unaltered. If we increase the decomposition tension of the cupric ions by reducing their numbers, we must, if we wish to keep the oxidizing power of the solution unaltered, reduce the decomposition tension of the oxygen by a corresponding amount, and this we do by increasing

¹ LEWIS: Journal of the American Chemical Society, 1906, xxviii, p. 170.

the number of these ions (\bar{O}) in the solution. It is only necessary to reduce the concentration of the hydrogen ions by an amount equal to the square root of the change in concentration of the cupric ions, since the oxygen ions increase as the square of the change in the hydrogen. Thus, if the cupric ions are reduced to one fourth of what they were, it is only necessary to reduce the hydrogen ions to one half their former concentration, since this increases the concentration of the oxygen ions four times, which will exactly counterbalance the change in the decomposition tension of the copper. This fact appears in all the tables, although it is masked somewhat by the fact that the number of hydrogen ions in the table in the concentrated solutions is certainly too high, as already explained. For example, if we compare Columns 3 and 4, Table VII, for levulose, it will be seen that between the first and last concentration of the cupric ions there is a wide difference. That is, the ratio is 0.01993: 0.002639, or 7.475. The corresponding decrease in the hydrogen ions is 1.882: 0.568, or 3.331. The real ratio between the hydrogen ions should be the square root of 7.475, or 2.735. That is, if the concentration of the hydrogen ions in the weakest solution is 0.568×10^{-4} , that in the strongest solution corresponding to it should be $0.568 \times 10^{-4} \times 2.735$, or 1.553×10^{-4} , instead of 1.882×10^{-4} , the value found. It is owing to the fact that the concentration of the oxygen ions varies inversely with the square of the concentration of the hydrogen, that a small difference in acidity produces so great a change in the oxidizing power of the solution, and it is one reason why the alkaline solutions are so much more powerful than the acid. Every time we decrease the concentration of the cupric ions to one tenth their former value, the decomposition tension of this ion increases by 0.0288 volt. But every time we increase the hydroxyl ions ten times, or diminish the hydrogen ten times, the decomposition tension of the oxygen falls 0.057 volt. In a solution 0.01 normal as regards hydroxyl ions, the decomposition tension of the oxygen would be 0.793 volt, that is, approximately, 0.459 volt less than the decomposition tension of the oxygen in the solution of cupric acetate, just incapable of oxidizing levulose. To increase the decomposition tension of the cupric ions to a corresponding amount it would be necessary to reduce their concentration roughly by 10^{15} times.

The fact that the decomposition tension of the dissociated cupric oxide in those solutions which oxidize at the same rate is a constant,

means that the product of the concentration of these two ions must be a constant. We are unfortunately not able to prove this directly because we cannot measure the concentration of the oxygen ions, but the constancy of the decomposition tension, which is a function of the concentration, shows that this product is constant. It may also be shown indirectly, if two solutions are compared, since the oxygen ions vary inversely with the square of the concentration of the hydrogen ions. If, therefore, we multiply the concentration of the cupric ions in solution one, with the square of the concentration of hydrogen ions in solution two, the product should equal the product of the square of the concentration of hydrogen ions in one into the concentration of the cupric ions in two, or,

$$C_{Cu}(C^1_H)^2 = C^1_{Cu}(C_H)^2.$$

E. THE QUESTION OF THE ABSOLUTE OXIDIZING POTENTIAL OF CUPRIC ACETATE-ACETIC MIXTURES.

If the essential act in oxidation is the transfer of a positive charge of electricity from the oxidizing substance to the oxidized, the absolute oxidizing potential of any solution must be determined by that constituent of it which gives up a positive charge with the highest voltage, *i. e.*, that constituent which has positive electricity at the highest pressure. In our copper solutions this constituent is undoubtedly the cupric ions in the solution. If, therefore, we knew with what tension a cupric ion would give up a positive charge of electricity, we could answer the question of the absolute oxidizing potential of any solution containing copper ions and no other ions of higher voltage.

This factor of ionic voltage must be independent of the concentration of the cupric ions, since it depends on the essential nature of the ion. It is the factor one of the authors has already called the potential of the ion, or "ionic potential."¹ For the cupric ion its value is approximately 0.668 volt.

That the oxidizing potential of a cupric salt solution is dependent upon the ionic potential of the cupric ions rather than upon the solution tension or decomposition tension of the ion is shown by the oxidizing powers of Fehling's or Haines' solution as contrasted with cupric sulphate.

¹ MATHEWS, A. P.: Biological studies of the pupils of W. T. Sedgwick, Boston, 1906, p. 92.

In both Haines' solution and Fehling's solution the concentration of the cupric ions is reduced to a remarkably small figure, — in Haines' solution, by combining the cupric hydrate with glycerine, in Fehling's by combining it with the tartrate. We tried to measure roughly the concentration of the cupric ions in Haines' solution by measuring the difference of potential between copper in such a solution and copper in a $\frac{M}{2}$ copper sulphate solution. The electromotive force was very large, being over 1 volt. This difference shows that the concentration of the cupric ions in Haines' solution is extremely small, being at least 10^{-30} times that of the cupric ions in $\frac{M}{2}$ cupric sulphate. If the decomposition tension of the cupric ion determined its oxidizing power, such a solution should have a much lower oxidizing potential than an acid solution, since the decomposition tension of the cupric ion is vastly greater in the more dilute solution. But notwithstanding this fact, such a solution oxidizes very rapidly and powerfully, showing no reduction of oxidizing potential. The oxidizing potential is therefore, independent of the concentration of the cupric ions, and is hence probably determined by the ionic potential. This has been determined in a provisional way for copper to be 0.668 volt. The absolute oxidizing potential of any solution which contains cupric ions and none other of higher pressure, is, therefore, approximately 0.668 volt. All the sugars can be oxidized by this pressure, but they oxidize at varying speeds.

F. THE DISSOCIATION OF THE VARIOUS SUGARS.

If we take a solution of cupric acetate containing $\frac{N}{3}$ cupric acetate and $\frac{N}{4}$ acetic acid and mix with one part of it an equal volume of levulose, 1 per cent solution, and with the other part an equal volume of a 1 per cent solution of glucose, place them both in sealed tubes, and heat uniformly, the levulose will be found to reduce the cupric solution at a rate much faster than the glucose. In fact, with such tubes after an hour the levulose tube will be found to be almost colorless and to contain a heavy precipitate of cuprous oxide; while the glucose tube contains but a faint red coating on the glass, and is hardly appreciably reduced. By making experiments of this sort, it may easily be ascertained that in solutions containing the same concentrations of cupric acetate-acetic acid, and the same concentration (molecular) of the various sugars, the oxidation proceeds at vastly different rates with the different sugars. In fact, with lactose and

maltose it may go so slowly as to escape detection unless the experiment is kept under way some time. For the latter two sugars it might seem impossible to say with certainty whether they are inverted before they are oxidized, but the fact that they oxidize much faster in alkaline than in acid solutions, although they invert much faster in the latter than in the former, shows that they are oxidized before inversion.

Why is it that in these solutions containing the same number of cupric ions, of oxygen ions, and sugar molecules of the same general nature, the speed of reaction differs so widely? The answer to this question is, we think, perfectly evident. We must be dealing with a reaction which involves not the sugar molecule as such, but probably some dissociated part of it. A glucose solution oxidizes less rapidly than a levulose solution of the same concentration, for the reason that the sugar breaks up to a smaller extent into the active particles which take part in the reaction. That this is undoubtedly the explanation is shown in part by the evidence Nef¹ has presented for similar phenomena in the glycerine series; and also by the recent publication by Schade² of the spontaneous fermentation of sugars. The latter author has shown for the sugars, as Nef has shown for the alcohols, that they decompose spontaneously into highly reactive groups (acetaldehyde and formic acid), and that this decomposition is enormously greater in alkaline than in acid solutions. This explains at once why it is that levulose and glucose and the other sugars oxidize with so much greater speed in the alkaline than in acid solutions, in spite of the fact that the number of cupric ions is so greatly reduced in these alkaline solutions.

To get some idea of the relative dissociation, expressed numerically, of the different sugars in slightly acid solutions, we made the not improbable assumption that in our solutions the speed of reaction was proportional to the product of the cupric and oxygen ions and the dissociated sugar molecules. Solutions were taken containing 1 per cent of the various sugars, the same amount of cupric acetate but varying amounts of acetic acid, so that in each solution the reduction would just appear if a certain amount of the solution was heated to boiling and then cooled. These solutions then all had roughly equal speeds of oxidation. The product of the three variables being a constant and the cupric concentration being a constant, evidently, on

¹ NEF: LIEBIG'S Annalen, 1904, cccxxxv, p. 192.

² SCHADE: Zeitschrift für physikalische Chemie, 1906, lvii, pp. 1-46.

the assumption we started with, the concentration of oxygen ions must vary inversely with the concentration of active sugar particles. Knowing the ratio of oxygen ions in two solutions, — one of levulose, the other of some other sugar, — the relative concentration of active particles of the different sugars may be easily computed.

The result was as follows, calculated from Column 4, Table VII, p. 215: Levulose contains 4.2 times as many active particles as galactose; 6.81 times as many as glucose; 122.4 times as many as maltose, and 450 times as many as lactose, when each is at molecular concentration at a temperature of 99° C. and in slightly acid solution. These figures are only rough approximations. They may be wide of the mark, but they give at least some numerical expression of the differences between these sugars. Whatever the actual numerical relationships may be, there seems no other probable explanation of the varying speeds of oxidation of such sugars as glucose, levulose, or galactose, other than a difference in their degree of dissociation.

This explanation makes it clear why levulose is oxidized so much more rapidly than the other sugars, and shows one reason why bacteria and other organisms are able to break up the sugars with such varying speeds. Evidently, if we grow a bacterium in a mixture containing levulose and glucose, it will necessarily oxidize that sugar most rapidly which presents the largest concentration of active particles. Thus it may happen that the levulose will be oxidized before the glucose is appreciably attacked. This will happen unless secondary reactions by which the glucose decomposition is accelerated come into play. Whether the decomposition of the sugars remains the same relative to each other in alkaline as in acid solution is not determined absolutely, but is rendered probable by the observations of Framm.¹ The enormous difference in the dissociation of the sugars in an alkaline as contrasted with an acid solution is a factor which cannot be neglected. Practically, all work done upon the comparative fermentative actions of yeasts and bacteria on the various sugars in which the reaction of the protoplasm and the culture medium is left out of account, is thereby made of doubtful significance.

For example, suppose a bacterial culture slightly acid is found to decompose a sugar very slowly, while in slightly alkaline or neutral solutions the decomposition goes on very fast. The inference generally drawn that the bacterium contains a ferment active in neutral or alkaline media but not in acid is entirely unjustified, since the

¹ FRAMM: *Archiv für die gesammte Physiologie*, 1896, lxiv, p. 597.

change in reaction of the medium will of itself enormously affect the rate of sugar decomposition. It is premature to ascribe the selective fermentation of any sugar to conditions in the organism until the dissociation of the sugar in various conditions shall have been worked out.

G. THE OXIDIZING POWER OF CUPRIC ACETATE-ACETIC, OF FEHLING'S, HAINES', BARFOED'S, AND OTHER REAGENTS CONTAINING CUPRIC SALTS.

From what has been said, it is clear that the differences between these various solutions is one only of the speed of oxidation, not of the potential. It has been shown that cupric acetate-acetic mixtures may be used, as Barfoed's reagent is used, for the differentiation of one sugar from another, but only within certain time limits. All such solutions will oxidize all sugars which have been tested. Even all cane sugars which we have tested have been found to reduce Fehling's solution, if given time enough and if concentrated enough; and there is no clear evidence that inversion precedes the reduction. In our opinion the ordinary statement that cane sugar will not reduce Fehling's solution will have to be modified by some clause defining the time and concentration within which visible reduction will not take place.

The addition of acid to any of these solutions reduces the speed of the reaction, first, by reducing the dissociation of the sugar molecules, and, second, by reducing the concentration of the oxygen ions. The great speed of oxidation of Fehling's solution, as compared with acid cupric solutions, depends on the great increase in dissociation of the sugar molecule in alkaline reaction, on the great increase in concentration of oxygen ions, and upon the rapidity with which cupric ions are formed from the non-ionized copper compound in the solution. It thus happens that although the cupric ions are present in such minute amounts, they are reformed so speedily that the speed of the reaction is very rapid.

ON THE ACTION OF CYANIDES ON THE HEART

By A. J. CARLSON.

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

I. THE LITERATURE.

THE view that the action of the hydrocyanic acid and the cyanide on living tissues is due to the inhibition of the oxidative processes within the tissues is commonly accepted by physiologists and pharmacologists. Paralyzing a tissue by hydrocyanic acid is considered the equivalent of paralyzing it by the deprivation of oxygen. In fact, many physiologists have made use of hydrocyanic acid and the cyanides of sodium and potassium interchangeably with deprivation of oxygen in studies designed to show the effects of lack of oxygen on various physiological processes.

In the course of my studies of the mechanisms of the action of drugs on the heart I made some observations on the action of the cyanides on the heart ganglion and the heart muscle of *Limulus* which seemed to contradict the above-stated theory of hydrocyanic acid action.¹ I found that in certain concentrations the cyanides have a primary stimulating action both on the heart ganglion and the heart muscle, instead of the depressant action of asphyxia.

On turning to the literature I find that this theory of the mechanism of the action of hydrocyanic acid on living tissues rests in the main on these three lines of evidence: (1) It was surmised by Bernard, and later apparently proved by Geppert² in an extended series of careful experiments, that the mammalian tissues under the influence of hydrocyanic acid use up less oxygen and produce less carbon dioxide than under normal condition of the tissues with the animal at rest. Geppert made direct determinations of the gases of the blood in the same animal when normal and when under the influ-

¹ CARLSON: This journal, 1906, xvii, p. 177.

² GEPPERT: *Zeitschrift für klinische Medizin*, 1889, xv, p. 307.

ence of non-lethal doses of the poison. He found that the diminished consumption of oxygen parallels the diminished output of carbon dioxide. In most of the experiments the poisoned animal produced much less carbon dioxide and used up much less oxygen than the normal animal, despite the fact that one stage in the hydrocyanic poisoning is characterized by violent convulsions. Geppert concludes, therefore, that the cyanides poison by inhibiting tissue oxidation.

It must be admitted, however, that Geppert's method is not accurate enough to detect a possible slight increase in the oxygen consumption and the carbon dioxide output if such an increase is confined to the first few minutes at the beginning of the drug action. Nevertheless, Geppert actually found a great increase in the oxygen consumption during the first stage of the poisoning in no less than five of his experiments, as shown by his Table I, which is herewith reproduced.

TABLE I.

(GEPPERT.)

Experiment.	Oxygen Consumption.		Animal.
	Normal.	After K CN.	
17	21	26.7	Rabbit
25	28.8	46.4	Cat
	33.7		
26	33.1	46.8	Cat
	31.6		
27-28	39.7	52.0	Dog
	39.3		
	35.7	65.0	
	42.1	46.0	

It is therefore evident that Geppert's observations do not show that the very beginning of the action of the cyanides on the tissues is marked by a diminution in the oxygen consumption and the carbon dioxide output. They do show such a decrease in the latter stages of the poisoning, but this is a common action of most depressant drugs, drugs that have by no means been shown to depress by direct inhibition of tissue oxidations.

On the basis of influence of hydrocyanic acid on the respiratory quotient of the isolated (and dead) kidney, Vernon¹ arrives at conclu-

¹ VERNON: Journal of physiology, 1906, xxxv, p. 70.

sions somewhat different from those of Geppert. According to Vernon, hydrocyanic acid paralyzes the mechanism of oxygen absorption in the tissues in direct proportion to the amount of the acid that combines with the tissues, while it has no direct action on the processes leading to the production of carbon dioxide. Vernon suggests that the oxygen, when absorbed by the tissues, unites with the aldehyde groupings, and it is this union that is prevented by the cyanides themselves uniting with these groupings, forming $-\text{CH}(\text{CN})\text{OH}$ groups. On this theory we would expect that anaerobic organisms would be immune to the cyanides, and also that the tissues containing the greater quantity of intra-molecular oxygen would exhibit the greater resistance to the action of the cyanides.

(2) In the second place, hydrocyanic acid has been shown to produce changes in many forms of protoplasm apparently similar to those produced by the lack of oxygen. Lack of oxygen leads to disintegration and solution of ova, embryos, and many unicellular organisms.¹ Similar changes are produced by the cyanides. But the same changes are produced by a number of other substances which have not been shown to interfere directly with the action of the tissue oxidases or with oxidative processes in vitro. Thus Budgett² found that antipyrine, veratrine, morphia, quinine, nicotine, and the hydrates of sodium and potassium produce the same changes in paramœcium and other protozoa that are effected by the cyanides. The list could undoubtedly be extended. The electrical current of a certain intensity, for example, will produce solution or disintegration of many protozoa. So does deprivation of oxygen. But I think it would be hazardous to infer from this analogy that the direct and primary action of the electrical current on protoplasm is the inhibition of the oxidative processes. Furthermore, Lyon³ found that the weakest concentration of the cyanides that produces an effect at all on the segmentation and development of the sea-urchin eggs accelerates these processes, and that it is only in the stronger solutions that retardation or depression appears. I know of no observation to the effect that deprivation or diminution of the normal oxygen supply accelerates cell division and growth.

(3) A further support of the theory is found in the fact that hydrocyanic acid inhibits many oxidative processes and catalytic actions in

¹ LOEB : Dynamics of living matter, 1906, pp. 16-28.

² BUDGETT : This journal, 1898, i, p. 200.

³ LYON : This journal, 1902, vii, p. 56.

vitro. It inhibits the oxidation of guaicum by the potato oxidase as well as by many inorganic oxides and peroxides.¹ But this is also done by many other substances that act as anæsthetics or antiseptics. Moreover, while the cyanides inhibit the oxidation of guaicum by most of the inorganic peroxides or oxides, Kastle and Loewenhardt found that they accelerate the oxidation of guaicum by silver oxide and lead peroxide, the acceleration being the greater the stronger the concentration of the cyanide.

Hydrocyanic acid inhibits the catalytic action of colloidal platinum, for example, its decomposition of hydrogen peroxide. But a similar inhibitory action is exhibited by many other substances, among others some of the electrolytes and non-electrolytes of the blood.² So far as I know, there is no evidence that urea, sodium chloride, or barium chloride has any direct inhibitory action on the absorption of oxygen or the oxidation of the cleavage products in living tissues.

The literature furnishes, therefore, no proofs that the primary action or the only primary action of the cyanides on protoplasm is of the nature of an inhibition of respiration.

II. THE ACTION OF THE CYANIDES ON THE LIMULUS HEART GANGLION.³

The primary action of the cyanides on the *Limulus* heart ganglion depends on the condition of the ganglion and the concentration of the cyanide. The weakest concentration of the cyanides of sodium or potassium that affects the heart ganglion at all augments the intensity of the nervous discharges from the ganglion without altering the rate of the discharges. In the case of heart ganglia in good condition this stimulating action is usually obtained in dilutions represented by one part isotonic KCN or NaCN to five to ten thousand parts of plasma or sea water. Weaker concentrations produce no distinct effect on the ganglion. In a solution of one part $\frac{6}{10}$ *n* KCN to five thousand parts of plasma the ganglion will continue in activity for many hours. There is a gradual diminution in the rate and intensity of the ganglionic rhythm, but this decline cannot be distinguished from that which takes place at the same time under the experimental condition,

¹ KASTLE and LOEWENHART: American chemical journal, 1901, xxvi, p. 539.

² NIELSON: This journal, 1903, x, p. 191; NIELSON and BROWN, *ibid.*, 1904, xii, p. 374; NIELSON and TERRY, *ibid.*, 1905, xiv, p. 248.

³ For the experimental method used, see this journal, 1906, xv, p. 207.

even when the ganglion is placed in plasma or sea water. I am therefore unable to demonstrate any depressor after-effects of the primary stimulating action of the cyanides in weak concentrations. A typi-

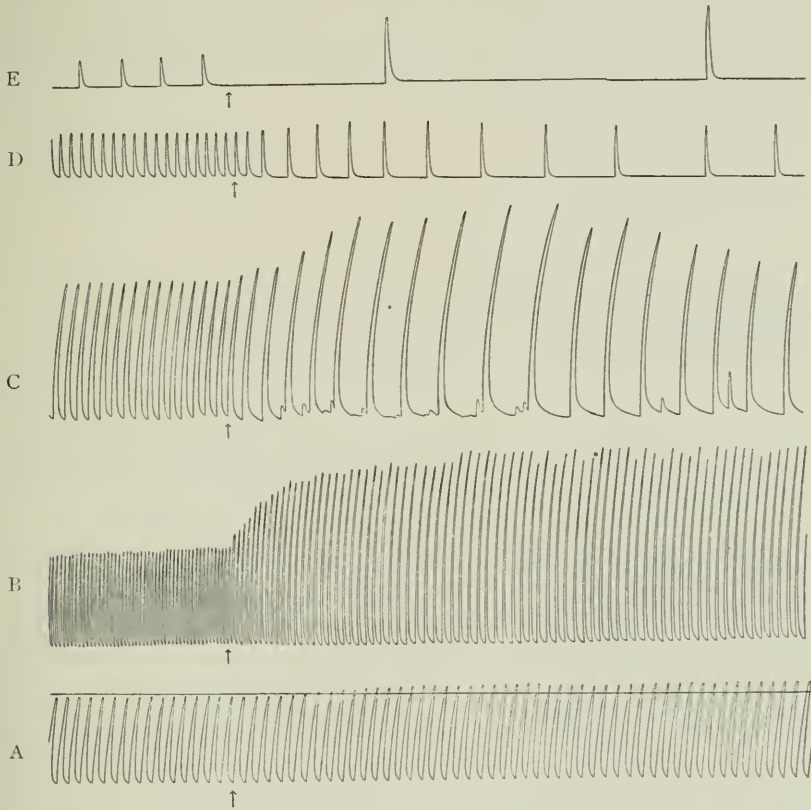


FIGURE 1.—About two thirds the original size. Tracings from the anterior end of the *Limulus* heart. The ganglion isolated posteriorly. ↑, application of KCN in plasma to the isolated ganglion. *A*, KCN(6/10 *n*) in plasma 1-5000. Showing primary augmentation of the nervous discharges without alteration in rate. *B*, KCN(6, 10 *n*) in plasma 1-1000. *C*, KCN(6 10 *n*) in plasma 1-100. Showing augmentation of the intensity of the nervous discharges accompanied by slowing of the rate and inco-ordination of the ganglion. *D* and *E*, KCN(6/10 *n*) in plasma 1-50. Showing great primary slowing of the rate accompanied by an apparent increase in the intensity of the nervous discharges.

cal tracing showing this primary stimulating action of the cyanides on the intensity of the ganglionic discharges is reproduced in Fig. 1*A*.

In stronger concentrations the primary action of the cyanides on the ganglion exhibits some variations. In the case of ganglia in good

condition one part of the isotonic cyanide in five hundred or one thousand parts of plasma produces great augmentation of the intensity of the nervous discharges coupled with a retardation of the rate, the rhythm continuing perfectly regular. These same effects may be obtained by concentrations up to one part of the cyanide to one hundred parts of plasma. But at this or greater concentrations the following irregularities appear. The primary augmentation of the

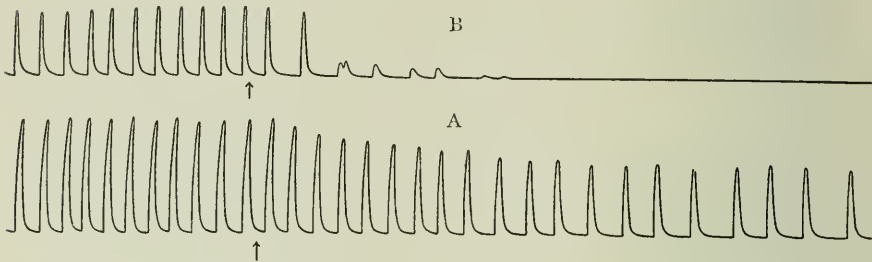


FIGURE 2.—Tracings from the anterior end of the *Limulus* heart. Ganglion isolated posteriorly. ↑, application of KCN(6/10 *n*) in plasma 1-10 to the isolated ganglion. Showing primary and permanent depression of the ganglionic rhythm (with some indication of inco-ordination in *B*) by this concentration of the cyanide.

nervous discharges appear as before, but it is of relatively brief duration and is followed by great depression of the intensity. The rate of the rhythm is greatly retarded and tends to become irregular. This irregularity appears to be due to a type of inco-ordination of the ganglionic activity rather than to an actual augmentation of the rate. The irregularities usually take the forms shown in tracing *C*, Fig. 1, and tracing *A*, Fig. 3. This irregularity does not always appear in the strong concentrations, even in the case of the ganglia in prime condition. In concentrations represented by one part of the cyanide to fifty parts of plasma the ganglion is brought to a standstill in a few minutes, the rate of the ganglionic rhythm being greatly retarded from the very beginning, but the few discharges that do appear before the cessation of the rhythm still exhibit the augmented intensity (Fig. 1, *D*, *E*).

In the case of ganglia in good condition I have succeeded in only four cases in obtaining a primary depression both of the rate and the intensity of the nervous discharges with very strong solution (one part to ten). Typical tracings from these experiments are reproduced in Fig. 2. So far as regards the primary reaction of a ganglion to the cyanides, a ganglion once brought to a standstill in a cyanide

solution and restored to activity by removal to plasma, may be said to be in poor condition, even though its rhythm may be both regular and vigorous, because the same strength of the cyanide that brought the ganglion to a standstill after a primary stimulation when applied the first time brings the ganglion to a standstill without any primary stimulation and almost instantaneously when applied the

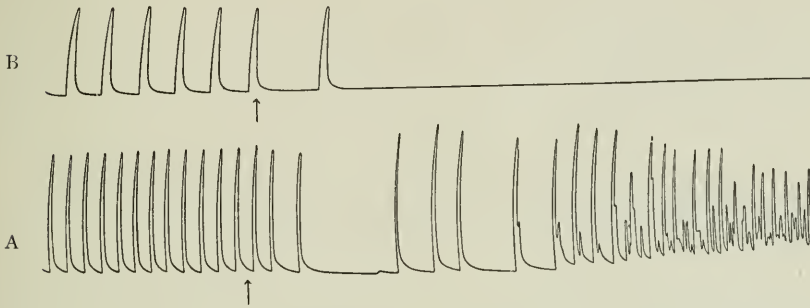


FIGURE 3.— Four fifths the original size. Tracings from the anterior end of the *Limulus* heart. Ganglion isolated posteriorly. ↑, application of KCN(6/10 μ) in plasma 1-10 to the isolated ganglion. Tracing B is taken after the ganglion had recovered from Experiment A. Showing that the same concentration of the cyanide may produce primarily opposite effects, depending on the condition of the ganglion.

second time, or to the restored ganglion. Tracings illustrating this difference in the action of the ganglion to the same concentration of the cyanide depending upon the different conditions of the ganglia are reproduced in Fig. 3. The difference, then, in the reaction of the ganglion in poor and in prime condition to the cyanides is mainly one of degree, the ganglion in poor condition being more sensitive to the cyanide.

It is thus evident that the cyanides have a uniform depressant action on the rate of the ganglionic rhythm, the greater the stronger the concentration. On the intensity of the ganglionic discharges the cyanides have a primary stimulating action up to a certain concentration, when the primary augmentation gives way to a primary depression, always in ganglia in poor condition, and sometimes in ganglia in prime condition.

Both the sodium and potassium cyanide were used in the experiments. No appreciable difference was observed in their mode or intensity of action.

III. THE ACTION OF THE CYANIDES ON THE LIMULUS HEART MUSCLE.

The primary action of the cyanides on the *Limulus* heart muscle is the same as that on the heart ganglion, that is, stimulating. A concentration of the cyanides that affects the heart muscles at all increases the amplitude of the contractions. The solution acting on the heart muscle alone does not affect the rate, as this is determined by the heart ganglion.

The heart muscle is, however, much less sensitive to the cyanide than the heart ganglion. This is shown by the fact that the heart

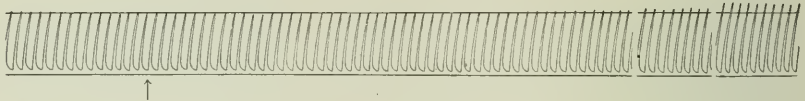


FIGURE 4.— One half the original size. Tracing from the anterior end of the *Limulus* heart. The ganglion extirpated in the recording segments, having the lateral nerves intact. ↑, application of $\text{NaCN}(6/10 \mu)$ in plasma 1-100 to the ganglion-free anterior end. Showing primary stimulating action of the cyanide on the heart muscle and motor nerve plexus.

muscle is not appreciably affected by dilutions of one to five or ten thousand, concentrations that act strongly on the ganglion. The stronger solutions that stimulate and paralyze all ganglia within three or four minutes maintain the heart muscles for hours. A graphic representation of this difference of degree of the sensitiveness of the heart muscle and the heart ganglion to these chemicals are given in Fig. 1 C and Fig. 4. At ↑ in the latter tracing a cyanide solution of one to one hundred was applied to the heart muscle. At ↑ in the former tracing the same strength of the cyanide was applied to the heart ganglion.

There appears, then, to be a close agreement between the primary action of the cyanides in *Limulus* and in the vertebrates. In the mammals the primary action is on the central nervous system and of the nature of stimulation, skeletal muscle and peripheral nerves being hardly at all affected by concentration of the drug that acts strongly on the nerve-centres. The heart is slowed both by the stimulation of the vagus centres in the medulla as well as by direct action on the heart. In *Limulus* we have seen that the strength of the cyanide that affects the rate at all retards it by its action on the ganglion, while the primary augmentation of the intensity of the nervous dis-

charges may be entirely absent when strong solutions are employed. In the case of the vertebrates some authors ascribe the primary stimulating action of the cyanides on the central nervous system to asphyxia due to the inability of the tissues to absorb oxygen. This explanation is probably insufficient, if not completely wrong. The following facts show that the explanation is not tenable for the *Limulus* heart.

1. As the stronger solutions of the cyanides paralyze the heart ganglion within a few minutes, this paralysis is not due to prevention of the absorption of oxygen from the surrounding liquids, because the *Limulus* heart ganglion and muscle continue in activity for hours in the total absence of free or atmospheric oxygen.¹

2. The primary stimulating and subsequent paralyzing action of weak solutions of the cyanides both on the heart muscle and the heart ganglion might be interpreted in accordance with the theory as due to stimulating actions of incompletely oxidized cleavage products, but this explanation is untenable in the case of the stronger solutions that may bring the ganglion to a standstill without having made a single nervous discharge after the application of the drug.

It is, therefore, improbable that the primary action of the cyanides on all forms of protoplasm is the prevention of tissue respiration or oxidation. These latter symptoms are probably the results of the direct or primary action, the mechanism of which is still unknown.

SUMMARY.

1. The cyanides have a primary stimulating action on the *Limulus* heart muscle.

2. In weak concentrations the cyanides augment the intensity of the nervous discharges from the heart ganglion, but in the strong concentration or in the case of ganglia in poor condition they may diminish the intensity of the nervous discharges from the beginning.

3. Solutions of the cyanides that affect the rate of the nervous discharges from the heart ganglion at all, retard the rate. Strong solutions produce various forms of inco-ordination of the ganglionic activity.

4. The *Limulus* heart muscle is much less sensitive to the action of the cyanides than the heart ganglion, but even the ganglion

¹ NEWMAN: This journal, 1906, xv, p. 371.

exhibits a much greater resistance to the drug than, for example, the central nervous system of mammals.

5. The primary action of the cyanides on the *Limulus* heart cannot be interpreted on the theory that the sole and primary action of these chemicals on protoplasm is the prevention of tissue respiration or oxidation.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS.— VII. SOLUTIONS OF ELECTROLYTES.

By TORALD SOLLMANN.

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INTRODUCTION AND DISCUSSION OF METHODS.

IN the preceding papers of this series¹ I have discussed the value of the perfusion of excised kidneys for studying the formation of a urine by purely physical processes, with the object of comparing these physical phenomena with the results obtained on living animals. A general agreement between the two was found to exist in the response of the circulation, ureter flow, and oncometer, toward a variety of conditions, such as changes in the arterial, venous, and ureter pressure, to changes of the osmotic pressure, and of the viscosity of the perfusing fluids. The phenomena observed in the excised kidneys were found to have relatively simple mechanical explanations. I also reported some experiments with equiosmotic solutions of non-electrolytes, showing that dextrose and cane sugar behaved like sodium chloride; alcohol and urea behaved as hypoisotonic solutions.² The present paper deals with similar investigations on a series of electrolytes, employing the methods described in the preceding paper.³ The excised dogs' kidneys were perfused at room temperature with the solutions under a uniform injection pressure of 130 cm. of solution.

¹ SOLLMANN: This journal, 1905, xiii, pp. 241-303.

² *Loc. cit.*, p. 288.

³ *Loc. cit.*, p. 247.

The vein and ureter flow from perfused kidneys is not perfectly constant, but is subject to a variety of changes,¹ some regular and gradual, others sudden, irregular, and accidental. These spontaneous changes do not present any serious difficulty if the experimental changes are prompt or extensive; but when the experimental variations are slight or slow, every precaution must be used to exclude deception by spontaneous variations. The disturbing element of the regular and gradual natural changes can be effectively excluded by plotting the readings on charts, where the natural tendencies can be seen and discounted. Accidental variations can only be ruled out by painstakingly guarding against accidents, and by multiplying experiments. Definite conclusions are only justified when a number of experiments yield qualitatively similar results. With some salts, however, the effects remain variable, even when accidents are effectively excluded.

Each kidney served for a number of experiments, the perfusions with the fluid under investigation being alternated with perfusions with 1 per cent sodium chloride, allowing sufficient time for any changes to reach a constant (generally half an hour or longer). This also permitted the study of the recovery or of any after-effects. As a rule, several substances were used, but this was done in varying sequence, to exclude the possibility of mutual interference and the effects of exposure. When the perfusion was performed on the same day as the excision, the kidneys are designated as "living," since I have shown in my previous paper that vital phenomena persist for a day; those kidneys used on the following day, or later (generally on the third or fourth day), are designated as "dead." The comparison of these results shows whether the solution acts differently on the living than on the dead kidney,—in other words, whether a "*vital factor*" is involved. The ureter flow in "dead" kidneys is generally unsatisfactory. In some cases, therefore, the vitality was lowered by mixing one volume of the solution with two volumes of 2 per cent sodium chloride, and comparing the results with a sodium chloride solution of equal concentration. These hyperisotonic solutions decrease the vital phenomena, whilst preserving a good ureter flow.

The equiosmotic solutions (which may be conveniently designated as Δ solutions) were made of the same freezing-point as the 1 per cent sodium chloride (1 gm. of sodium chloride dissolved in sufficient

¹ *Loc. cit.*, p. 249.

water to make 100 c.c.). The percentage strengths of these solutions, as given in the text, are only approximately correct; the precise adjustment of the solutions being made by direct cryoscopic determination, and not by the weight of dissolved substance. The anions were used in combination with sodium, the cathions in combination with chloride.

On studying the perfusion phenomena, it soon became evident that solutions of the same freezing-point as 1 per cent sodium chloride are not necessarily isotonic to the kidney cells, since these have a selective permeability. Whenever the solutions produced a definite effect, it was therefore investigated whether this was not simply osmotic, by noting the change of the weight of kidney sections in the solution, using the method previously described.¹ These alterations in weight correspond to changes in the blood and urine channels,² and when they occurred, it was considered whether they could account for the perfusion phenomena. If this was the case, a concentration was sought experimentally which did not change the weight of the sections, and the perfusions were repeated with this concentration. This would effectively exclude phenomena due to changes in the volume of the cells. It must be borne in mind, however, that the method for determining changes in weight is not as sensitive as the observation of perfusion phenomena; so that slight changes in the latter might be referable to osmosis even if the weight of sections is unchanged. Nor are the alterations in weight always osmotic. Chemical changes may occur, as indicated by the altered appearance and consistency of the sections, and by the failure of the sections to return to normal conditions when replaced in sodium chloride, or of the perfusion phenomena when sodium chloride is resumed.

Further experiments were tried with dilutions of the solution with 1 per cent sodium chloride. If the changes persisted after considerable dilution, they cannot be osmotic, but must be vital or chemical.

A few perfusion experiments were made on organs other than kidneys; these are not so well adapted to this method, and the results are inserted only for the sake of completeness.

In the following section I shall condense the results and conclusions for each ion which was investigated; these will be compared in the final summary.

¹ *Loc. cit.*, p. 280.

² *Loc. cit.*, p. 284.

IONS CAUSING PRACTICALLY NO CHANGE.

This class comprises K, Li, I, Br, NO_3 , ClO_3 , and $\text{C}_2\text{H}_3\text{O}_2$.

1. **Potassium.** — Four perfusions with Δ solutions were made on living kidneys. The *vein flow* was unchanged in two and slightly decreased in two. The *ureter flow* was unchanged in one and diminished a trifle in one. The *oncometer* was unchanged in one and fell slightly in one. On resuming NaCl, the vein and oncometer recovered, and rose slightly above normal.

Conclusions. — The effects are so small and inconstant that they may be judged as negative.

2. **Lithium.** — $\Delta = 0.726$ per cent of LiCl, determined experimentally.

Four perfusions with the Δ solution were made on living kidneys. Two showed no change in vein, ureter, or oncometer. In the third the vein flow was slightly decreased, the ureter flow slightly increased, with the oncometer unchanged. The fourth showed some irregular and evidently accidental variations. One perfusion on an excised spleen showed a slight decrease of vein flow.

There were no after-effects.

Conclusions. — Lithium produces practically no effect.

3. **Iodid.** — $\Delta = 2.542$ per cent of NaI, calculated.

Six perfusions with Δ solution were made on living kidneys. Two showed no changes in the vein or ureter flow, or oncometer. The vein flow was slightly increased in one, fairly increased in one, slightly decreased in two, recovering during the perfusion. The ureter flow showed a fair increase in four. The oncometer was slightly increased in two. One perfusion of an excised spleen gave a fair decrease of the vein flow. The conditions returned to normal on resuming NaCl.

Conclusions. — The effects are small and inconstant, but tend to increase the vein and ureter flow and oncometer.

4. **Bromid.** — $\Delta = 1.746$ per cent NaBr, calculated.

Four perfusions with Δ solution on living kidneys.

The results were entirely negative.

5. **Nitrate.** — $\Delta = 1.535$ per cent of NaNO_3 , determined experimentally.

Eight perfusions with Δ solution on living kidneys. The vein flow was unchanged in three, increased in five (trifling in one, slight in one, fair in three). The ureter flow was unchanged in one, increased

in five (slight in four, fair in one). The oncometer was unchanged in five, and increased in three (slightly in two, fair in one). The conditions return to normal on resuming NaCl.

Conclusions. — The changes are inconstant and small, but tend to increase of vein, ureter and oncometer.

6. **Chlorate.** — $\Delta = 1.795$ per cent of NaClO_3 , experimentally determined.

Four perfusions with Δ solutions on living kidneys. The results were entirely negative.

7. **Acetate.** — $\Delta = 1.275$ per cent of $\text{Na C}_2\text{H}_3\text{O}_2$, experimentally determined.

Five perfusions with Δ solutions on living kidneys. The vein flow was unchanged in one and decreased in four (two slight, two fair). The ureter flow was unchanged in one, decreased in two (one trifling, one fair), and variable in two (rise followed by fall). The oncometer was unchanged in one and decreased in three (slight in two, fair in one). On resuming NaCl the conditions tend to return to normal, but the recovery is sometimes delayed. A kidney section laid in the solution does not change in weight.

Conclusions. — The vein and ureter flow and oncometer tend to fall, but the effect is small and inconstant. The unchanged weight of the kidney section shows that it is not osmotic. When a fall has occurred, the recovery of the vein and ureter flow is imperfect and slow, whilst the oncometer returns promptly.

IONS WHICH INCREASE THE VEIN AND URETER FLOW.

The oncometer is also increased by $\text{C}_6\text{H}_5\text{O}_7$ and Mg, not by SO_4 and NH_4 .

8. **Citrate.** — $\Delta = 2.737$ per cent of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, experimentally determined.

Four perfusions with Δ solution on living kidneys invariably increased the vein and ureter flow and oncometer, the effect being generally quite large and recovering promptly when NaCl was substituted. The same results were obtained in five perfusions on dead kidneys (two further perfusions being negative). It is therefore evident that the main action is not vital. Three sections of kidney, laid in the solution, lost considerable weight; in other words, the solution is hyperisotonic, which would explain the increased flow.

To exclude the osmotic factor, the solution was diluted until it left

the weight of sections unchanged. This required the addition of one volume of water to three volumes of solution (two experiments; in equal volumes of water and solution, a section gained weight, showing that this dilution is hypoisotonic).

This isotonic ($\frac{3}{4} \Delta$) solution was perfused through two living and two dead kidneys. It now caused only a slight increase of vein flow, a fair decrease of ureter flow (in the living kidneys) and no change in the oncometer.

The hypoisotonic solution ($\frac{1}{2} \Delta$) decreased the vein and especially the ureter flow, leaving the oncometer unchanged (two perfusions of living kidneys).

Conclusions.—The increase of vein and ureter flow and oncometer are osmotic. When osmosis is excluded, the citrate has practically no effect.

9. **Magnesium.**— $\Delta = 2.115$ per cent $MgCl_2$, dried at $110^\circ C.$, determined experimentally.

Six perfusions with Δ solutions on living kidneys showed a constant and marked increase, returning to normal when NaCl was resumed. The same results were obtained in four perfusions of dead kidneys, but four other perfusions showed no change. The effects therefore are not vital. Three sections lost weight in the Δ solution, which is therefore hyperisotonic, explaining the increased flow. The osmotic factor was excluded by diluting three parts of the Δ solution with one part of water (two sections). Perfusion with this dilution, on two living and four dead kidneys, did not increase the flow, but on the contrary diminished the vein and ureter flow, and generally the oncometer. This diminution is not vital, and I am inclined to attribute it to incomplete elimination of osmosis; for the changes in the weight of sections cannot be determined as accurately as the changes in the other functions.

Conclusions.—The increased vein flow, ureter flow and oncometer are osmotic. When osmosis is largely excluded, these phenomena are rather decreased; the decrease is not vital, and may be due to the incomplete elimination of osmosis.

10. **Sulphate.**— $\Delta = 2.10$ per cent of Na_2SO_4 , determined experimentally.

Six perfusions with Δ solutions on living kidneys produced a constant and pronounced increase of the vein flow; the increase in ureter flow and oncometer being less marked and less constant. On resuming NaCl the conditions return promptly to normal. With dead kid-

neys (four perfusions) the effects are much less, so that there may be a vital factor. However, the main action is osmotic, the solution being hypertonic (decrease of weight of two sections). It becomes nearly isotonic (weight of two sections unchanged) when 3 volumes are diluted with one volume of water. Four perfusions with this diluted solution on living kidneys still showed some increase of vein flow, with decrease of ureter flow and unchanged oncometer. Incomplete elimination of osmosis could not account for the divergent behavior of the vein and ureter flow; there must be some other factor. Perfusing this solution through two dead kidneys caused practically no change, suggesting again that there is a vital element involved. Perfusion of four living kidneys with a hypotonic solution (equal volumes of Δ solution and water) decreased the vein and ureter flow markedly and constantly, the oncometer being unchanged. The vital factor is therefore eliminated or overcome by the swelling of the cells. It is also eliminated by mere dilution, apart from osmosis, for the perfusion of a mixture of 1 volume of Δ Na_2SO_4 and 19 volumes of Δ NaCl (equals 0.1 per cent Na_2SO_4) through six living kidneys is practically negative.

Conclusions. — The increased vein and ureter flow are mainly osmotic; but there is a weak vital factor, tending to increase the vein and to diminish the ureter flow. This factor is eliminated when the sulphate is reduced to 0.1 per cent, or when the solution is rendered hypoisotonic.

11. **Ammonium.** — $\Delta = 0.913$ per cent NH_4Cl , deduced by analogy.

During the perfusion of living kidneys (six experiments) with the solution the vein flow is scarcely altered, but tends to a trifling and inconsistent increase. In the ureter flow this tendency is somewhat stronger. The oncometer tends to fall. The outflowing liquids are tinged with laked hemoglobin.

On resuming the perfusion with NaCl the conditions returned to normal in three of the experiments, but in the other three the vein flow, the ureter flow, and oncometer fell considerably below the normal.

Of eight dead kidneys perfused by the Δ solution, two gave negative results. The other six showed the following:

During the perfusion the vein flow increased somewhat more constantly and markedly than in the living kidney. The oncometer was unchanged in three, and fairly increased in three — the reverse of the living kidney. On resuming NaCl the vein flow tended to remain

high, or even to rise further; the oncometer returned to normal, but did not fall below this.

It is seen that the phenomena differ considerably in the living and dead kidneys, so that a vital factor must be assumed.

Six sections laid in the solution appear as if cooked, indicating a chemical change. The weight was increased a trifle in five, diminished in one. The solution is therefore slightly hypotonic, which would tend to decrease the flow, whereas an increase was generally observed. The changes in the perfusion are therefore not osmotic. On transferring these sections to 1 per cent NaCl, they lost not only what they had gained in the ammonium chlorid, but something in addition.

Conclusions. — The changes produced by ammonium are too complex for analysis. They are not due to osmosis: vital and probably chemical factors are involved.

IONS WHICH DIMINISH THE VEIN AND URETER FLOW.

This class comprises Ba, H, Ca, C_2H_4 , F, OH, CO_3 , HCO_3 , and HPO_4 .

12. **Barium.** — $\Delta = 2.562$ per cent, determined experimentally. Seven perfusions of the Δ solution through living kidneys gave inconstant results. In one there was practically no effect. Two perfusions of another kidney produced a very marked increase of vein flow without marked change in the oncometer; the conditions returned to normal on resuming sodium chlorid. The four other kidneys showed a marked decrease of the vein and ureter flow, and a lesser fall of the oncometer. In one of these kidneys the vein flow and oncometer recovered somewhat during the perfusion. In another kidney the oncometer after a slight preliminary fall, rose above normal. On resuming the perfusion of sodium chlorid all four kidneys exhibited a further decrease of the vein flow and oncometer.

The effects are quite different in dead kidneys. Of five perfusions, one showed no change. In the other four the vein flow was slightly increased; the oncometer also tended to rise a trifle, but less constantly. The changes on resuming sodium chlorid were very irregular, but quantitatively insignificant.

In view of these differences the diminished vein and ureter flow in the living kidneys must be considered vital. The increased vein flow and oncometer in dead kidneys is, at least mainly, osmotic; for

the Δ solution proved hypertonic to three out of five sections. This explanation probably applies also to the occasional primary or secondary increase in kidneys which were recorded as living, but which had probably been injured by the barium. The sections did not show any visible chemical changes.

The decreased vein and ureter flow in the living kidneys might be ascribed to the well-known vaso-constrictor action of barium. This would be partly counteracted by the osmotic vasodilation. This would explain why the subsequent perfusion of sodium chlorid (which would remove the osmotic dilatation) causes a further fall.

Perfusion of three living kidneys with a diluted solution (1 volume of Δ barium chlorid with 9 of Δ sodium chlorid = 0.256 per cent BaCl_2) decreased the vein flow a trifle, without altering the ureter flow or oncometer.

Perfusion of two living kidneys with a 0.128 per cent solution of BaCl_2 in Δ NaCl was ineffective. Perfusion of a 0.005 per cent solution in blood was also ineffective.

Perfusion of the vessels of an excised intestine with a 0.1 per cent solution of BaCl_2 in Δ NaCl caused a small, doubtful diminution of the vein flow. Injection of 5 c.c. of a 1 per cent BaCl_2 solution into the fluid perfusing through an excised spleen lowered the vein flow and oncometer very markedly.

Conclusions.—Barium chlorid is somewhat hypertonic, and therefore quickens the vein flow in dead kidneys. In living kidneys this effect is overcome by the vasoconstriction, which lowers the vein and ureter flow.

13. **Acids.**— $\Delta = 0.572$ HCl , determined experimentally.

Six perfusions of living kidneys with the Δ solution all show very marked decrease of vein and ureter flow and oncometer. On resuming NaCl , the recovery is slow and very imperfect. Six perfusions of dead kidneys gave similar results, although not quite as marked or constant. Recovery is again imperfect; indeed, the vein flow and oncometer often continue to fall after the NaCl is resumed. The same phenomena are seen when the acid is added to 2 per cent NaCl . It is, therefore, evident that the decrease is not vital.

Three sections laid in the Δ solution behaved differently: one lost in weight, another gained, and a third gained at first and later lost. These inconstant results show that the phenomena are not osmotic. This is also confirmed by the persistence of the effects on dilution: Two perfusions of living kidneys with a mixture of one volume of Δ HCl

and 9 volumes of 1 per cent NaCl (= 0.057 per cent HCl) gave quantitatively the same results as the Δ HCl. Even a dilution of 1 Δ HCl to 50 Δ NaCl (= 0.011 per cent HCl) decreased the vein flow and oncometer in two living and two dead kidneys.

Conclusions. — Hydrochloric acid decreases the vein and ureter flow and oncometer in living and dead kidneys, so that the action is not vital. The decrease also occurs in very dilute solutions, and the behavior of sections is variable, so that the action is not osmotic. The slow and imperfect recovery indicates that the effects are chemical.

14. **Calcium.** — $\Delta = 1.633$ per cent CaCl_2 , determined experimentally.

Of eight perfusions of the Δ solution through living kidneys, three were negative, the other five produced uniformly a very marked fall of the vein and ureter flow and oncometer. These effects are vital, for of five perfusions through dead kidneys, two were entirely negative, and the other three showed only small and inconstant changes; the effects were similarly small and inconstant in three experiments in which the vitality was injured by perfusion with 2 per cent NaCl.

Four kidney sections showed a trifling loss of weight in the Δ CaCl_2 . The osmotic effects would therefore be insignificant, but would be opposed to the results actually observed.

Perfusion of living kidneys with dilute solutions give results very similar to the undiluted: in perfusing with a mixture of one volume Δ CaCl_2 and five volumes 1 per cent NaCl (= 0.272 per cent CaCl_2), the effects were practically identical with the Δ CaCl_2 . The decrease was somewhat less constant and less marked in four perfusions with 1 Δ CaCl_2 to 9 Δ NaCl (= 0.163 per cent CaCl_2), and in two perfusions with 1 Δ CaCl_2 to 19 Δ NaCl (= 0.082 per cent CaCl_2). The occurrence of the changes with these dilute solutions indicates that they are chemical. This is supported by the observations that recovery on the perfusion of NaCl is slow and often imperfect.

Conclusions. — The decrease of vein and ureter flow and oncometer does not occur in dead kidneys, and is therefore vital; its occurrence in dilute solutions, and the imperfect recovery, indicate that it is chemical. The osmotic effects are insignificant, but would be opposed to the vital action.

15. **Oxalate.** — $\Delta = 2.3$ per cent $\text{Na}_2\text{C}_2\text{O}_4$, determined experimentally.

Perfusions of two living kidneys with the Δ solution show very marked decrease of the vein and ureter flow and oncometer, and no recovery on resuming NaCl. The results are so pronounced that no

further experiments were made. Two perfusions on dead kidneys gave quantitatively identical results, so that the action is not vital. Two kidney sections showed a very marked loss of weight in the Δ solution: the osmotic action would therefore be opposed to the observed effects, and cannot explain them. The failure of the perfusion to recover with NaCl indicates that the action is chemical. However, it appears to require a fairly high concentration of the oxalate, for four perfusions of living kidneys with a mixture of 1 Δ $\text{Na}_2\text{C}_2\text{O}_4$ and 19 Δ NaCl (= 0.115 per cent $\text{Na}_2\text{C}_2\text{O}_4$) produced inconstant and small results.

Conclusions. — The decrease of vein and ureter flow and oncometer occurs also in dead kidneys, and is therefore not vital. The failure to recover indicates that it is chemical, but it does not occur in dilute solutions.

The Δ solution is strongly hypertonic, so that the osmotic effects would be opposed to those which were actually observed.

16. **Fluorid.** — $\Delta = 0.721$ per cent, deduced by analogy.

In three perfusions of living kidneys with the Δ solution the vein flow was unaffected in two, markedly decreased in the third. The ureter flow showed a marked decrease, and the oncometer a somewhat lesser fall, in all three. On perfusing NaCl the recovery was slow, but perfect.

In four perfusions of dead kidneys the vein and ureter flow were uniformly increased, although not very markedly. The oncometer generally remained unchanged. The recovery with NaCl was perfect. The changes are therefore quite different in dead and living kidneys, so that they must be vital.

Two kidney sections showed a trifling gain in weight in the Δ solution. The osmotic effects would therefore be insignificant, but opposed to those observed in the perfusions of dead kidneys.

The vital changes are not very strong, for when 0.3 per cent NaF was added to 1 per cent NaCl (thus raising the osmotic pressure), the results were very variable in four perfusions of living kidneys. In two dead kidneys the vein flow and oncometer were decreased.

Perfusions of two living kidneys with a mixture of 1 Δ NaF and 19 Δ NaCl (= 0.036 per cent NaF) gave a trifling decrease of the vein and ureter flow and oncometer; but more experiments would be needed to confirm this.

Conclusions. — The decrease of the vein and ureter flow and oncometer are vital, dead kidneys giving the opposite response. Δ solutions are a trifle hypoisotonic, but not sufficiently so to affect the

results. The mechanism of the action on living and dead kidneys is not explained by the experiments; it may be chemical.

17. **Hydrate.** — $\Delta = 0.7$ per cent NaOH, deduced from tables.

Five perfusions of living kidneys with the Δ solution all showed a very large decrease of the vein and ureter flow, the oncometer being variable. The fluid flowing from the vein and ureter was very viscid and mucinous, and tinged with hemoglobin. On resuming the perfusions of NaCl there was generally no recovery; on the contrary, the changes continued and often progressed.

Two kidney sections laid in the Δ solution gained markedly in weight, but did not recover in NaCl, so that the change was not osmotic. The appearance of the sections undergoes a marked change; they become gelatinous and transparent, and the surface is partly dissolved into a very viscid fluid. These appearances persist when the sections are changed to NaCl.

The effects are evidently chemical, and since they agree with those produced by sodium carbonate, no further experiments were made.

18. **Carbonate.** — $\Delta = 1.454$ per cent, deduced from tables. This was tried only on living kidneys.

Of four perfusions of the Δ solution one gave anomalous and complex results. The other three showed a very large decrease of the vein and ureter flow, which did not recover with NaCl. The vein and ureter fluid has the viscid character described under sodium hydrate. Two perfusions of a dilution of 1 Δ Na_2CO_3 with 2 Δ NaCl (= 0.485 per cent Na_2CO_3) also decreased the vein and ureter flow and oncometer to the same extent. On resuming NaCl the ureter flow did not recover, the oncometer imperfectly, but the vein flow increased markedly above normal. Three perfusions of 1 Δ Na_2CO_3 and 9 Δ NaCl (= 0.145 per cent Na_2CO_3) showed the same decrease, but the recovery was more perfect, although slow. Two perfusions with 1 Δ Na_2CO_3 and 49 Δ NaCl (= 0.03 per cent Na_2CO_3) also produced a marked decrease of the vein and ureter flow and oncometer, but the recovery was more prompt.

Two kidney sections laid in the Δ solution lost slightly in weight and became semi-gelatinous. They recovered their weight when replaced in Δ NaCl.

Conclusions. — The decrease of vein and ureter flow and oncometer occurs even in very dilute solutions, indicating that the cause is chemical. The chemic change is also shown by the gelatinous appearance of the sections. This solution of the cells and the result-

ing viscosity of the perfusing solution are doubtless important factors in the perfusion phenomena.

19. **Bicarbonate.** — $\Delta = 0.966$ per cent NaHCO_3 , determined experimentally.

Of five perfusions of the Δ solution through living kidneys, three were negative; two showed a small decrease of the vein and ureter flow and oncometer. The fluid was tinged yellow by hemoglobin. On resuming the NaCl these did not recover typically.

Four perfusions of dead kidneys all decreased the vein flow quite markedly; the changes in the ureter flow and oncometer were less marked and less constant. The recovery was imperfect.

Three kidney sections gained markedly in weight in the Δ solution. This would agree with the lessened flow, although it may not be the main cause.

The following experiments were made on living kidneys with dilutions with 1 per cent NaCl:

Five perfusions with 0.394 per cent NaHCO_3 gave variable results, tending to a decrease of the phenomena.

Four perfusions with 0.03 per cent NaHCO_3 were negative.

Conclusions. — The effects on living and dead kidneys and sections agree qualitatively. However, the slow recovery indicates a chemical rather than an osmotic factor. There is probably some solution of proteid and thus increased viscosity. The smaller decrease in living kidneys points to an opposing vital factor.

20. **Phosphate.** — $\Delta = 2.10$ per cent Na_2HPO_4 , determined experimentally.

Five perfusions of the Δ solution through living kidneys decreased the vein flow slightly in one (a) and increased it markedly in the other four. In two of these (b and c) it remained high during the perfusion; in the other two (d and e) it fell considerably below normal. When NaCl was resumed, it continued to fall for a time in all the four, and then rose again toward normal. This is evidently a persistence of the effect, which may therefore be described as a very marked increase, followed by a marked diminution of the vein flow. The ureter flow was markedly diminished. Both the vein and ureter fluid were viscid and turbid and tinged with hemoglobin.

The oncometer fell somewhat in (a) and rose somewhat in (b); (c) showed a marked fall; in (d) and (e) there was a fall, followed by a rise. The oncometer therefore varied in the inverse direction to the vein flow during the perfusion. On resuming NaCl the

changes of the oncometer were in the same direction as those in the vein.

The Na_2HPO_4 was then again perfused through the kidneys (b) and (c) with practically the same results as before, both during the perfusion and in the after-period.

Four perfusions were made through dead kidneys. The vein flow was at first increased in all. In two it remained high, in the third it fell to the normal, and in the fourth below normal. The ureter flow increased in one and diminished in one; in a third it was first increased and then diminished. The oncometer was scarcely changed. The functions generally recovered when NaCl was resumed.

The primary increase of vein flow is therefore common to both living and dead kidneys; but the secondary decrease and the oncometric changes are less conspicuous or absent in dead kidneys.

Four kidney sections lost weight in the Δ solution, indicating that this is hypertonic and would produce an increased flow. However, the sections appeared partly gelatinous and did not recover their weight in Δ NaCl, so that the change is probably largely chemical. In a dilution of three volumes of Δ Na_2HPO_4 with one volume of water the weight remained almost unchanged, one section gaining, and two losing a trifle. This solution was perfused in order to exclude the gross changes of volume. Four living and five dead kidneys were used. All of the living kidneys showed an increased vein flow without secondary fall. The oncometer was unchanged. In the dead kidneys the changes were very slight and inconstant, perhaps with a tendency to increase of vein flow.

The dilution, therefore, modified the phenomena considerably, but since the vein flow was increased in the living kidney this effect is not osmotic.

A dilution of the Na_2HPO_4 with fifty volumes of 1 per cent NaCl (Δ 0.042 per cent Na_2HPO_4) was perfused through four living and five dead kidneys. In all the living kidneys the vein and ureter flow were increased somewhat, the oncometer remaining practically stationary. Of the dead kidneys, two showed a slight increase, and two a slight decrease, of vein flow, the oncometer being again stationary.

Two perfusions of spleens with the Δ solution decreased the vein flow markedly.

Conclusions. — In living kidneys the Δ solution produces a primary increase and secondary diminution in the vein flow; the oncometer showing the opposite changes, and the ureter flow being diminished.

The opposite tendency of the oncometer and vein flow indicates that the changes are peripheral to the glomeruli. The decreased vein flow is not due to injury of the kidney, for on flushing the renal vessels with NaCl, and then resuming the phosphate, the primary increase is again obtained. Dead kidneys show the primary increase of vein flow so that it is not exclusively vital. The secondary decrease and the oncometric changes are absent in dead kidneys, so that there must be a vital factor.

The Δ solution appears hyperisotonic to kidney sections, but there is also a chemic change. Diluting the solution so that it does not change the weight of sections, it still increases the vein-flow in living kidneys, so that this phenomenon is not explained by osmosis. In dead kidneys the changes were much less, so that there may be an osmotic factor.

Dilute isotonic solutions caused a fair increase of vein and ureter flow in living kidneys; in the dead this was much less pronounced. The action is therefore mainly vital, and differs from that caused by more concentrated solutions.

MIXTURES OF ELECTROLYTES.

In this connection I have only investigated whether calcium and citrate are antagonistic in the kidney, as they are in other organs; and the influence of the components of Ringer's solution. The experiments were restricted to living kidneys.

21. *Calcium and Citrate.*—In a first series three solutions were compared, containing:

Solutions Ca: 1 volume Δ CaCl₂ and nine volumes Δ NaCl.

Solutions Ci: 2.13 volume Δ Na₃C₆H₅O₇ and 7.87 volumes Δ NaCl.

Solutions Ca-Ci: 1 volume Δ Ca Cl₂, 2.13 volume Δ Na₃C₆H₅O₇, and 6.87 Δ NaCl.

Solution Ca = 0.1483 M¹ of CaCl₂; solution Ci = 0.1068 M.

The ratio of Ca to C₆H₅O₇ molecules in solution Ca-Ci is as 1:1.55.

Four perfusions with Ca showed a fair and constant decrease of the vein and ureter flow, and a slight and inconstant fall of the oncometer.

Four perfusions with Ci showed a fair but inconstant increase of

M = gram molecules per litre.

vein flow; a somewhat larger and more constant increase of ureter flow, and a fair but inconstant increase of the oncometer.

The two solutions are therefore fairly balanced; and their admixture should produce but little effect. However, four perfusions with Ca-Ci caused practically the same effect as Ca alone, so that no antagonism could be demonstrated.

In a second series the proportion of citrate was chosen higher.

Solution Ca = 1 volume Δ CaCl_2 and 19 volumes Δ $\text{NaCl} = 0.0742$ M CaCl_2 .

Solution Ca-Ci = 1 volume Δ CaCl_2 and 5 volumes Δ $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ and 14 volumes Δ NaCl . The molecular ratio of Ca and $\text{C}_6\text{H}_5\text{O}_7$ is as 1 : 3.6.

Two perfusions with the Ca solution decreased the vein and ureter flow, the oncometer being unchanged. Two perfusions with the Ca-Ci solution left the vein flow and oncometer unchanged, the ureter flow being slightly increased.

Conclusions. — In so far as these experiments go, they indicate that the citrate can antagonize the effects of calcium, but that a considerable excess is required.

22. *Ringer's solution.* — Various modifications of this mixture were investigated, taking Locke's fluid as a type. The following mixtures were used:

	A (Locke's fluid).	B (double calcium).	C (no calcium).	D (only bicarbonate)
CaCl_2 . .	0.024%	0.048%
KCl . .	0.042 "	0.042 "	0.042 %	...
NaHCO_3 .	0.03 "	0.03 "	0.03 "	0.03 %

These salts were dissolved with sodium chloride so as to make them approximately isotonic with 1 per cent NaCl .

Solution A. — Twelve perfusions of living kidneys were made. The vein flow was unchanged in four, somewhat increased in two, somewhat diminished in three, and doubtful in three. The ureter flow showed some increase in eight, in the other four it was too small for observation. The oncometer remained stationary in four; three showed a trifling fall, and three were doubtful.

The only definite effect, therefore, consisted in a small increase of the ureter flow.

Solution B (double calcium). — Four perfusions were made. The vein flow was not changed in two, somewhat diminished in two.

The ureter flow was somewhat increased in three. The oncometer was stationary in three, and indefinite in the fourth.

It appears that doubling the calcium does not affect the result. This quantity of calcium is about one half of the lowest concentration which was tried in pure solution, and was there found to decrease the vein and ureter flow somewhat.

Solution C (without calcium). — This was only tried on one kidney, giving the same results as solution A.

Solution D (bicarbonate alone). — Four perfusions gave results practically identical with solution A.

Conclusions. — The perfusions with Locke's salt solution gave about the same results whether the calcium was doubled or omitted, or when only the bicarbonate was used. The only difference from plain sodium chloride is a slightly better ureter flow.

Perfusions with boiled blood. — In order to test the effects of the blood salts, the defibrinated blood was diluted with 3 to 5 parts of Δ NaCl, boiled and filtered.

The filtrates were perfused through three kidneys, resulting in a slight decrease of the vein and ureter flow and oncometer. This result may be referred to the viscosity from the proteids remaining in the solution.

SUMMARY AND CONCLUSIONS.

The details of the actions of the several ions have been sufficiently summarized in the preceding section, and it only remains to point out some of their relations.

Gross effects on perfusion of solutions of the freezing-point of one per cent NaCl. — The following ions produced practically no effect: K; Li; Br, and ClO_3 . I and NO_3 caused a trifling increase, and $\text{C}_2\text{H}_3\text{O}_2$ a trifling decrease of the vein and ureter flow and oncometer.

$\text{C}_6\text{H}_5\text{O}_7$, Mg, SO_4 , and NH_4 caused a more marked increase of the vein and ureter flow; the first two raise the oncometer, SO_4 and NH_4 do not. The phenomena with NH_4 are peculiarly complex.

The vein and ureter flow, and generally the oncometer, are diminished by Ba, H, Ca, C_2H_4 , F, OH, CO_3 , HCO_3 , and HPO_4 . The phenomena with the last are complex.

Changes of weight of kidney sections in these solutions. — The weight of the section is:

Diminished.	Unchanged.	Increased.	Variable results.
$\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$	$\frac{3}{4} \Delta \text{Na}_3\text{C}_6\text{H}_5\text{O}_7$	$\frac{1}{2} \Delta \text{Na}_3\text{C}_6\text{H}_5\text{O}_7$
MgCl_2	$\frac{3}{4} \Delta \text{MgCl}_2$
Na_2SO_4	$\frac{3}{4} \Delta \text{Na}_2\text{SO}_4$
Na_2HPO_4	$\frac{3}{4} \Delta \text{Na}_2\text{HPO}_4$
Na_2CO_3	NaOH
....	NaHCO_3
CaCl_2	BaCl_2
$\text{Na}_2\text{C}_2\text{O}_4$	$\text{NaC}_2\text{H}_3\text{O}_2$	NaF	HCl
....	NH_4Cl

The decrease in weight indicates that the solution is hyperisotonic, and *vice versa*. It is worthy of notice that $\text{C}_6\text{H}_5\text{O}_7$, Mg , SO_4 , and HPO_4 are rendered isotonic by about the same degree of dilution ($\frac{3}{4} \Delta$). The changes in weight may also be chemic; this is indicated by the failure of the sections in NH_4 , OH , and HPO_4 to return to their normal weight in 1 per cent NaCl ; and by the altered appearance of the sections in these solutions and in HCl and Na_2CO_3 . In the other solutions the changes of weight are probably osmotic, due to the selective permeability of the kidney cells.

The osmotic, vital, and chemical factors in the changes noted during perfusion. — The influence of osmosis predominates with Mg , $\text{C}_6\text{H}_5\text{O}_7$, SO_4 ; is subordinate or doubtful with HCO_3 and HPO_4 ; is unimportant with NH_4 , Ba , Ca , H , OH , CO_3 , C_2O_4 , F , $\text{C}_2\text{H}_3\text{O}_2$.

The vital factor predominates with NH_4 , Ba , Ca , F ; is subordinate or doubtful with HPO_4 , SO_4 , HCO_3 , $\text{C}_2\text{H}_3\text{O}_2$; is unimportant with Mg , H , CO_3 , OH , $\text{C}_6\text{H}_5\text{O}_7$, C_2O_4 .

The chemical factor predominates with H , OH , CO_3 , HPO_4 , C_2O_4 ; is subordinate or doubtful with Ca , Mg , HCO_3 , $\text{C}_2\text{H}_3\text{O}_2$, NH_4 , F ; is unimportant with SO_4 , Ba , $\text{C}_6\text{H}_5\text{O}_7$.

Changes occur in dilute solutions with: Ba , Ca , H , CO_3 , HPO_4 , F ; not with HCO_3 , SO_4 , C_2O_4 .

The effects of calcium can be antagonized by a considerable excess of citrate. The mixture of ions in Ringer's fluid has but little effect.

These results throw but little light on the relative diuretic effect of salts in the body. A general agreement between the gross results can be seen in the case of citrate, sulphate, magnesium, calcium, and barium and some others. The first three, however, act mainly by osmosis, which can scarcely occur to any great extent in the body; the action of barium is accounted for by its known vaso-constrictor

action. This leaves, thus far, the calcium action as the most interesting example of the analogy. It must be remembered, however, that the comparative diuretic action of electrolytes has been but little investigated in living animals; when this is done, the present results may become of greater practical value.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. —
VIII. THE EFFECTS OF SOLUTIONS ON THE HISTO-
LOGICAL APPEARANCE OF KIDNEY SECTIONS.

By W. W. WILLIAMS.

AT the suggestion of Dr. Sollmann the following experiments were undertaken by me with the idea of determining which phenomena described in the preceding papers might be explained by cytologic changes in the kidney. We were inclined to anticipate very definite results after learning the marked changes which Schmitter¹ obtained after macerating kidney sections in distilled water and varying strengths of sodium chlorid solution.

METHODS.

1. **Fresh sections.** — A kidney was removed immediately after killing a dog, and a specimen was frozen and sections cut. The sections were placed in various solutions for one hour. After examination the sections were replaced in the same solutions, to which a few drops of Loeffler's methylene blue had been added, for sixteen hours, and again examined.

2. **Hardened sections.** — A fresh kidney was cut into small pieces, about 5 mm. or less in thickness, and two specimens were placed in each of the various solutions for one hour and then removed — one to Orth's fluid and the other to Zenker's fluid — and allowed to remain for twenty-four hours. They were then washed in running water and afterwards passed through 80 per cent, 90 per cent, and absolute alcohol, alcohol and ether, and imbedded in celloidin. The sections were stained with diluted Delafield's hematoxylin and eosin.

DESCRIPTION OF FRESH SECTIONS.

I. *Tap water.* — The epithelial cells of the convoluted tubules are slightly enlarged, the cytoplasm granular and containing numerous

¹ SCHMITTER: Centralblatt für die gesammte wissenschaftliche Anatomie, 1905, xxvi, Nos. 11 and 12.

reflective globules of various sizes (fat?).¹ The nuclei are slightly enlarged and are very vesicular.

II. 1 per cent sodium chlorid. — The epithelial cells of the convoluted tubules are apparently of normal size, with slightly granular protoplasm containing numerous fat globules. The nuclei are normal, outlined by a distinct membrane and containing a definite nucleolus.

III. 10 per cent sodium chlorid. — The epithelial cells of the convoluted tubules are markedly decreased in size, and are separated from the basement membrane by a distinct clear space. The nuclei are small, and the chromatin condensed into an irregular mass surrounded by a clear space within the membrane. The glomerular tufts are shrunken and are enclosed in a large, clear capsular space.

After being in the solution for sixteen hours, the sections are very gelatinous and practically no structure can be made out.

IV. Δ^2 sodium sulphate. — The epithelial cells of the convoluted tubules are apparently slightly decreased in size, and are usually separated somewhat from the basement membrane. The cytoplasm is granular and the fat globules are conspicuous. The nuclei are slightly shrunken, but there is no condensation of the chromatin. The glomeruli are apparently normal.

After sixteen hours the methylene blue in the solution was slightly decolorized.

V. Δ sodium citrate. — The epithelial cells of the convoluted tubules are slightly decreased in size, containing granular cytoplasm and distinct fat globules. The nuclei are apparently normal.

The sections became somewhat gelatinous after being in the solution for sixteen hours, and the methylene blue was almost completely decolorized.

VI. Δ sodium carbonate. — The epithelial cells of the convoluted tubules are slightly increased in size. The nuclei are very pale, but are apparently normal in size.

The methylene blue was slightly decolorized, and the sections became somewhat gelatinous after being in the solution for sixteen hours.

VII. *Caffein-sodium-benzoate*. — 1 : 5000 in 1 per cent sodium chlorid. The epithelial cells of the convoluted tubules are increased

¹ The sections were not stained for fat. Whenever the word "fat" is used, these refractive globules are referred to.

² Δ equals solution of the freezing-point of 1 per cent sodium chlorid.

in size and the cytoplasm slightly granular. The nuclei are very indistinct, but are apparently normal in size.

The methylene blue was slightly decolorized in this solution.

DESCRIPTION OF HARDENED SECTIONS.

I. 10 per cent sodium chlorid. — The epithelial cells, especially of the convoluted tubules, are markedly shrunken in size and are usually separated from the basement membrane. Many of the cells are separated from each other and lie loosely in the lumen. The cytoplasm is densely granular, and many of the cells take a homogeneous reddish tint with the eosin. Numerous cells are markedly vacuolated. The chromatin of most nuclei is grouped into an irregular, deeply staining mass which is separated from the distinct nuclear membrane by a clear space. In some places there is a distinct brush-border lining the lumen of the convoluted tubules, and in others it is represented by a hyalin-appearing narrow band. The glomeruli are considerably reduced in size, and the capsular space is either entirely empty, or filled with a finely granular material which is frequently vacuolated.

II. 2 per cent sodium chlorid. — The epithelial cells are slightly decreased in size, having cytoplasm which is fairly granular and often showing clefts. They are often separated from the basement membrane and from each other. The lumen is frequently filled with granular detritus and vesicle-appearing structures. The nuclei are apparently normal. A definite brush-border is distinctly visible in many places. The glomeruli are unaffected.

III. 1 per cent sodium chlorid. — The epithelial cells are of normal size, although many of them are finely vacuolated and are disintegrating on the lumen surface. The cytoplasm is finely granular. The nuclei and glomeruli are normal. A distinct brush-border is seen in many tubules.

IV. 0.75 per cent sodium chlorid. — Same as III.

V. 0.25 per cent sodium chlorid. — The epithelial cells are enlarged, completely obliterating the lumen in many places. The cytoplasm is very finely granular, and often contains small vacuoles. No changes can be made out in the nuclei or glomeruli. The brush-border is distinctly visible in many places.

VI. Δ sodium sulphate. — The epithelial cells are slightly increased in size, thereby diminishing the size of the lumina. In all other respects the appearance is identical to V.

VII. 0.75 per cent Δ sodium sulphate. — No noticeable changes from VI.

VIII. Δ sodium citrate. — The epithelial cells are diminished in size, and many of them are separated from the basement membrane and from each other, and lie loosely in the lumen. The cytoplasm is more granular, and here and there takes on a homogeneous reddish stain. The nuclei and glomeruli are apparently normal. The brush-border is easily made out in many places.

IX. 0.75 per cent Δ sodium citrate. — The epithelial cells are normal in size and appearance.

X. 2 per cent Δ hydrochloric acid in 1 per cent sodium chlorid (equals 0.01 per cent HCl). — The epithelial cells are decreased in size, and many are separated from the basement membrane and from each other. The cytoplasm is granular and is considerably vacuolated. The lumen is increased in size and contains granular detritus and vesicles. The brush-border can be seen in many places. The nuclei and glomeruli are apparently normal.

XI. Urea, 0.947 gm. in 50 c.c. distilled water. The epithelial cells are slightly decreased in size. They are generally adherent to the basement membrane, and many are disintegrating on the lumen edge. The cytoplasm is finely granular. The nuclei and glomeruli present no changes.

XII. Caffein-sodium-benzoate 1 : 5000 in 1 per cent sodium chlorid. The epithelial cells are somewhat increased in size. In other respects all the structures appear normal.

XIII. Δ di-sodium phosphate. — The epithelial cells are increased in size, more or less obliterating the lumen. They are, as a rule, adherent to the basement membrane. The cytoplasm is finely granular. The nuclei and glomeruli are normal.

XIV. Δ calcium chlorid. — The epithelial cells are diminished in size, usually adherent to the basement membrane, although occasionally separated from each other. The cytoplasm is very granular and vacuolated. The nuclei are somewhat smaller and take a deep stain. The glomeruli are unaffected. The brush-border is present in places.

XV. Δ ammonium chlorid. — The epithelial cells are normal in size and appearance.

XVI. Δ sodium carbonate. — The epithelial cells are increased in size, adherent to the basement membrane, and occluding the lumen of many tubules. The cytoplasm is finely granular. The nuclei are

very vesicular, and about normal in size. The brush-border is prominent. The glomeruli are normal.

XVII. Δ $MgCl_2$.—No noticeable changes in size or structure of the epithelial cells.

SUMMARY.

The above descriptions are based on a careful examination with oil immersion and camera lucida sketches of the sections. It will be noted that, with four or five exceptions, the same description is applicable to all the sections. That is the impression I want to make. Taking the sections immersed in 1 per cent and 0.75 per cent sodium chlorid, as the ones showing the least deviation from normal, then those in 10 per cent sodium chlorid and Δ sodium phosphate represent the extremes, the former showing the most marked diminution in the size of the epithelial cells, with a condensation of the chromatin material; the latter, the greatest increase in the size of the cells. Between these extremes and the normal there are all degrees of changes, and in most cases it is very difficult to decide what changes predominate in a given section. With the exception of the extremes, it is easily possible to sketch a field from any section which would answer all the essential details of the description of any other section. For instance, in all of the sections may be found cells separated from the basement membrane and from each other; cells filled with vacuoles, and others taking a homogeneous, deep red tint; nuclei very vesicular, or irregular masses of deeply staining material. Nevertheless, I think the descriptions are correct, since they were made purely objectively, without any preconceived ideas as to what should be expected.

Size of cells.—*Increased* in: Tap water, 0.25 per cent sodium chlorid, 1:5000 caffen-sodium-benzoate, Δ sodium phosphate, Δ sodium carbonate, Δ sodium sulphate.

Decreased in: 10 per cent and 2 per cent sodium chlorid, Δ sodium citrate, 2 per cent Δ hydrochloric acid, urea 0.947 gm. in 50 c.c. distilled water, and Δ calcium chlorid.

Unchanged in: 1 per cent and 0.75 per cent sodium chlorid, $\frac{3}{4}$ Δ sodium citrate, Δ ammonium chlorid, and Δ magnesium chlorid.

Cells separated from basement membrane in: 10 per cent and 2 per cent sodium chlorid, Δ sodium citrate, and 2 per cent Δ hydrochloric acid.

Cytoplasm takes a homogeneous reddish tint in: 10 per cent sodium chlorid; in all the others it varies from finely granular to coarser granular, with more or less vacuolation.

Nuclei decreased in size in 10 per cent sodium chlorid and Δ calcium chlorid. No change in size in the others.

In the lumen in all cases there are *granular detritus and vesicles*.

Glomeruli are shrunk in 10 per cent sodium chlorid. No change in others.

Brush-border present in all.

Interstitial tissue. — No apparent change.

DISCUSSION BY TORALD SOLLMANN.

As stated by Dr. Williams, these experiments were undertaken with the object of throwing some light on the changes which underlie the perfusion phenomena. In this they failed entirely. For instance, as regards the size of the cells, the histological examination agreed with the changes in weight in seven cases: Water and 0.25 per cent NaCl (increase); 10 per cent and 2 per cent NaCl, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ (no change). But in six cases the two methods led to different results; namely, with Na_2HPO_4 , Na_2SO_4 , and Na_2CO_3 (weight diminished, microscopically increase); urea (weight increased, microscopically decrease); NH_4Cl (weight increased, microscopically no change); and MgCl_2 (weight decreased, microscopically no change). Again, in several cases in which the gross appearance of pieces of kidney is manifestly altered by solutions (NH_4Cl , Na_2HPO_4 , Na_2CO_3), the microscopical appearance presented nothing abnormal. Since the changes in weight and gross appearance are perfectly definite and unmistakable, we can only conclude that the microscopical examination is not capable of furnishing a correct picture of these changes. The explanation of this failure has been pointed out by Dr. Williams; namely, the difficulty of selecting the typical picture in each section, since every cell presents a different degree of alteration.

SOLUTION TENSION AND TOXICITY IN LIPOLYSIS.

By RAYMOND H. POND.

[From the Chemical Laboratory of the New York Botanical Gardens.]

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INTRODUCTION

AN effort has been made to ascertain whether lipolysis is affected by toxic agents in a manner corresponding to that found by Mathews¹ for fertilized eggs of *Fundulus*, by McGuigan² for diastatic

¹ MATHEWS, A. P.: American journal of physiology, 1904, x, pp. 290-323.

² MCGUIGAN, H.: American journal of physiology, 1904, x, pp. 444-451.

activity, and by Caldwell¹ for proteolytic digestion. Mathews announced as a general law that the toxic action of cations as such and of anions as such is an inverse function of, and is determined by, their solution tension. The toxic action of any salt is, then, an inverse function of the decomposition tension of that salt which is the sum of the solution tension of the cation and of the solution tension of the anion of that salt, both values being regarded as having the same sign.

Solution tension is really the affinity of an ion for its charge, and may be measured and expressed in volts. Thus mercury, having a low solution tension, readily yields its charge to another substance, and hence is more potent in disturbing the organization of that substance than sodium, for example, which has a high solution tension and is consequently relatively inert toward such a substance. Disregarding for the present the exceptions observed by Mathews,² it may be noted that he based his law upon the fact that when the metals are arranged according to their solution tension they are also arranged in the order of their relative toxicity toward the eggs of *Fundulus*. The same general relation was found by McGuigan³ and Caldwell,⁴ as mentioned, and so far as I know the validity of the law has never been questioned.

It may be said that it is a matter of interpretation as to whether the experimental data gathered by any one or by all of those men warrants such a conclusion. If there were no exceptions (some were found in each research) and if the results of the three papers were in perfect agreement, would the evidence be conclusive for the law formulated? This is merely a matter of logic, and in another form the question is, When does circumstantial evidence constitute a demonstration? Since the work by those men seems inconclusive, I have attempted to remove existing doubt in the matter. In the catalytic saponification of esters we have a reaction which can be measured perhaps more accurately than any so far tested, and of course more satisfactorily than in the case of living organisms.

The original intention was to extract a lipase from the Castor Bean, and then to ascertain the effect of various salts upon the saponification of castor oil by this lipase. Comparative tests with a commercial product (Holadin) having lipolytic power revealed the fact that much

¹ CALDWELL, S. J.: *Botanical gazette*, 1905, xxxix, pp. 409-419.

² MATHEWS: *Loc. cit.*

³ MCGUIGAN: *Loc. cit.*

⁴ CALDWELL: *Loc. cit.*

sharper end points could be obtained when ethylbutyrate was saponified by this commercial product than when castor oil and lipase were used. It is much easier to obtain pure ethylbutyrate than pure castor oil. For those reasons the Holadin was used.

All of the work here presented has been done in the laboratory of the New York Botanical Garden, and I am indebted to this institution for financial support and liberal encouragement in the investigation. To Professor Wm. J. Gies I wish to make special acknowledgment for frequent and valuable counsel.

METHODS.

Holadin. — This substance is a commercial product prepared from pancreatic glands by Fairchild Brothers & Foster of New York City. The lipolytic activity of this preparation is regarded as relatively less than its proteolytic or its amylolytic power. It comes to market as a powder which is very hygroscopic and soon decomposes with a strong odor if exposed. If kept in a properly stoppered container, the lipolytic efficiency remains undiminished for a period of six months, at least, this being the limit of my experience. On forming a colloidal solution with water the reaction is at once appreciably acid if the solution is sufficiently concentrated. If 2 c.c. of the filtrate obtained by filtering a colloidal solution containing 1 gm. of the powder in 100 c.c. of water be added to 2 c.c. of water, it is found that about 0.30 c.c. of M-20 KOH is required to neutralize the total volume of 4 c.c. After four hours' incubation at 40° C. this initial acidity will be doubled. To what the initial acidity is due, or why there is an increase during incubation or digestion at room temperature, is not known. Perhaps there is some fat or other acid-yielding substance in the powder. Boiling inhibits the progress of acid formation during incubation. The initial acidity of a solution of the concentration mentioned is not changed by boiling. In stronger solutions, however, there is possibly a slight increase in acidity due to boiling, but it is too slight to be accurately measured by M-20 KOH and is therefore negligible.

On heating a filtered solution of 1 gm. of the powder in 100 c.c. of water it is noted that coagulation commences at about 60° C. Heavy flocculation soon occurs at higher temperature. The filtrate of this boiled solution contains protein substance, and gives the reaction for phosphate with molybdic solution. Salts of the heavy metals will

give precipitates upon addition to this filtrate. It is possible, however, to have a solution so dilute that there is no initial acidity, that acid formation will not occur during an incubation period of four hours, that the merest opalescence appears on boiling and yet the lipolytic activity of the unboiled solution is sufficient to produce from neutral ethylbutyrate during an incubation of four hours enough acid to require 0.20 c.c. of M-20 KOH for neutralization. Only salts of the heavy metals will precipitate anything from a solution of such concentration, though the incubation be prolonged to eighteen hours. We thus can have lipolytic activity under conditions which allow its determination with very pleasing accuracy.

An aqueous solution is prepared as follows: The weighed quantity of Holadin is triturated in a mortar with just enough water to form a paste. More water is then added, and thorough mixture is secured by merely rotary motion. If shaken, an abundant froth forms which greatly interferes with filtration. The efficiency of the solution is decreased by filtration, but accuracy requires a homogeneous colloidal condition. All samples were therefore filtered.

Solutions of toxic salts.— In order to determine the relative toxicity of the various salts with accuracy, it seemed desirable to have the same anion in as many cases as possible. The nitrates were selected. The nitrate of mercury decomposes with water to form an insoluble basic salt so that a substitution had to be made on this account. Just how this difficulty was met by Caldwell¹ is not understood as he records the use of both mercurous and mercuric nitrate. For this reason I used the chlorid of mercury instead, but with this exception all of the salts used were nitrates. Mathews² and McGuigan³ used the chlorid of mercury in comparison with the nitrate of silver. Caldwell⁴ states that "the nitrates uniformly inhibit the action of the enzyme (bromelin) in somewhat greater dilution than the corresponding sulfates and chlorides which agree closely." The chlorid of mercury is not, however, in his list, and this may still be an exception to the relation found by him. Kahlbaum's C. P. chemicals were used in all cases except that of lead, which was J. T. Baker's guaranteed purity. Weighings were made from new and original packages. Salts not carrying water of crystallization were regarded as anhydrous and weighed as such. The molecular weight of salts containing water of crystallization was calculated to include the water. No al-

¹ CALDWELL: *Loc. cit.*

² MATHEWS: *Loc. cit.*

³ MCGUIGAN: *Loc. cit.*

⁴ CALDWELL: *Loc. cit.*, p. 415.

lowance was made for deliquescence during weighing. However the weighing was done as rapidly as possible. The deliquescent salts were all obtained in the crystalline form. Error due to deliquescence is negligible in view of the concentrations used. The molecular weights were based upon the international table of atomic weights with oxygen as sixteen. All the solutions were made up molecular, so that M-32 silver nitrate is equivalent with M-64 of barium nitrate. The actual values used are as follows:

Nitrates.	Mol. weight.	Nitrates.	Mol. weight.
Ammonium	80.112	Magnesium + 6 water	256.536
Barium	261.480	Mercury (chlorid)	270.900
Cadmium + 4 water	308.544	Potassium	101.190
Cobalt + 6 water	291.176	Silver	169.970
Copper + 3 water	241.728	Sodium	85.090
Lead	330.980	Strontium	211.680
Lithium	69.070	Zinc + 6 water	297.576

The use of ethylbutyrate.— This ester is suitable because it can be used pure. The butyric acid arising from its saponification readily dissolves in water containing the alcohol which forms simultaneously with the acid. Being lighter than water and only slightly miscible, it floats upon the surface so that the saponification products diffuse downward as rapidly as formed, leaving the residual butyrate exposed to the action of the enzyme. As obtained under the label "absolute," ethylbutyrate contains saponification products and has an acid reaction. A supply of pure and hence neutral butyrate was prepared and kept as follows: On shaking with water several times in a separatory funnel the acid and the alcohol can be removed. Further shaking with dilute alkali to which some litmus has been added completes the preparation. Thus a stoppered cylinder contains two separate liquids. The lower one is blue if alkaline, and the upper one, the butyrate, is clear, and may be regarded as neutral as long as the lower liquid separates blue after thorough shaking. If the container is carefully stoppered, the spontaneous saponification is not rapid, and by shaking each evening one can have each morning a supply of neutral butyrate which may be withdrawn with a pipette. Such a neutral butyrate will not develop an acid reaction in distilled water during an incubation of four hours at 40° C.

Titration with phenolphthalein.— This indicator proved very satisfactory, and was used in every case except that of ammonium. The same

amount of indicator was used in each titration (one drop from an expansion stopper). The amount of lipolytic activity was measured by the number of c.c. of M-20 KOH required to make the preparation neutral after digestion. Suitable controls were carried in each test and will be described. The indicator was of course added at the close of the incubation period.

Sources and control of error. — The control preparations were exactly like the others, except in one particular, namely, the enzyme solution used had been boiled over the free flame. Boiling was continued only a few seconds, as the activity is practically destroyed at a lower temperature. That this treatment was adequate is shown by the fact that the controls were neutral after digestion, which means that the butyrate was beyond doubt also neutral.

The individual digestive mixtures were made up in the following manner: Six were used for each concentration of the toxic agent, of which three were controls. To each vial were first added 2 c.c. of the toxic solution of a concentration twice as great as was intended for the test. Then to the three controls were added for each individual 2 c.c. of the *boiled* enzyme solution. Then to the other three individually were added 2 c.c. of the *unboiled* enzyme solution. A total volume of 4 c.c. was thus present in each individual of every test. Then, to each individual were added 0.10 c.c. of neutral butyrate. This order of procedure was strictly followed in every test. After the incubation the difference in acidity between the controls and the others as found by titration was attributed to the activity of the enzyme. In the concentration of the enzyme used there was no initial acidity, and the boiling left the reaction neutral, so there was no error from those sources. If the butyrate had happened to be slightly acid, the same amount would have been added to each vial. The butyrate was measured accurately from a 1 c.c. pipette graduated to one-hundredths. To ascertain the full activity of the enzyme a set of six mixtures was prepared, in which distilled water replaced the toxic agent.

Optimum conditions. — Since it seemed more important to eliminate disturbing factors than to provide conditions for the maximum activity of the enzyme, all of the tests were carried out with the reaction neutral. Some of the toxic solutions have an initial acidity as, for example, zinc nitrate M-64 is so acid that 3.40 c.c. of M-20 KOH are required to bring 4 c.c. of it to neutral reaction. In such cases the natural acidity of the reagent was not neutralized. It can

hardly be assumed that the addition of acid or of alkali even in completely dissociated condition does not alter the toxicity of the reagent. Since the activity of the enzyme varies with acidity, with alkalinity, with temperature, etc., it is more important to maintain uniform conditions with disturbing factors reduced to a minimum than to establish certain optimum conditions with such factors present. The temperature of 40° C. was selected for all the experiments. The actual concentration of the enzyme was not known. The following was the procedure in the preparation of the colloidal solution of the enzyme. One tenth of a gram of the enzyme powder was triturated with a little water and diluted to 100 c.c. This was filtered from a small residue which was discarded with the filter. Of this filtrate 50 c.c. were diluted to 200 c.c. Then of this volume 2 c.c. were pipetted to each vial which already contained 2 c.c. of the toxic agent or of distilled water. If all the powder had dissolved, and if the powder had been dry at the beginning, the concentration of the enzyme ready for incubation would have been 0.0125 per cent. It must, of course, have been less than that. The concentration of the enzyme solution, when boiled, was 0.025 per cent. There was no flocculation. After the incubation there was sediment in only one case, mercury.

RELATIVE TOXICITY WITH THE CONCENTRATIONS
OF ENZYME THE SAME.

Sodium, lithium, and potassium in contemporaneous test. — The figures in the tables which follow express the quantity in c.c. of M-20 KOH required for the neutralization of the liquid in each individual vial at the close of the incubation period. If the acidity of the three individuals of a given group varied, the average was taken. If more than one was wrong, the test was repeated. As a rule only very trivial variation appeared. In the column headed "M" is given the concentration of the toxic salt, present during the incubation, in terms of fractional molecular concentration. Thus 64 in the column headed "M" means a concentration of one sixty-fourth molecular. The word "Water" in this column means that 2 c.c. of distilled water replaced the 2 c.c. of the toxic salt, so that the uninhibited activity of the enzyme is shown. The column headed "Reagent" shows the initial acidity of 4 c.c. of the toxic salt solution of the concentration indicated by the corresponding figures in the column

headed "M." The column headed "Increase" shows the difference between the acidities of the control and of the enzyme solution at the close of the incubation period, and expresses the activity of the enzyme under the conditions tested for the concentration of the corresponding toxic solution indicated in the column headed "M." If one drop of alkali produced a deep purple, the acidity was called zero.

TABLE I.

SODIUM, LITHIUM, AND POTASSIUM.

M ¹	Reagent. ¹	Control. ¹	Enzyme. ¹	Increase. ¹
Water	...	0.00	0.20	0.20
64	0.00	0.00	0.20	0.20
32	0.00	0.00	0.15	0.15
16	0.00	0.00	0.10	0.10
8	0.00	0.00	0.07	0.07
4	0.00	0.00	0.03	0.03

Enzyme, 0.0125 per cent.¹ Incubation period, 4 hours. 40° C.

This test was repeated the following day with exactly the same result, so that the relative toxicity of these three nitrates is based upon the agreement of six individuals for each concentration of the toxic salt both for the controls and for the enzyme. The salts were neutral in all concentrations tested. The controls were all neutral after incubation, so it is evident that the butyrate added to the control must have been neutral at the beginning and did not perceptibly become saponified.

A concentration of M-4 does not totally inhibit, while in M-64 there is no inhibition at all, as is shown by the figure, for the enzyme in the absence of toxic agent. Since the same figures were obtained for all three salts in a contemporaneous test, it seems clear that they are practically equal in toxicity under the conditions tried. Since M-4 allows only 0.03, it is reasonably certain, though not proved, that M-2 is the approximate point of total inhibition. All the preparations were water clear after digestion and with no sediment whatever present; the end point was very sharp.

Barium, strontium, and magnesium in contemporaneous test. — It may be noted, by referring to Table I, that since all the controls were neutral and the reagents also neutral in the concentrations indicated, the figures for the column headed "Enzyme" are identical with the

¹ See pages 263 and 264.

corresponding ones in the column headed "Increase." The same relation was found in this contemporaneous test of barium, strontium, and magnesium. To avoid repetition, the figures expressing "Increase" are alone indicated under the name of the corresponding metal.

TABLE II.
BARIUM, STRONTIUM, AND MAGNESIUM.

M.	Barium.	Strontium.	Magnesium.
64	0.15	0.10	0.10
32	0.07	0.05	0.05
16	0.03	0.03	0.05
8	0.00	0.00	0.00
Water = 0.20			

Enzyme, 0.0125 per cent. Incubation period, 4 hours. 40° C.

All the preparations were water clear after digestion and free from sediment. Practically the same figures were obtained in a subsequent test. These three salts are undoubtedly, under these conditions, practically equal in toxicity.

The figure showing the uninhibited activity of the enzyme is identical with that obtained in the test given in Table I. In comparison with sodium, lithium, and potassium these three are certainly more toxic, and upon an equimolecular basis the ratio is 1 : 4 if the limit for the former is assumed to be M-2.

Ammonium. — Since phenolphthalin cannot be used in the case of ammonium, liquid litmus was used instead, being added of course after the incubation. In other respects the conditions were exactly the same as in the preceding test. To avoid error due to change of indicator, the barium test was repeated at the same time. The litmus was used in the place of the phenolphthalin. The titration with litmus is not sharp, as is the case with phenolphthalin, but in this test it happened that the figures for barium corresponded exactly with those obtained in the preceding test, so that it is unnecessary to repeat them. This means that the result with litmus in this case is just as satisfactory as with phenolphthalin, and the figures for ammonium are strictly comparable with those for barium and the rest. Since all the controls were neutral and the reagents were also neutral in all dilutions of the test, only the figures showing the acidity developed during incubation are given.

TABLE III.

AMMONIUM.

M.	Increase.
32	0.10
16	0.05
8	0.03
4	0.00
Water	0.20

Enzyme, 0.0125 per cent. Incubation period, 4 hours. 40° C.

The limit for ammonium is evidently at 4, and allowing for the fact that it is univalent makes it equal in toxicity with barium, strontium, and magnesium. All the preparations were water clear after digestion and free from sediment. On titration a precipitate did not appear at or near the neutral point, which is also true of the salts thus far tried, but is not true of some of those to be referred to presently.

Cadmium, cobalt, and zinc in contemporaneous test. — Since in higher concentrations the solutions of these salts are acid, it seemed necessary to know the actual acidity of the plain salt solution for each dilution tried. In the table, therefore, there is the column headed "Reagent," which shows the acidity of 4 c.c. of the toxic salt corresponding to the concentration indicated in the column headed "M." It may be noted, as might be expected, that the acidity increases directly with the concentration. The acidity of the control does not vary with such precision. In this test the incubation period was lengthened, so that digestion could be going on during the night to save time. This was regarded as safe, since the salts are toxic enough to prevent any bacterial action during the incubation. But even granting some fermentation, which is very improbable, it would be equally distributed in the control and in the enzyme solution.

All the preparations were water clear and free from sediment after the incubation. On titration with alkali a precipitate appeared at the neutral point, or in some cases shortly before. The concentration at which this precipitate first appeared agreed closely with that of the plain reagent showing the first trace of acidity. The plain reagent also throws down a precipitate at or nearly the same concentration. The coincidence of these points was closer, the greater the dilution of the enzyme.

TABLE IV.

CADMIUM.				
M.	Reagent.	Control.	Enzyme.	Increase.
2048	0.00	0.00	0.20	0.20
1024	0.00	0.03	0.20	0.17
512	0.00	0.05	0.20	0.15
256	0.00	0.10	0.20	0.10
128	0.05	0.20	0.20	0.00*
64	0.10	0.30	0.30	0.00**
COBALT.				
2048	0.00	0.10	0.20	0.10
1024	0.00	0.15	0.25	0.10
512	0.00	0.20	0.25	0.05*
256	0.02	0.25	0.30	0.05**
128	0.05	0.30	0.30	0.00***
ZINC.				
2048	0.10	0.15	0.25	0.10
1024	0.20	0.30	0.40	0.10*
512	0.45	0.50	0.50	0.00**
256	0.90	0.90	0.90	0.00***

Enzyme, 0.0125 per cent. Incubation period, 18 hours. 40° C.

This test was repeated the following day with only insignificant variations, the digestion period being only twelve hours.

It is very clear that zinc is more toxic than cadmium or cobalt. The amount of saponification allowed by zinc and cobalt at 2048 and at 1024 is, however, exactly the same, while cadmium at 2048 does not at all inhibit. On this basis zinc and cobalt are equal in toxicity, and both are more toxic than cadmium. In other cases it will be seen that relative toxicity based upon the concentration of total inhibition gives a different relation from that based upon the maximum concentration not inhibiting. This fact has been disregarded in concluding as to the relative toxicity and only the points of total inhibition compared.

* Indicates the first appearance of a precipitate on titration, and the extra stars mean increasing quantity of precipitate.

Zinc, lead, and copper in contemporaneous test. —

TABLE V.

ZINC.				
M.	Reagent.	Control.	Enzyme.	Increase.
1024	0.20	0.30	0.35	0.05
512	0.45	0.45	0.45	0.00
256	0.90	0.90	0.90	0.00
128	1.70	1.70	1.70	0.00
LEAD.				
1024	0.10	0.20	0.25	0.05
512	0.20	0.30	0.30	0.00
256	0.45	0.60	0.60	0.00
128	0.90	1.10	1.10	0.00
COPPER.				
1024	0.20	0.20	0.30	0.10
512	0.40	0.40	0.40	0.00
256	0.70	0.80	0.80	0.00
128	1.40	1.50	1.50	0.00

Enzyme, 0.0125 per cent. Incubation period, 5 hours. 40° C.

In this test the three salts are certainly of equal toxicity. The acidity of the control tends to be greater than that of the plain reagent, but this will be discussed later. It will be noted that the point of total inhibition for zinc is the same as in the preceding test, in which the incubation period was eighteen hours. This change in the incubation period does not materially change the relative toxicity even of different groups. A precipitate fell in each case at or near the neutral point.

Copper and zinc in contemporaneous test. — To show how closely the figures in successive tests will agree the results of two other tests with these salts will be given.

TABLE VI.

COPPER.				
M.	Reagent.	Control.	Enzyme.	Increase.
2048	0.10	0.10	0.15	0.05
1024	0.20	0.20	0.20	0.00
512	0.40	0.40	0.40	0.00
256	0.70	0.80	0.80	0.00

TABLE VI (continued).

ZINC.				
2048	0.10	0.15	0.25	0.10
1024	0.20	0.30	0.33	0.03
512	0.45	0.45	0.45	0.00
256	0.90	0.90	0.90	0.00

Enzyme, 0.0125 per cent. Incubation period, 4 hours. 40° C.

In this case 1024 of copper totally inhibits, while the corresponding concentration of zinc allows slight acidity. The difference is too small, however, to establish a positive difference in toxicity between the two salts.

Copper and lead in contemporaneous test. — A long time test is given here to show that the relative toxicity of these two salts is not modified, although the point of total inhibition is different.

TABLE VII.

COPPER.				
M.	Reagent.	Control.	Enzyme.	Increase.
4096	0.05	0.07	0.20	0.13
2048	0.10	0.12	0.25	0.13
1024	0.20	0.20	0.30	0.10
512	0.40	0.40	0.45	0.05
256	0.70	0.75	0.78	0.03
128	1.40	1.50	1.50	0.00
LEAD.				
4096	0.00	0.10	0.25	0.15
2048	0.05	0.20	0.35	0.15
1024	0.10	0.30	0.40	0.10
512	0.20	0.40	0.45	0.05
256	0.45	0.60	0.65	0.05
128	0.90	1.20	1.20	0.00

Enzyme, 0.0125 per cent. Incubation period, 17 hours. 40° C.

The difference in acidity between the control and the plain reagent tends to be greater in the case of the lead than of the copper. This is also noticeable in other tables, so that it would seem as though the action of lead on the enzyme is such as to generate acid products, more than in the case of copper.

Mercury, copper, and lead in contemporaneous test. — This test is to show that mercury exceeds copper and lead in toxicity.

TABLE VIII.

MERCURY.				
M.	Reagent.	Control.	Enzyme.	Increase.
8192	0.00	0.05	0.08	0.03
4096	0.05	0.13	0.13	0.00
2048	0.10	0.15	0.15	0.00
1024	0.20	0.25	0.25	0.00
COPPER.				
4096	0.05	0.08	0.30	0.22
2048	0.10	0.15	0.25	0.10
1024	0.20	0.20	0.25	0.05 *
512	0.40	0.40	0.40	0.00 **
LEAD.				
4096	0.00	0.10	0.30	0.20
2048	0.05	0.10	0.30	0.20 *
1024	0.13	0.20	0.30	0.10 **
512	0.20	0.30	0.30	0.00 ***

Enzyme, 0.0125 per cent. Incubation period, 17 hours. 40° C.

In the case of mercury there was some sediment in each preparation, and apparently more in the control than in the unboiled. In the case of copper 2048 and 4096 preparations were free from sediment, but some was present in 512 and 1024, and apparently more in the boiled than in the unboiled solution. In the case of mercury a positive precipitation on titration could not be observed.

It will be noted that copper and lead yield practically the same figures as in preceding cases. Mercury is far more toxic than any of the others so far tried. In both mercury and lead the acidity of the control, as compared with that of the plain reagent, seems to be greater than in the case of copper.

Mercury and silver in contemporaneous test. — Since the acidity of the plain reagent in the case of both silver and mercury is zero for all the concentrations tried, this column is omitted from the tables.

The silver preparations were free from sediment, while every one of the mercury preparations contained a settled precipitate. It is to be noted that all the silver controls are neutral. Two of the mercury controls are acid in spite of the fact that the plain reagent is neutral.

* Indicates the first appearance of precipitate on titration, and the extra stars mean increasing quantity of precipitate.

TABLE IX.

MERCURY.			
M.	Control.	Enzyme.	Increase.
32768	0.00	0.00	0.00
16384	0.05	0.05	0.00
8192	0.07	0.07	0.00
SILVER.			
32768	0.00	0.10	0.10
16384	0.00	0.05	0.05
8192	0.00	0.03	0.03

Enzyme, 0.0125 per cent. Incubation period, 4 hours. 40° C.

Since this test did not locate the limit for either salt, another was made the following day. In this latter test a repetition of the preceding (all conditions being the same), the mercury showed total inhibition in concentrations 8192-65536 inclusive. The figures for the silver are as follows:

TABLE X.

SILVER.			
M.	Control.	Enzyme.	Increase.
32768	0.00	0.10	0.10
16384	0.00	0.05	0.05
8192	0.00	0.02	0.02
4096	0.00	0.00	0.00

This leaves the limit for mercury still undetermined while the figures for silver are almost identical with those found in the preceding test. The following experiment locates the limit for mercury and confirms antecedent figures for silver. All conditions are repeated as in the preceding test.

TABLE XI.

MERCURY.			
M.	Control.	Enzyme.	Increase.
262144	0.00	0.05	0.05
131072	0.00	0.02	0.02
65536	0.00	0.00	0.00
32768	0.00	0.00	0.00
SILVER.			
65536	0.00	0.10	0.10
32768	0.00	0.10	0.10
16384	0.00	0.05	0.05
8192	0.00	0.02	0.02
4096	0.00	0.00	0.00

Even when the fact that silver is univalent is allowed for, it is very evident that in these tests mercury is much more toxic than silver. Of course the chlorid of mercury is compared with the nitrate of silver, but this was also the case when Mathews found silver more toxic than mercury toward *Fundulus* eggs. Here is also the interesting observation that total inhibition may occur in neutral solutions at least neutral to the indicator under the conditions tried.

Zinc with ethylbutyrate absent. — That no question may arise as to the validity of my controls in the foregoing experiments, the following test is given here. The figures show that in the concentration used boiling does not change the acidity of the enzyme solution or introduce a disturbing factor. The filtered enzyme solution (0.025 per cent) was divided into two portions. One of them was boiled a moment over the free flame. There was no flocculation and the merest trace of opalescence. The initial acidity of the boiled and of the unboiled portions was zero. Preparations were made of the various concentrations of zinc nitrate, as indicated in the table, and after five hours' incubation the acidity again determined. The concentration of the enzyme was reduced one half in making the preparations as usual.

TABLE XII.

ZINC.			
M.	Reagent.	Boiled.	Unboiled.
32768	0.00	0.00	0.00
16384	0.00	0.00	0.00
8192	0.00	0.00	0.00
4096	0.05	0.05	0.05
2048	0.10	0.10	0.10
1024	0.20	0.25	0.25
512	0.45	0.45	0.45
256	0.90	0.80	0.80
128	1.70	1.40	1.40
64	3.40	3.40	3.40

Enzyme, 0.0125 per cent. Incubation period, 4 hours. 40° C.
Ethylbutyrate absent.

It is to be observed that the boiled and the unboiled solutions of the enzyme are identical in acidity in all the concentrations of the reagent. The control is therefore perfectly reliable, since, when the butyrate is added, any trace of acidity it carries is equally distributed. Also noticeable is the almost uniform agreement of the enzyme solutions

with the plain reagent, indicating that the concentration of the enzyme product is too slight to displace the point of neutralization. A comparison of the figures for the boiled solution in this test with those for the control in preceding zinc tests shows that the latter tend to run a little higher. This must be due to slight acidity of the ethylbutyrate rather than to any enzyme action of the boiled extract.

By way of recapitulation it may be said that the tests show a very marked grouping of the metals according to toxicity, and that the limits are sharp and do not overlap even when the extreme allowance for error is made. Thus it does not seem quite certain that lead and zinc are of equal toxicity; however, copper can hardly be classed by itself and certainly not as approaching equality with silver. On the equinormal basis mercury is certainly eight times more toxic than silver and probably sixteen times. Silver is sixteen times as toxic as copper, lead, or zinc. Cadmium and cobalt are only one fourth as toxic as copper, lead, or zinc, and only twice as toxic as barium, strontium, magnesium, or ammonium. This arrangement of the metals according to toxicity disagrees with that of Mathews, McGuigan, and Caldwell in several particulars, most notably in the relative toxicity of mercury and silver. All three of those men found silver to be more toxic than mercury.

If the degree of toxicity of a series of metals varies inversely with the solution tension of those metals, the relative toxicity of any pair would be theoretically and approximately, at least, the reciprocal of the relative solution tension. Thus, copper, having about one fifth the solution tension of lead, should be five times more toxic. In the following table, in the column headed "Theory," are given a few of the ratios derived from the table of solution tension as quoted by Mathews. In the column headed "Found" are given the ratios resulting from my own experiments. The figures are based upon equinormal concentration, since Mathews's table of the values for the respective solution tensions of the metals is for those metals in equinormal solutions.

	Theory.	Found.
Mercury and silver	1 : 1	8 : 1
Copper and lead	5 : 1	1 : 1
Lead and cobalt	3 : 1	4 : 1
Cadmium and zinc	3 : 1	1 : 4
Zinc and cadmium	5 : 1	64 : 1

The solution tension of mercury and silver is about equal, and hence according to Mathews's theory they should be about equal in toxicity. When the other ratios are also considered, it is evident that the disagreement between theory and experiment is greater than the agreement, and one would be reluctant to formulate a law upon such slight conformity.

In this connection it is interesting to note the general agreement given by Mathews¹ in a comparison of the actual toxicity, as found by him, and the theoretical, as computed according to his own formula for the use of solution tension as a basis of computation. Mathews's Table VI. gives the computed and found values of the minimum fatal dose in terms of a normal solution. Thus, the computed value for KCL is given as 0.948, and the found as 0.507. This is a considerable difference, and expressed upon the percentage basis means that the computed is greater than the found by 87 per cent. The following summary gives the difference between the computed and found, as calculated from Mathews's table :

Calcium chlorid	Computed is greater than found by	50 per cent.
Barium chlorid	“ “ “ “ “ “	55 “ “
Potassium chlorid	“ “ “ “ “ “	87 “ “
Zinc chlorid	“ “ “ “ “ “	266 “ “
Aluminum chlorid	Computed is less than found by	86 “ “
Magnesium chlorid	“ “ “ “ “ “	102 “ “
Manganese chlorid	“ “ “ “ “ “	133 “ “
Ferrous chlorid	“ “ “ “ “ “	531 “ “

The following agree sufficiently well: Cobalt, ferric iron, nickel, copper, mercury, sodium, and lithium. Of the fifteen here considered, four deviate more than 100 per cent, and four as much or more than 50 per cent.

Here, again, it is merely a matter of judgment as to the conclusion to be drawn. Of course, the difference between theory and experiment expressed in per cent is often large, even in ordinary reactions with inanimate matter; but I think it would be perfectly safe to say that such figures leave solution tension, as a determining factor in toxicity, in considerable doubt.

¹ MATHEWS : *Loc. cit.*

RELATIVE TOXICITY WITH THE CONCENTRATIONS
OF ENZYME DIFFERENT.

One of the conclusions reached by Galdwell is that the metals maintain their *relative* toxicity regardless of the concentration of the enzyme or of protein impurities. A relatively small increase of protein impurity was found by him to very much increase the concentration of the toxic agent necessary for inhibition. The relative toxicity of the metals remained the same, although the concentrations of inhibition were different. My own experience does not agree with this, as the following tests show. In the preceding tests I found cobalt and cadmium to be equal, while zinc was four times more toxic under the conditions tried than either. From the following tables it will be seen that when the concentration of the enzyme is increased the three metals are about equal in toxicity.

Zinc. — In this and in subsequent tests the controls were not boiled, because in the higher concentrations of the enzyme solution there is a flocculation on boiling, and in pipetting from such a boiled solution the coagulum cannot be as evenly distributed to the members of a given series as in the method used. This was simply to pipette the unboiled enzyme solution to each vial containing the toxic salt solution, and then to place the vials in cold water and bring the water to boiling. The controls, therefore, were not actually boiled, but the heat and the toxic salt combined were certainly adequate to destroy all activity of the enzyme. The tables show that this treatment insured a reliable control. The concentration of the enzyme, as recorded in the table, was 0.125 per cent. It was, however, actually less. The paste formed by the trituration of 0.5 gm. in a few drops of water was diluted to 200 c.c. and filtered. Of that filtrate 2 c.c. were used in each vial, thus reducing the concentration of the enzyme by one half. Since so much residue remained upon the filter, the actual concentration of the enzyme must have been less than the percentage figures given. The coagulum on heating shows that the concentration was at least greater than that used in the earlier tests, where coagulation did not occur:

TABLE XIII.

ZINC.				
M.	Reagent.	Control.	Enzyme.	Increase.
16384	0.00	0.15	1.10	0.95
8192	0.00	0.15	1.05	0.90

TABLE XIII (*continued*).

4096	0.05	0.15	1.00	0.85
2048	0.10	0.20	1.00	0.80
1024	0.20	0.30	1.00	0.70
512	0.45	0.50	1.00	0.50
256	0.90	0.90	1.40	0.50
128	1.70	1.80	2.10	0.30
64	3.40	3.40	3.60	0.20
32	6.70	6.70	6.70	0.00
Water	0.15	1.10	0.95

Enzyme, 0.125 per cent. Incubation period, 4 hours. 40° C.

This shows that 32 is the toxic limit under the conditions of the test. There is only slight inhibition at 8192, and none at 16384. Notable is the fact that, while the enzyme has an initial acidity of 0.15, and the control also has this value of acidity in the concentrations of the reagent having no acidity, as the acidity of the reagent increases by concentration, the acidity of the control and reagent become more nearly equal, and finally are the same. This is interesting in connection with the results of tests soon to be given here, which show that the toxicity of the metals tends toward equality with increasing concentration of the enzyme. The following table shows that there is a reaction between the toxic agent and the substance of the enzyme solution. In this test the ethylbutyrate was omitted.

TABLE XIV.

ZINC.

M.	Reagent.	Boiled.	Unboiled.
32768	0.00	0.15	0.25
16384	0.00	0.15	0.25
8192	0.00	0.15	0.25
4096	0.05	0.15	0.25
2048	0.10	0.20	0.28
1024	0.20	0.25	0.36
512	0.45	0.50	0.50
256	0.90	0.85	0.85
128	1.70	1.70	1.70
64	3.40	3.40	3.40
Water	0.15	0.30

Enzyme, 0.25 per cent. Incubation period, 4 hours. 40° C. Initial acidity of the boiled and unboiled enzyme solution with toxic agent absent and before incubation = 0.15.

Before titration, at the close of the incubation period, it was noted that in the unboiled preparations having the toxic agent present in concentration of above 1024 there was no sediment or precipitate in suspension. In 1024 there was no sediment, but there was some precipitate in suspension. With increasing concentration of the agent the quantity both of sediment and of precipitate in suspension increased perceptibly to the eye. The sediment seems to be identical with the precipitate in suspension. The final acidity of the unboiled is just double the initial acidity. The final and initial acidity of the boiled enzyme solutions is, however, identical. Boiling, therefore, stops autolysis. The enzyme solution used was the filtrate from 1 gm. of powder mixed with 200 c.c. of water. On heating this filtrate over the free flame there was slight turbidity at about 45° C., flocculation at about 70°. Boiling was continued for only a moment.

Several points of interest are to be noted here. The acidity of 4 c.c. of the reagent in concentrations 8192-32768 inclusive is 0. The acidity of the boiled enzyme containing the toxic agent in those same concentrations is 0.15, or just exactly what it is if the toxic agent is not present. In the unboiled preparations corresponding to these concentrations, the autolysis, though very slightly checked, is almost the same as though the agent were not present. Comparison with other tables shows that the very same relations are sustained here as in those cases where the ethylbutyrate is present during the incubation.

Cobalt. —

TABLE XV.

M.	Reagent.	COBALT.		
		Control.	Enzyme.	Increase.
8192	0.00	0.15	1.10	0.95
4096	0.00	0.15	1.10	0.95
2048	0.00	0.15	1.10	0.95
1024	0.00	0.20	1.10	0.90
512	0.00	0.30	1.10	0.80
256	0.02	0.50	1.30	0.80
128	0.05	1.40	1.80	0.40
64	0.10	2.60	2.90	0.30
32	0.20	3.20	3.20	0.00
Water	...	0.15	1.10	0.95

Enzyme, 0.125 per cent. Incubation period, 4 hours. 40° C.

The point of total inhibition is 32, just the same as for zinc. In the concentrations greater than 512 the end reaction on titration is

not sharp. The reagent itself, however, has a very sharp end reaction. In the higher dilutions of the plain reagent there is no precipitation, even at the neutral point. In greater concentrations of the plain reagent there is a precipitate at the neutral point. If the enzyme is present the precipitate appears long before the neutral point is reached, and as the alkali is added the precipitate takes up the purple for some time before the liquid shows alkaline. This accounts for the fact that the acidity of the control is so greatly in excess of the sum of the acidity of the enzyme and of the plain reagent. Compare with mercury in this particular:

Cadmium. —

TABLE XVI.

CADMIUM.

M.	Reagent.	Control.	Enzyme.	Increase.
8192	0.00	0.10	1.10	1.00
4096	0.00	0.10	1.00	0.90
2048	0.00	0.15	0.95	0.80
1024	0.00	0.20	1.00	0.80
512	0.00	0.25	0.95	0.70
256	0.00	0.40	0.85	0.45
128	0.05	0.70	1.20	0.50
64	0.10	1.20	1.25	0.05
32	0.20	1.60	1.60	0.00
Water	...	0.10	1.10	1.00

Enzyme, 0.125 per cent. Incubation period, 4 hours. 40° C.

The same remarks concerning the end reaction are applicable here as in the case of cobalt, although I could not see that the precipitate turned purple before the liquid, as was the case with cobalt. The point of total inhibition is at 32, which is identical with cobalt and zinc. Since these tests were not contemporaneous, and, therefore, not strictly comparable, the following test is given:

Cadmium, cobalt, and zinc in contemporaneous test. —

TABLE XVII.

CADMIUM.

M.	Control.	Enzyme.	Increase.
32	1.40	1.40	0.00
64	1.00	1.30	0.30
COBALT.			
32	3.20	3.20	0.00
64	2.50	2.80	0.30

TABLE XVII (continued).

ZINC.			
32	6.70	6.70	0.00
64	3.30	3.60	0.30

Enzyme, 0.125 per cent. Incubation period, 5 hours. 40° C.

This shows the approximately equal toxicity of these metals under these conditions. If the concentration of the enzyme is reduced, but not so much as in Table IV, it is seen that the greater toxicity of zinc reappears.

Cadmium and zinc in contemporaneous test. —

TABLE XVIII.

CADMIUM.			
M.	Control.	Enzyme.	Increase.
128	0.20	0.50	0.30
64	0.50	0.50	0.00

ZINC.			
M.	Control.	Enzyme.	Increase.
128	1.80	1.80	0.00
64	3.30	3.30	0.00

Enzyme, 0.02 per cent. Incubation period, 17 hours. 40° C.

Here, in spite of the long incubation period, the greater toxicity of zinc is apparent.

Mercury, copper, and lead show the same tendency to be of equal toxicity with increasing concentration of the enzyme.

TABLE XIX.

MERCURY (CHLORID).				
M.	Reagent.	Control.	Enzyme.	Increase.
32768	0.00	0.15	1.10	0.95
16384	0.00	0.15	0.90	0.75
8192	0.00	0.15	0.80	0.65
4096	0.05	0.15	0.60	0.45
2048	0.10	0.15	0.50	0.35
1024	0.20	0.20	0.60	0.40
512	0.35	0.30	0.60	0.30
256	0.65	0.70	0.80	0.10
128	1.30*	1.10	1.10	0.00
64	2.50*	2.20	2.20	0.00
Water	...	0.15	1.10	0.95

Enzyme, 0.25 per cent. Incubation period, 4 hours. 40° C.

* A red precipitate.

The point of total inhibition for this concentration is thus seen to be 128. Under conditions similar to these lead and copper will show exactly the same point. Notable here is the fact that the acidity of the control is less than that of the plain reagent at the concentration of the plain reagent at which a red precipitate appears upon titration. In the control and in the enzyme solution a red precipitate does not appear on titration either at 128 or at 64. The protein of the enzyme solution prevents the formation of the precipitate, and some of the natural acidity of the reagent is probably united to it.

TABLE XX.

COPPER.				
M.	Reagent.	Control.	Enzyme.	Increase.
65536	0.00	0.20	1.60	1.40
32768	0.00	0.20	1.60	1.40
16384	0.00	0.20	1.60	1.40
8192	0.00	0.20	1.60	1.40
4096	0.05	0.20	1.50	1.30
2048	0.10	0.20	1.50	1.30
1024	0.20	0.25	1.00	0.75
512	0.40	0.30	0.90	0.60
256	0.70	0.50	0.90	0.40
128	1.40	1.40	1.40	0.00
64	2.80	2.60	2.60	0.00
Water	...	0.20	1.60	1.40

Enzyme, 0.25 per cent. Incubation period, 4 hours. 40° C.

Notable is the fact that dilutions above 4096 are indifferent, but there is no trace of stimulation. As in the case of mercury, the acidity of the control is less than that of the plain reagent near the point of total inhibition.

TABLE XXI.

LEAD.				
M.	Reagent.	Control.	Enzyme.	Increase.
16384	0.00	0.15	1.40	1.25
8192	0.00	0.15	1.30	1.15
4096	0.00	0.15	1.10	0.95
2048	0.05	0.20	1.10	0.90
1024	0.10	0.20	1.00	0.80
512	0.20	0.30	1.00	0.70
256	0.45	0.50	0.90	0.40
128	0.90	1.00	1.00	0.00
64	1.80	1.80	1.80	0.00

Enzyme, 0.25 per cent. Incubation period, 4 hours. 40° C.

The same point of total inhibition is found here as in the case of mercury and copper. The relative acidity of the reagent and control is different, however. The difference in acidity between the control and the reagent steadily increases to zero at the point of total inhibition. There is something of individuality in the behavior of the toxic salts to the protein of the enzyme solution. Since the tests for mercury, lead, and copper were conducted separately, the following contemporaneous test for mercury and lead is given.

Mercury and lead in contemporaneous test. —

TABLE XXII.

M.	MERCURY.			Increase.
	Control.		Enzyme.	
256	0.80		0.90	0.10
128	1.50		1.50	0.00
64	2.60		2.60	0.00
	LEAD.			
256	0.60		0.90	0.30
128	1.20		1.20	0.00
64	2.00		2.00	0.00

Enzyme, 0.25 per cent. Incubation period, 4 hours. 40° C.

Just why the acidity of the controls in this test is so much higher than in the preceding separate tests is not known. However, both the mercury and copper controls are higher, so that perhaps the ethylbutyrate was not quite neutral. Even so, the same relative toxicity is maintained in a contemporaneous test.

DISCUSSION.

The solutions of sodium, lithium, potassium, ammonium, barium, strontium, and magnesium, are all neutral, and so are the corresponding controls for the concentrations of the enzyme tried. In those cases there is no evidence of chemical reaction between the substance of the enzyme solution and the reagent. With the other reagents tried (Cu, Pb, Cd, Co, Zn, Hg, and Ag) there is evidence of such chemical action, and it seems but natural to suppose that the cause of toxicity is very different in the one group from that in the other. The latter solutions show natural acidity, and this is more or less affected by the substance of the enzyme solution, even though the latter be too dilute to coagulate upon boiling, or have any initial acidity.

In the case of zinc the reagent and the control acidity are equal at the point of total inhibition. This same relation of control and reagent acidity is again seen with greater concentration of the enzyme for cadmium in Table XVI, cobalt in Table XV, and zinc in Table XVII. This indicates a difference in the action between zinc and the other two, and a similarity between cobalt and cadmium. Referring to Tables XIII, XX, and XXI, we see that copper, lead, and zinc are alike, in that the acidity of control is less than the sum of reagent acidity and the initial acidity of the enzyme solution, but that the control acidity is greater than that of the reagent, and that the difference decreases to zero at the point of total inhibition. That this relation holds in the case of zinc, at least in the absence of butyrate, is seen in Table XIII. In Table XIX mercury seems to be different in behavior from any of the others. At the point of total inhibition the acidity of the control is less than that of the reagent. Mercury is the only one which precipitates the enzyme during incubation in the 0.0125 per cent concentration of the enzyme. These relations acquire significance when we find that the similarities are correlated with relative toxicity, at least in the case of the highest dilution of the enzyme tried. Thus copper, lead, and zinc are equal, which is also true of cobalt and cadmium. The more acidity consumed in the union of the reagent and the enzyme substance, the greater the toxicity.

It is the intention to further investigate some of the correlations apparent in this study, but so far as ionic potential as a determining factor in toxicity is concerned, my results, in accord with those of Berg and Gies,¹ not only fail to furnish support to such a law, but show that in the lipolysis tested toxicity does not vary inversely with the solution tension. On the other hand, a reaction, in the case of electrolytes at least, in which solution tension is not a factor, is hardly imaginable, but the same is true of temperature, etc.

CONCLUSION.

The toxicity of the salts tested does not under the conditions herein specified, vary inversely with the decomposition tension of those salts.

¹ BERG, W. N., and GIES, W. J.: *Journal of biological chemistry* 1907, ii, pp. 489-546.

THE NUTRITIVE VALUE OF GELATIN. — I. SUBSTITUTION OF GELATIN FOR PROTEID, WITH MAINTENANCE OF NITROGEN EQUILIBRIUM AT THE FASTING LEVEL.

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INTRODUCTION.

METABOLISM of protein substances is an accompaniment of all vital phenomena. The interest of physiologists in the processes comprehended under this term has been greatly stimulated by the new knowledge, accruing through the researches of Emil Fischer, P. A. Levine, T. B. Osborne, and their co-workers, as to the chemical constitution of the proteins. Analysis of the nitrogenous excreta affords a means of determining the ultimate fate of such bodies occurring in the food of the organism, and already many of the intermediate transformations which the proteins undergo in the adult mammalian body have become fairly clear. In view of the differences by which gelatin is distinguished chemically from the proteids, its behavior when ingested has been regarded by many as highly significant. As these chemical differences are now capable of pretty exact statement, it becomes important to have, if possible, an exact determination of the part gelatin may play in nutrition.

The experiments reported in this paper were made nearly three years ago, but as many points immediately suggested by them remained unclear, publication was deferred with the hope of being able later to give a more satisfactory explanation of the results obtained than was at that time possible. Experiments recently carried out have, I believe, fulfilled that hope to a measurable degree, and in another paper shortly to appear they will be fully set forth.

The immediate object at the beginning of the study was to determine the minimum amount of proteid which could be fed under the combined sparing-action of gelatin, fat, and carbohydrate without

disturbance of nitrogen equilibrium; and to express this minimum in terms of the fasting metabolism of proteid in the manner adopted by E. Voit and Korkunoff,¹ for proteid alone, proteid and carbohydrate, and proteid and fat. A preliminary report on this phase of the subject was made at the meeting of the American Physiological Society in 1904.² The subject was suggested to me by Professor Graham Lusk, to whom I am indebted for much valuable counsel throughout the course of the work.

HISTORICAL.

Exact knowledge of the nutritive value of gelatin had its beginning in the researches of Carl Voit,³ published in 1872. As early as 1860 Voit and Bischoff⁴ had established experimentally the truth first perceived by Donders,⁵ that gelatin reduces the proteid requirements of the body; but they were of the opinion at this time that it could take over all the work of proteids and could replace them entirely in the diet. After Voit⁶ had shown that a part only of the nitrogenous excreta is derived from the proteids bound up in the organs, a part coming from the "circulating" proteids, he once more took up the rôle of gelatin as a substitute for proteid, with the following results: "Gelatin exercises its sparing-power on the proteids both with large and with small quantities of proteid (meat) fed at the same time, and with small quantities in much higher degree than either fat or carbohydrates. It can be shown that large quantities of gelatin spare more proteid from combustion than do small quantities; that, however, proteid is lost from the body even if with large quantities of gelatin the greatest possible amount of fat be given. A direct laying-on of gelatin, either in the glutin-yielding tissues or in the proteid-forming tissues, is not possible, and it must therefore be assumed that when gelatin is formed in the body it is at the expense of proteid. Gelatin, for this reason, is capable of replacing proteids of the food only in part."⁷

Voit made no special attempt to set the limits within which proteid

¹ E. VOIT and KORKUNOFF: *Zeitschrift für Biologie*, 1895, xxxii, p. 58.

² See Proceedings, this journal, 1905, xiii, p. xxix.

³ VOIT: *Zeitschrift für Biologie*, 1872, viii, p. 297.

⁴ VOIT u. BISCHOFF: *Die Gesetze der Ernährung des Fleischfressers*, 1860.

⁵ DONDEERS: *Die Nahrungstoffe*, 1853, p. 72.

⁶ VOIT: *Zeitschrift für Biologie*, 1869, v, p. 344.

⁷ *Ibid.*, 1872, viii, p. 330.

may be so replaced but gives for a large dog these figures: 168 gm. dry gelatin spared, 84 gm. dry flesh.¹

The next investigation bearing on the comparative value of gelatin and proteid was that of Oerum,² who placed a dog on a daily diet of meat, starch, butter, and meat extracts; then replaced all of the meat with enough gelatin to maintain the same nitrogen supply. He records a considerable increase in the nitrogen of the urine in the latter case.

Pöllitzer,³ in the course of some experiments undertaken to prove that the products of proteid digestion are to be classed with the proteids themselves, and not with the proteid-sparing foods merely, compared the effects of gelatin on the nitrogen output with those of horse-flesh and its products of gastric digestion. He concludes that peptone and hemialbumose (prepared by Kühne's methods) have a nutritive value which is in "sharp contrast with the considerable loss of nitrogen which takes place after feeding an equivalent amount of gelatin."

Ganz⁴ fed Paal's glutin-peptone and was able⁴ to cover more than half of the total nitrogen requirements therewith. Gerlach⁵ also prepared a "glutin-peptone," and found that it is a good sparing-agent, but is not of itself able to replace proteid.

I. Munk⁶ in a brief series of experiments attempted to find the "upper limit for the substitution of food proteid with gelation," and reached the conclusion that at least half as much proteid must be fed as is destroyed by the animal in fasting, if nitrogen equilibrium is to be maintained.

Kirchmann,⁷ in a very painstaking research with proteid-free gelatin, determined that the proteid destruction may be reduced under the influence of gelatin alone as much as 35 per cent, and that this maximum effect is obtained when 62 per cent of the body's energy requirement is supplied by the gelatin.

Krummacher,⁸ carrying the work begun by Kirchmann still farther,

¹ VOIT: *Zeitschrift für Biologie*, p. 347.

² OERUM: *Nordiskt medicinskt Arkiv*, 1879, xi — reviewed by HAMMARSTEN in MALY's *Jahresbericht*, 1879, p. 308.

³ PÖLLITZER: *Archiv für die gesammte Physiologie*, 1885, xxxvii, p. 301.

⁴ GANZ: Quoted by KIRCHMANN, *loc. cit.*

⁵ GERLACH: *Die Peptone von Gerlach*, Hamburg and Leipzig, 1891.

⁶ MUNK: *Archiv für die gesammte Physiologie*, 1894, lviii, p. 309.

⁷ KIRCHMANN: *Zeitschrift für Biologie*, 1900, xl, p. 54.

⁸ KRUMMACHER: *Zeitschrift für Biologie*, 1901, xlii, p. 242.

found that when the entire energy requirement of the dog was covered by gelatin the total sparing was only 37.5 per cent of the fasting nitrogen. Applied to a man whose energy requirement is 2500 Cal., Krummacker calculates that if 5 per cent of his requirement were supplied in gelatin (*i. e.*, about 33 gm. dried and purified gelatin), the proteid destruction in his body would be reduced from 70 gm. to about 56 gm., or, in other words, the 33 gm. of gelatin would replace 14 gm. of proteid.

Gregor¹ used gelatin in feeding infants in certain cases where excess of proteid was contraindicated, and concluded, by comparing his analyses for nitrogen in the excreta with those of Kellar² on a starving child of about the same age, that with a diet containing 4.8 gm. N per day (of which "nearly all" was gelatin N), not more than half as much nitrogen was lost from the body as in starvation.

Brat³ prepared a gelatose, which he identifies by Chittenden's⁴ method as a deuterogelatose, and fed it to convalescent patients as a substitute for a portion of the proteid in their diets. For example, a patient who had had *Erythema nodosum* was given for five days a preliminary diet containing 107.7 gm. proteid, 126 gm. fat, and 236 gm. carbohydrate, yielding altogether 2581 Cal., or 52.6 Cal. per kgm. He then replaced one-half of the proteid and 50 gm. of the carbohydrate with his deuterogelatose for four days, and followed this period with another five days of the all-proteid diet. The all-proteid diet contained 17.23 gm. N, and there was in the preliminary period a daily retention amounting to 2.9 gm. N, or 18.12 gm. proteid. The gelatose diet contained 24.62 gm. N, and the daily retention amounted to 10.16 gm. N, or 62.5 gm. proteid.⁵ In the subsequent proteid period, with the same nitrogen intake as before, the retention was 4.15 gm. N, or 25.93 gm. proteids.

Mancini⁶ studied the nitrogen balance of five convalescents from typhoid fever, while giving "large quantities" of gelatin. He observes a considerable retention of nitrogen, but doubts whether proteid nitrogen is replaceable by gelatin nitrogen.

Kauffmann⁷ studied the replacing power of gelatin in a diet con-

¹ GREGOR: Zentralblatt für innere Medicin, 1901, xxii, p. 65.

² KELLAR: Zeitschrift für physiologische Chemie, xxvi, p. 158.

³ BRAT: Deutsche medicinische Wochenschrift, 1902, p. 21.

⁴ CHITTENDEN: Journal of physiology, xii.

⁵ Singularly enough, the patient gained in weight exactly 62 gm. per day during this period.

⁶ MANCINI: Reale Accademie dei Fisiocritici, 1905, sed. 29, Dec.

⁷ KAUFFMANN: Archiv für die gesammte Physiologie, 1905, cix, p. 440.

taining "only as much proteid (mainly casein) as is necessary with a sufficient supply of energy for maintenance of the body's condition." He concludes from his experiments on dogs that not more than one-fifth of the proteid in such a diet can be replaced by (pure) gelatin if nitrogen equilibrium is to be maintained. With one-fourth of the proteid nitrogen so replaced a small minus balance occurs. Kauffmann's paper is concerned chiefly with the attempt to bring gelatin up to the full nutritive value of proteid by adding to it the amino-acids which it lacks, but which casein contains. With this aspect of the problem it is my purpose to deal in a later paper.

Rona and Müller,¹ in attempting to confirm Kauffmann's results with gelatin, tyrosin, and tryptophan, found first "the smallest quantity of proteid nitrogen with which the animal could well get along," and then replaced one-fifth of this proteid (casein) with gelatin nitrogen. Their observation as regards the amount which would be replaced was quite in accord with Kauffmann's, for when gelatin was substituted for two-fifths of the casein there was a distinct minus balance. One incidental observation of theirs is especially significant in this connection. "It is very remarkable," they say, "that the same animal in an experiment with two-fifths gelatin N, lasting from May 26 to June 1, exhibited a minus balance of 0.45 gm. daily, whereas now (June 22 to 27) showed a balance of only - 0.16 gm. N." I shall return to this point again after presenting my own observations in detail. It will suffice at present to state that in my experience the results depend in no small degree on the condition of the animal at the time of the experiment.

The above is, I believe, a complete résumé of the researches which have been made hitherto on the proteid-replacing power of gelatin. Their results may be summarized briefly as follows: gelatin can replace proteid only in part (Voit, Oerum, Pöllitzer); it has, however, a high proteid-sparing effect, whether fed alone (Kirchmann, Krummacher), or with other foods (Voit, Oerum, I. Munk, Kauffmann, Rona and Müller), in infant feeding (Gregor), or in convalescence (Brat, Mancini); this proteid-sparing effect is exerted also by gelatin-peptones (Ganz, Gerlach), and gelatoses (Brat).

METHOD.

The nutritive value of gelatin can be expressed in various ways. The experiment of I. Munk, in which he replaced "five-sixths of the

¹ RONA and MÜLLER: *Zeitschrift für physiologische Chemie*, 1907, 1, p. 263.

proteid of a proteid-rich diet" with gelatin, without loss of nitrogen equilibrium, has been widely quoted, yet but very little exact information is conveyed by the statement, for the reason that "proteid-rich diet" is a very vague term. The recent experiments of Kauffmann and of Rona and Müller seem to me to be open to the same objection. The "quantity of proteid necessary with a sufficient supply of energy to keep the animal in condition" is also very indefinite; for, as indicated by the experiments in this paper, that quantity must vary with the amount of carbohydrate present in the food. It may also vary with the condition of the animal at the time of the experiment. Besides, it is well known that an animal can be kept in good condition on widely different quantities of proteid.

Munk himself realized the insufficiency of such a designation as "five-sixths of the proteid of a proteid-rich diet," and therefore sought to express the nutritive value of gelatin, as we have seen (p. 287), also in terms of the fasting metabolism.¹ This use of the fasting proteid metabolism as a basis of comparison is likewise open to the objection that the quantity so determined is not absolutely constant from day to day in the same animal, much less so in different animals chosen without reference to their previous nutritive condition. But we must suppose, I think, that the metabolic capabilities of the organism bear a much more constant relationship to the absolute nutritive requirements as determined by fasting, whatever the condition of the body as regards fat, than to the requirements for any so-called "satisfactory condition"; and it is the particular metabolic capability of the organism with respect to a certain article of food, or, in other words, the use which the body can make of it as a resource from fasting, that best expresses the absolute nutritive value of the article. In a study of this kind it is of course the absolute or theoretic nutritive value with which we are concerned.

The method adopted by E. Voit and Korkunoff² of determining the "proteid minimum" in terms of the fasting proteid metabolism which must be fed alone, or with carbohydrate, or with fat in order to keep the body in nitrogen equilibrium, expresses the relative nutritive values of the different classes of foodstuffs, as regards their influence on proteid metabolism, in the clearest possible manner. Thus

¹ MUNK later gave one-fifth of the fasting requirement as the maximum amount which could be supplied by gelatin. *Realenzyklopoedia der gesammten Heilkunde*, 1897, xiii, p. 401.

² VOIT and KORKUNOFF: *Loc. cit.*

they found that 3.5 times the proteid destroyed in fasting must be fed if proteid is given alone; from 1.6 to 2.1 times, if fat is given with the proteid; and 1 to 1.2 times, if carbohydrate is given with the proteid. What would be the minimal quantity necessary if gelatin only were fed with proteid? It would not be difficult to determine; but such a proteid minimum would not possess any very great interest, because gelatin is not merely a proteid-sparing food in the sense in which that term may be applied to fat or carbohydrate. It is from the nutritive standpoint what one might call a part-proteid, and we are concerned far more to know its claims as a substitute or a rival, let us say, of proteid than as a source of energy merely.

For the reasons just indicated the following experiments were planned to test the proteid-replacing power of gelatin rather than the proteid-sparing power. It matters little whether one feed gelatin first and then replace portions of it with proteid, or proteid first and replace various fractions with gelatin. In the experiments with dogs A and B, the former was the method followed; in those with dog C and a man, proteid was fed first. The amount of nitrogen fed in all cases was gauged by the fasting nitrogen, though not always exactly equal to that quantity. The results are expressed both as a + or - balance and as the percentage of body proteid spared.

The amount of energy supplied by the non-nitrogenous foods varied in the different series of experiments, but was for the most part the same in the different feeding periods of the same series. Exceptions to this rule will be noted at the proper places.

The gelatin used was practically proteid-free, giving only a very faint turbidity with acetic acid and potassium ferrocyanide, and no colored precipitate at all with Millon's reagent. Attempts were made to improve on the purity of the gelatin as determined by these tests, by Kirchmann's¹ method, but without success. The product at the end gave just as much turbidity with acetic acid and potassium ferrocyanide, and just as deep a color in the fluid with Millon's, as before. The nitrogen impurity found by precipitating with HCl-alcohol was so small as to be deemed entirely negligible.

The gelatin was prepared for feeding by dissolving in a small quantity of warm water, then mixing thoroughly with the other constituents of the diet, or allowing it to harden if the quantity given was large. One feeding a day was given in the experiments with dogs A and B,

¹ KIRCHMANN: *Loc. cit.*, p. 58.

and the first series of those with dog C; two a day in the second series with dog C, and in the experiment on a man. Only where the quantity of gelatin was predominant in the diet was it necessary to feed the dogs by hand. Usually it was all eaten freely, and the appetite remained good to the end of the experiment.

The excreta were collected in the manner commonly followed in metabolism experiments. The daily period was always closed by catheterizing and washing the bladder thoroughly. Much of the time all the urine was collected by the catheter, but in no experiment with the dogs was it possible, owing to the polyuria induced by gelatin, to avoid altogether the necessity of using also the cage washings. Frequent tests were made for albumin, and the urine always discarded if found contaminated. The fæces of the dogs were separated into periods by means of amorphous silicic acid, those of man by powdered charcoal. All nitrogen determinations were made by the Kjeldahl method.

EXPERIMENTS.

Dog A. — A female mongrel in good physical condition, weighing at the beginning of the experiment 13.0 kgm. The fasting nitrogen was determined in a four-day period, and the following diets, containing approximately the same amounts of nitrogen, were then given in successive periods of three days each.

ALL GELATIN N (3 DAYS).

20 gm. gelatin (15.34 % N) = 3.06 gm. N and 76 Cal.	
60 gm. lard	558 "
Total	3.06 gm. N and $\frac{558}{634}$ Cal. = 48.7 Cal. per kgm.

HALF CRACKER-MEAL N, HALF CASEIN N (3 DAYS).

100 gm. cracker-meal (1.48 % N) = 1.48 gm. N and 392.4 Cal.	
10 gm. casein (13.6 % N) = 1.36 gm. N " 41.3 "	
30 gm. lard	279.0 " [per kgm.]
Total	2.80 gm. N and $\frac{279.0}{712.7}$ Cal. = 58.4 Cal.

HALF CRACKER-MEAL N, HALF GELATIN N (3 DAYS).

100 gm. cracker-meal (1.48 % N) = 1.48 gm. N and 392.4 Cal.	
10 gm. gelatin (15.34 % N) = 1.53 gm. N " 38.0 "	
30 gm. lard	279.0 " [per kgm.]
Total	3.02 gm. N and $\frac{279.0}{709.4}$ Cal. = 54.5 Cal.

The food in each case was made into a homogenous mass by dissolving the gelatin or casein in warm water and mixing with the other constituents while still fluid. One feeding only (at 8.50 A.M.) each day was given. A small quantity of water in addition to that contained in the food was allowed. A summary of the results, expressed as the mean for the second and third days of each period, is given below.

SUMMARY.

(FOR FULL DETAILS SEE TABLE I.)

Source of nitrogen.	Gm. N fed.	Gm. N excreted.	N dif.	Fasting N for the period.	Percentage of body proteid spared.
All gelatin N	3.06	5.17	- 2.11	3.00	30.5
Half cracker-meal N . . . }	2.84	3.28	- 0.44	2.50	82.5
Half casein N }					
Half cracker-meal N . . . }	3.02	3.71	- 0.69	2.00	65.0
Half gelatin N }					

The nitrogen excreted on the fasting day (fourth) just previous to the all-gelatin period amounted to 2.99 gm., and on the second fasting day following the third period, to 1.99 gm. The body requirement for nitrogen, therefore, is placed at 3.00 gm. for the first period, 2.5 gm. for the second, and 2.00 gm. for the third.

The energy requirement of the animal, calculated by Rubner's¹ method, employing v. Meeh's formula ($11.2 \sqrt[3]{(13000)^2} \times .105$), is 623 Cal.

We see that covering the nitrogen requirement of the animal with gelatin and making up the supply of energy to something over the actual requirement results in an average loss, for the second and third days, of 2.11 gm. nitrogen, *i. e.*, 69.5 per cent of the amount excreted on the fourth fasting day. The sparing effect of this diet, therefore, is 30.5 per cent. This is but slightly better than Kirchmann's results with gelatin alone, where about the same percentage of the energy requirement was furnished by the gelatin. His curve² would indicate a sparing of about 27 per cent. The sparing effect of the fat added to the gelatin in the above diet might be placed, therefore, at 3 per cent.

The nitrogen balance which was aimed at in the next period is not perfect, the second and third days showing an average loss of 0.44 gm.

¹ RUBNER: Zeitschrift für Biologie, 1885, xxi, p. 370.

² *Loc. cit.*, p. 81.

This is 17.5 per cent of the nitrogen requirement, estimated for this period at 2.5 gm. (the mean between the last two days of the two fasting periods).

Replacing the 10 gm. of casein (1.36 gm. N) with 10 gm. of gelatin (1.53 gm. N), although it supplies 0.17 gm. more nitrogen, results in a loss of 0.68 gm., *i. e.*, 0.24 gm. more than in the preceding period. Under the conditions of this experiment we must conclude, therefore, that gelatin nitrogen is not capable of replacing casein nitrogen to the extent of one-half.¹

Dog B.—Female bull-terrier in good condition, weighing at the beginning 9.8 kgm. The procedure was the same as in the former experiment, except that a fasting period was introduced after each feeding period. The foods were the same and were prepared in the same manner. Following are the diets for the successive feeding periods:

ALL GELATIN NITROGEN (3 DAYS).

13.2 gm. gelatin (15.34 % N)	= 2.02 gm. N and 40.1 Cal.	
55.0 gm. lard		511.5 " [per kgm.]
Total	2.02 gm. N and	551.6 Cal. = 62.6 Cal.

HALF CRACKER-MEAL, HALF CASEIN NITROGEN (3 DAYS).

75.0 gm. cracker-meal (1.48 % N)	= 1.11 gm. N and 294.0 Cal.	
8.5 gm. casein (13.6 % N)	= 1.15 gm. N " 35.5 "	
30.0 gm. lard		279.0 " [per kgm.]
Total	2.26 gm. N and	608.5 Cal. = 74.2 Cal.

ALL CRACKER-MEAL NITROGEN (3 DAYS).

152 gm. cracker-meal (1.48 % N)	= 2.25 gm. N and 592.8 Cal.	
30 gm. lard		294.0 " [per kgm.]
Total	2.25 gm. N and	871.8 Cal. = 114 Cal.

HALF CRACKER-MEAL, HALF GELATIN NITROGEN (3 DAYS).

75.0 gm. cracker-meal (1.48 % N)	= 1.11 gm. N and 294.0 Cal.	
7.5 gm. gelatin (15.34 % N)	= 1.15 gm. N " 28.5 "	
30.0 gm. lard		279.0 " [per kgm.]
Total	2.26 gm. N and	601.5 Cal. = 82 Cal.

¹ See later experiments for a discussion of the relation of nitrogen supply to nitrogen requirement.

SUMMARY.

MEAN OF SECOND AND THIRD DAYS (FOR FULL DETAILS SEE TABLE II.).

Source of nitrogen.	Gm. N fed.	Gm. N excreted.	N dif.	Fasting N for period.	Percentage of body proteid spared.
All gelatin N	2.02	3.69	- 1.67	2.21	24
Half cracker-meal N	2.26	2.92	- 0.66	2.31	71
Half casein N					
All cracker-meal N	2.25	2.78	- 0.53	2.05	74
Half cracker-meal N	2.26	2.91	- 0.65	1.80	60
Half gelatin N					

It will be seen that the fasting requirement for nitrogen was not quite fully met in the first two periods, but was a little more than met in the two subsequent feeding periods. The difference was not quite so great as appears, however, for the value taken for the fasting nitrogen in the first period is probably too high. Owing to the appearance of albumin in the urine in the fasting period immediately following the three days of gelatin, the urine was discarded. Whenever it has been possible, the mean between the amounts of nitrogen excreted on the last fasting day just preceding, and the amount on the second or third day following the feeding period is taken to represent the average amount which would have been excreted had the dog fasted during the feeding days, — *i. e.*, to represent the body proteid which would have been katabolized. For example, the amount, 2.31 gm., taken to represent the fasting nitrogen in the second feeding period in the summary above, is the mean between the amounts excreted on February 25, the day just preceding the feeding period (see Table II) and on March 1, the second day immediately following.¹ If the urine had not been contaminated with albumin on the fasting days immediately following the all-gelatin period, the nitrogen excretion would have been considerably lower than on the days just preceding, and the mean would therefore have been less than 2.21 gm.

The energy requirement of the dog at the beginning of the experiment, with a weight of 8.2 kgm., was $(11.2 \sqrt[3]{(8200)^2} \times .105)$, in round numbers 500 Cal. The amount available (it is presumed the fat was all digested) was 551.6 Cal., or 62.6 Cal. per kilogram.

¹ This is the method used by E. VOIT and KORKUNOFF.

The sparing of the body proteid in this period, taking the mean for the second and third days, was only 24 per cent, almost the same as Kirchmann obtained with a like amount of gelatin fed alone.

The next two feedings, with half cracker-meal nitrogen and half casein nitrogen in the second period and all cracker-meal nitrogen in the third, require little explanation. Nitrogen equilibrium was not obtained in either case, — a fact not attributable to a deficiency of energy in the food, but rather to the unavailability of the nitrogen compounds present. The period with all cracker-meal nitrogen, in fact, was introduced for the purpose of testing this question of availability. The result is not favorable to this form of nitrogen supply for dogs, for with the high amount of calorific energy supplied we should have obtained nitrogen equilibrium if the nitrogen of the cracker-meal had been as available as, for example, meat nitrogen is. The higher percentage of body proteid spared is probably due to the increased sparing effected by the carbohydrates contained in the cracker-meal.

In the next feeding period, where half of the cracker-meal was replaced by gelatin, the sparing is not so great as where half cracker-meal nitrogen and half casein nitrogen were given, notwithstanding that the energy supplied in the latter period was 82 Cal. per kilogram, as compared with 74.2 Cal. per kilogram in the former.

The result with half gelatin nitrogen is, therefore, even less favorable from the standpoint of the body proteid than in the case of dog A. The two series of experiments differ, in that with dog B fasting periods intervene between the feeding periods, thus enabling us to say more exactly what is the absolute requirement of the body in each period. We see that this requirement has gradually and pretty uniformly declined in the course of eleven days (see Table II) from 2.38 gm. to 1.87 gm. The significance of this fact will be commented on after the next series, where the same phenomenon is observed even when meat was used as the source of proteid nitrogen.

Dog C. — Female fox-terrier in good physical condition, permitted to fast two days before the excreted nitrogen was determined. Nitrogen equilibrium was first established with proteid, then various portions of the proteid nitrogen were replaced by gelatin nitrogen without intervening fasting periods.

Beef-steak, cut as free as possible from fat, was used as the source of proteid, the non-nitrogenous calories being supplied from corn-starch (.26 per cent N), nitrogen-free lard, and pure cane-sugar. The food was pre-

pared by cooking the corn-starch for at least thirty minutes over boiling water, mixing the other constituents of the diet with this while soft and then permitting the whole to "set" into a cake. It was eaten very eagerly at every feeding, of which there was one only each day.

The calorific requirement of the dog at the beginning of the experiment, with a body weight of 8.2 kgm. ($11.2 \times \sqrt[3]{8200^2} \times .105$), is again, in round numbers, 500 Cal.

Diets. — After three days with 80 gm. meat, equilibrium (see Table III) was established on the following diet :

ALL PROTEID N (1 DAY).

85 gm. beef-steak	(3.4 % N) = 2.89 gm. N and	108.9 Cal.	
55 gm. corn-starch	(0.26 % N) = 0.14 gm. N	"	184.5 "
30 gm. lard			279.0 "
15 gm. cane-sugar			58.5 " [per kgm.
Total	3.03 gm. N and	630.9 Cal.	= 71 Cal.

ONE-FOURTH GELATIN N (3 DAYS).

62.0 gm. beef-steak	(3.4 % N) = 2.10 gm. N and	82.1 Cal.	
4.7 gm. gelatin	(15.34 % N) = 0.72 gm. N	"	17.8 "
55.0 gm. corn-starch	(0.26 % N) = 0.14 gm. N	"	184.5 "
30.0 gm. lard			279.0 "
15.0 gm. cane-sugar			58.5 " [per kgm.
Total	2.96 gm. N and	621.9 Cal.	= 70 Cal.

ONE-THIRD GELATIN N (3 DAYS).

56.7 gm. beef-steak	(3.4 % N) = 1.92 gm. N and	75.2 Cal.	
6.3 gm. gelatin	(15.34 % N) = 0.97 gm. N	"	23.9 "
55.0 gm. corn-starch	(0.26 % N) = 0.14 gm. N	"	184.5 "
30.0 gm. lard			279.0 "
15.0 gm. cane-sugar			58.5 " [per kgm.
Total	3.03 gm. N and	621.1 Cal.	= 70 Cal.

ONE-HALF GELATIN N (3 DAYS).

42.5 gm. beef-steak	(3.4 % N) = 1.44 gm. N and	56.4 Cal.	
9.3 gm. gelatin	(15.34 % N) = 1.43 gm. N	"	35.3 "
55.0 gm. corn-starch	(0.26 % N) = 0.14 gm. N	"	184.5 "
30.0 gm. lard			279.0 "
15.0 gm. cane-sugar			58.5 " [per kgm.
Total	3.01 gm. N and	613.7 Cal.	= 70 Cal.

TWO-THIRDS GELATIN N (5 DAYS).

28.3 gm. beef-steak	(3.4 % N) = 0.96 gm. N	and	37.6 Cal.
12.7 gm. gelatin	(15.34 % N) = 1.94 gm. N	"	48.2 "
55.0 gm. corn-starch	(0.26 % N) = 0.14 gm. N	"	184.5 "
30.0 gm. lard			279.0 "
15.0 gm. cane-sugar			58.5 " [per kgm.]
Total	3.04 gm. N	and	607.8 Cal. = 70 Cal.

TWO-THIRDS GELATIN N IN TWO FEEDINGS (2 DAYS).

Same as above given in equal portions at 9.30 A. M. and 6.30 P. M.

SUMMARY.

MEAN OF ALL DAYS IN EACH PERIOD (FOR DETAILS SEE TABLE III).

Source of nitrogen.	No. of days.	Gm. N fed.	Gm. N excreted.	N dif.	Fasting N for period.	Per cent sparing.
All proteid N	1	3.03	3.03	0.0	2.8	100.0
One-fourth gelatin N . . .	3	2.96	2.85	+ 0.11	2.7	100.0
One-third gelatin N . . .	3	3.03	2.90	+ 0.13	2.6	100.0
One-half gelatin N . . .	5	3.01	3.02	- 0.01	2.4	99.5
Two-thirds gelatin N . . .	5	3.04	3.20	- 0.16	2.2	94.0
Two-thirds gelatin N ¹ . . .	2	3.04	3.15	- 0.11	2.0	94.5

¹ Two feedings per day.

Exact equilibrium was obtained, after three trial days, with 2.89 gm. of proteid nitrogen and 0.14 gm. in the corn-starch. Replacing one-fourth of the proteid nitrogen with gelatin nitrogen resulted in a slight daily retention; likewise with one-third gelatin nitrogen. The loss from the body was extremely small when one-half of the proteid nitrogen was replaced by gelatin nitrogen, but when two-thirds were given in this form it became significant, and was not measurably improved by two feedings per day.

It is interesting to observe (Table III) that the body weight suffered a loss of only 0.3 kgm. in the eighteen days of gelatin feeding.

The fasting requirement of nitrogen in this series, as in the former two, declined to a value at the end very much lower than it was at

the beginning. In view of the fact that the supply of energy throughout the series, as well as the supply of nitrogen, was practically the same, we are justified in assuming that this decline was a uniformly gradual one. Accordingly, the values 2.8, 2.7, 2.6, 2.4, 2.2, and 2.0 gm. have been given successively as the average nitrogen requirements for the different feeding periods. This means that if a fasting period had been introduced, say, immediately after the one-third gelatin period, the value found on the third day would have been 2.4 gm.

Whether these figures are absolutely correct or not, there is no mistaking the fact of the decline in the fasting proteid metabolism. The fact is one of some importance since, because of it, the dog in the later periods, although receiving practically the same absolute amount of nitrogen as in the first, was getting fully one-third more than he "absolutely" required to make good his actual body waste. The results (calculated as percentages of the body proteid spared) with one-half and two-thirds gelatin nitrogen, compared with those obtained with smaller fractions, are in reality, therefore, still less favorable than appears from the summary above.

In view of the fact that there must have been a considerable storage of glycogen in the three weeks and more of continuous feeding, it is possible that a fourth fasting day would have shown a somewhat higher nitrogen excretion. Since, however, the animal was in a minus nitrogen balance most of this time (the net loss for the entire twenty-three days being 1.48 gm.), it is clear that the "fasting requirement" ought to become smaller, just as it does during the intermediate stages of a prolonged fast, only more slowly.¹ If the body had been in a state of perfect nitrogen equilibrium throughout, the presumption is that the excretion would have been practically the same on the third, or at most the fourth, fasting day following, as it was on the last day preceding the feeding period.

Dog C. Series II. (Two feedings each day.)—In this series beef-heart was used as the source of proteid nitrogen instead of beef-steak. The interior portion of the thick ventricular wall near the apex was always freshly cut just before feeding, was freed as far as possible from visible connective tissue, and was entirely devoid of visible fat. Six determinations from three different hearts agreed perfectly in showing a n-content

¹ The influence of the minus balance on the rate of this decline may be seen by comparing Dog A (Table I), where the total loss in nine days is 9.47 gm., with Dog C (Table III), where the net loss in twenty-three days is 1.48 gm. The decline in fasting metabolism is about the same in the two.

in this portion of the organ of 3.07 per cent. Rosenfeld's¹ analyses of this portion of the fresh dog's heart show an average of 5 per cent fat. This figure is used in calculating the calories for the heart muscle. While Rosenfeld's analyses show a very considerable variation in the percentage of fat, I believe, from my own analyses, that the percentage of N is more uniform than it is in the beef-steaks which can be chosen from day to day in the markets. With the heart one can always choose the same part of the organ for feeding. The variation in daily excretion of nitrogen is not, however, noticeably affected.

After three trial days, immediately succeeding the fasting period, the following diet was settled upon and continued for two days :

ONE-HALF (47 %) GELATIN N (2 DAYS).

34 gm. heart	(3.07% N) = 1.04 gm. N and	42.4 Cal.	
7 gm. gelatin	(15.15% N) = 1.06 gm. N	"	26.6 "
55 gm. corn-starch	(0.26% N) = 0.14 gm. N	"	184.5 "
15 gm. cane-sugar			58.5 "
30 gm. lard			279.0 " [per kgm.
Total	2.24 gm. N and	591.0 Cal.	= 71 Cal.

TWO-THIRDS (63 %) GELATIN N (4 DAYS).

23.0 gm. heart	(3.07% N) = 0.70 gm. N and	28.1 Cal.	
9.2 gm. gelatin	(15.15% N) = 1.40 gm. N	"	34.9 "
55.0 gm. corn-starch	(0.26% N) = 0.14 gm. N	"	184.5 "
15.0 gm. cane-sugar			58.5 "
30.0 gm. lard			279.0 " [per kgm.
Total	2.24 gm. N and	585.0 Cal.	= 70 Cal.

This was followed by a fasting period of three days, the nitrogen output being found reduced to 1.8 gm. The diet was accordingly adjusted to the lower level.

HALF (46 %) GELATIN N (3 DAYS).

32.0 gm. heart	(3.07% N) = 0.98 gm. N and	39.8 Cal.	
6.2 gm. gelatin	(15.15% N) = 0.94 gm. N	"	23.5 "
55.0 gm. corn-starch	(0.26% N) = 0.14 gm. N	"	184.5 "
15.0 gm. cane-sugar			58.5 "
30.0 gm. lard			279.0 " [per kgm.
Total	2.06 gm. N and	585.3 Cal.	= 73 Cal.

¹ ROSENFELD: Archiv für experimentelle Pathologie und Pharmakologie, 1906, lv, p. 179.

In passing to two-thirds gelatin the calorific energy supplied by fat and carbohydrate was reapportioned so that fully two-thirds of the entire amount was furnished by carbohydrate and only one-seventh by fat.

TWO-THIRDS (58 %) GELATIN N (3 DAYS).

22.0 gm. heart	(3.07 % N) = 0.67 gm. N and	27.3 Cal.
8.4 gm. gelatin	(15.15 % N) = 1.27 gm. N	“ 31.9 “
100.0 gm. corn-starch	(0.26 % N) = 0.26 gm. N	“ 410.0 “
15.0 gm. cane-sugar		58.5 “
10.0 gm. lard		93.0 “ [per kgm.
Total 2.20 gm. N and		620.7 Cal. = 75 Cal.

Again a fasting period was taken, this time of four days, and a still smaller amount of nitrogen was found in the excreta. Apportioning the calories as in the last experiment, the following diet was made up :

TWO-THIRDS (58 %) GELATIN N (4 DAYS).

21.0 gm. heart	(3.07 % N) = 0.64 gm. N and	25.7 Cal.
8.2 gm. gelatin	(15.15 % N) = 1.24 gm. N	“ 31.1 “
100.0 gm. corn-starch	(0.26 % N) = 0.26 gm. N	“ 410.0 “
15.0 gm. cane-sugar		58.5 “
10.0 gm. lard		93.0 “ [per kgm.
Total 2.14 gm. N and		618.3 Cal. = 80 Cal.

SUMMARY.

(FOR DETAILS SEE TABLE VI.)

MEAN PER DAY.

Source of nitrogen. Actual per cent of total nitrogen fed.	No. of days.	Cal. per kilo.	Gm. N fed.	Gm. N excreted.	N dif.	Fasting N deter- mined.	Per cent of body proteid spared.
47 per cent gelatin N	2	71	2.24	2.07	+ 0.17	1.85	100
63 per cent gelatin N	4	70	2.24	2.31	- 0.07	1.82	96
46 per cent gelatin N	3	73	2.06	2.30	- 0.24	1.80	87
58 per cent gelatin N	3	75	2.20	2.13	+ 0.07	1.78	100
58 per cent gelatin N	4	80	2.14	1.92	+ 0.22	1.75	100

The intention was to give exactly one-half and two-thirds, respectively, of the fasting requirement for nitrogen in gelatin. Subsequent analysis of the corn-starch, however, revealed more nitrogen

than had been suspected. Consequently the actual percentage of the total nitrogen fed in gelatin was considerably less than one-half and two-thirds. The same corn-starch was used in the previous series of experiments, but so long as only 55 gm. of it were fed, its nitrogen constituted no very great percentage of the total. In the latter part of this series, where 100 gm. were given, the nitrogen of the corn-starch amounts to more than 10 per cent of the total amount fed, and must therefore be taken into account as a source of supply for the body. To what extent this nitrogen replaces the nitrogen of proteid in metabolism, I am not prepared to say. The general belief has been that the non-protein nitrogen¹ of plants has no significance in animal metabolism. It is possible, in the light of most recent investigations,² that this view is no longer tenable; but until the newer ideas of Lüthje are more firmly established we may assume that the nitrogen of the corn-starch, in so far as it is non-proteid nitrogen, has not influenced the metabolism one way or the other.

At any rate, it is clear that there is a retention of nitrogen in three out of the five feeding periods. This result is so strikingly at variance with the results usually obtained with gelatin that attention must be directed to one or two significant conditions.

First, then, with regard to the animal itself. This dog took gelatin unusually well. At no time in the two months and more during which the dog was being fed was it necessary to give the food by hand. All of the different diets were eaten voluntarily and quantitatively. The dog was of an unusually playful disposition, so that any adverse effect on the general feelings would have been easily detected. This was rarely the case, even in the two-thirds gelatin periods. The usual playful spirit was maintained, and the general bodily health was perfectly good throughout. The favorable results which were obtained in the later periods are, therefore, not to be ascribed to any abnormality in the organism. There may have been some idiosyncrasy in the matter of taste. I see no reason why idiosyncrasy might not figure in dogs as well as in men, especially considering the very diverse conditions under which laboratory animals live previous to confinement in the experiment cage. In fact, I have observed the greatest possible differences in this respect. The conditions for good digestion, therefore, were perhaps better than is ordinarily the case with experiment animals.

¹ See, for example, TIGERSTEDT'S text-book of physiology, English ed., p. 110.

² See, for example, LÜTHJE, *Archiv für die gesammte Physiologie*, 1906, cxiii, p. 547.

The chief factor, however, in obtaining the very unusual retention of nitrogen with so much gelatin in the food during the last two periods, is the coincident sparing action of carbohydrate. This is best seen by comparing the results in the fourth period with those in the third. A negative balance of 0.24 gm. per day was changed to a positive balance (a retention) of .07 gm. per day. Previously, in passing from one-half to two-thirds (approximately) gelatin nitrogen without changing the absolute amount of nitrogen in the food or the total supply of energy, a positive balance of 0.17 gm. per day was changed to a negative balance of 0.07 gm. per day. The favorable result after this transition from 46 to 58 per cent gelatin nitrogen cannot be due, therefore, to the increase in the percentage of gelatin nitrogen; nor can it be explained by the slight increase in the total nitrogen fed (occasioned by the increase in the corn-starch from 55 to 100 gm.). It must be due to the reapportionment of the non-nitrogenous calories. The advantage of high carbohydrate calories over high fat calories is obvious.¹

In the next feeding period without any increase in the absolute amount of energy there was, owing to the loss in weight of the animal during the four-day fasting period which intervened, an increase from 75 to 80 Cal. per kilogram. The retention (0.22 gm. N per day) is considerably greater.

INFLUENCE OF PROTEID CONDITION OF THE ORGANISM.

In the first two feeding periods of this series there is again a noticeable decline in the fasting metabolism. In the previous series, with twenty-three successive days of feeding, the decline amounted to 0.9 gm. (see Table III), counting from the third fasting day of the period just preceding to the third fasting day following. Here, by the same sort of reckoning, the decline is only 0.14 gm. (see Table IV) in nine days — considerably slower therefore. By inspection of the diets of the two series under comparison it will be seen that up to this point (May 30) the total energy supplied per kilogram of body weight, as well as the apportionment of the energy supply between the non-nitrogenous foodstuffs, was the same. The difference in the rate of decline of the fasting metabolism must be due therefore to the difference in the nitrogen supply or to some difference in the condition of the animal itself. The former difference is considerable, both as re-

¹ Cf. E. VOIT and KORKUNOFF, *loc. cit.*, p. 102.

gards quantity and kind, but in both respects is just the reverse of what would be required to explain the facts. Higher nitrogen ingestion, and especially higher proteid nitrogen, in the longer series, would serve, by replacing the body's store of food nitrogen more rapidly, to keep the fasting nitrogen, as determined by any given day after the feeding period, higher. We reach the conclusion that the greater proportional decrease in the fasting nitrogen from April 26 to May 21 than from May 21 to May 30 is attributable to a difference in the condition of the animal's body. What is this difference?

The rapid decline in the amount of nitrogen excreted during the first fasting period (Table III) shows that the dog had in its body at the beginning a plentiful supply of food proteid. This, however, was already pretty well depleted by the time the second series of feedings began; so that during the succeeding nine days the organism was drawing upon its own living substance to make good the deficiency of food proteid to a greater extent than before; that is, the body was in what we may call a *lower proteid condition*. The living substance not being so readily consumed as lifeless proteid, the fasting metabolism tends to become more nearly constant.¹

What influence would this lower proteid condition of the animal have on nitrogen retention? In the historical review of the subject (page 289) attention was drawn to a chance observation of Rona and Müller that the same dog in one experiment had exhibited on two-fifths gelatin N at one time a negative balance of 0.45 gm. and three weeks later a negative balance of only 0.16 gm. The authors quoted offer no explanation of this difference. I believe it is to be found in what we have just called the proteid condition of the animal. Their tables show that their dog at the beginning of the second period with two-fifths gelatin N had not yet returned to the proteid condition which he had at the beginning of the first experiment, although he had recovered his weight. Comparison of my Tables III and IV will show several such differences. For example, in the one-half gelatin period lasting from May 7 to 11 inclusive there is a net loss of 0.05 gm. N, while in the 47 per cent gelatin period lasting from May 22 to

¹ This is seen again in the next fasting period (June 9-12), where the decline, counting to the third day, is 0.18 gm. as against 0.14 in the period ending June 2. A slightly greater decline would be expected in view of the larger net loss (0.53 gm.) in the six days of feeding. A slower recovery of the fasting level might also be caused by a larger storage of glycogen, owing to the increase of carbohydrates in the diet. The fasting level is actually recovered on the fourth day of the period.

26 inclusive there is a net gain of 0.04 gm., notwithstanding that the amount of nitrogen supplied in the former case is much greater in comparison with the "requirement" for the period than in the latter. Or again compare the last two days of the two-thirds gelatin period extending from May 27 to 30, where the amount of nitrogen fed was within 0.42 gm. of the fasting requirement, with the two-day period of May 17 and 18, where the amount of nitrogen supplied was more than 1.0 gm. greater. The loss in the two cases is the same, although, the energy supply and apportionment being the same, it ought to be greater in the period first mentioned. Since both these periods came immediately before a fasting period, the only assignable difference is with reference to the proteid condition. The same could be said in explanation of the greater retention on June 15 and 16 than on June 7 and 8.¹ The conditions, other than that of the animal's body itself (the total supply and apportionment of both nitrogen and energy), are identical. I believe we are justified, therefore, in saying that the power of utilizing gelatin to reduce the waste of proteid from the body is greater, other things being equal, the more proteid the body has already lost.

EXPERIMENT ON MAN.

In order to test the results obtained in the last experiment still further, the writer carried out the following experiment on himself:

After undergoing a fast of three days a diet containing proteid nitrogen slightly in excess of the fasting requirement was eaten for three days. Nearly two-thirds of this proteid was beef-steak. Then for two days the meat was replaced by gelatin, the total nitrogen supplied, however, being 0.3 gm. more than in the previous diet, and the total energy supplied being increased, by addition of cane-sugar, from 45 Cal. per kgm. to 51 Cal. per kgm. This was followed again by the all-proteid diet, containing practically the same amount of nitrogen and potential energy as before. The daily ration was divided into two nearly equal meals eaten at 8 A. M. and 5 P. M.

Following are the daily diets:

¹ It should be observed that in this series, with two feedings each day, there is a considerable loss of nitrogen on the first day of each feeding period immediately following a fasting period. The reason is that the gelatin fed at 6.30 in the evening was probably not all available for its sparing effect before the end of the experiment day. Where this loss is very large, as on June 13, the day is neglected in making up the summary.

ALL-PROTEID NITROGEN (3 DAYS).

266 gm. beef-steak	(3.4 % N) =	9.04 gm. N and	268 Cal.
56 gm. oat-meal	(2.3 % N) =	1.28 gm. N	“ 218 “
1 egg		0.96 gm. N	“ 69 “
147 gm. "Triscuit" ¹	(1.6 % N) =	2.35 gm. N	“ 538 “
200 gm. cream	(0.3 % N) =	0.60 gm. N	“ 972 “
25 gm. butter	(0.1 % N) =	0.02 gm. N	“ 198 “
220 gm. cane-sugar			858 “
Total		14.25 gm. N and	3208 Cal. = 46 Cal. per kgm.

TWO-THIRDS (63 %) GELATIN NITROGEN (2 DAYS).

60 gm. gelatin	(15.34 % N) =	9.20 gm. N and	174 Cal.
30 gm. oat-meal	(2.3 % N) =	0.69 gm. N	“ 116 “
2 eggs		1.92 gm. N	“ 138 “
125 gm. "Triscuit"	(1.6 % N) =	2.10 gm. N	“ 462 “
200 gm. cream	(0.3 % N) =	0.60 gm. N	“ 972 “
25 gm. butter	(0.1 % N) =	0.02 gm. N	“ 198 “
400 gm. cane-sugar			1560 “ [per kgm.
Total		14.53 gm. N and	3620 Cal. = 51 Cal.

The second all-proteid diet differed slightly from the first, containing 14.26 gm. N and 3220 Cal., or 46 Cal. per kgm.

SUMMARY.

(FOR DETAILS SEE TABLE V.)

MEAN PER DAY (FASTING N, 3D DAY, 13.23 GM.).						
Source of nitrogen.	No. of days.	Total Cal. ingested.	Cal. per kgm.	Gm. N ingested.	Gm. N excreted.	N dif.
All-proteid N	3	3208	47	14.25	13.33 ¹	+ 0.87
Two-thirds (63 %) gelatin N	2	3620	51	14.53	13.82	+ 0.71
All proteid N	2	3220	46	14.26	13.52	+ 0.74

¹ Mean of second and third days only.

Neglecting the first day of the all-proteid diet, there was a gain to the body of 0.88 gm. N per day. It is perfectly fair to neglect a first day immediately following a fasting period, particularly when two

¹ A whole-wheat preparation.

meals a day are eaten, for the reason that the digestive secretions have not yet become adjusted to the diet. To count this first day would be to favor the gelatin period unduly. Inspection of Table V will show that the second gelatin day is likewise much better than the first gelatin day, but the first gelatin day is much better also than the first all-proteid day, not because gelatin is superior to proteid, but because gelatin, other things being equal, is much more easily digested and therefore more promptly burned.¹ Besides this difference in availability of the gelatin itself, the secretions on the first gelatin day had already been adjusted to a diet similar in amount and similar enough in character that the other constituents of the diet were more immediately attacked and placed at the disposal of the body.

Suspecting that the more favorable effect with Dog C, 2d series, in the last period over that just preceding might be due in part to the larger number of calories per kilogram which had come about through the loss in weight during the fasting period, the supply of energy in the gelatin period of this experiment was deliberately increased four calories per kilogram by addition of cane-sugar. The gelatin diet is of course favored by this excess of carbohydrate, but to what extent I have no means of knowing. The result, as will be seen from the summary above, is practically the same with two-thirds gelatin nitrogen as with all-proteid nitrogen. The experiment is too short to be cited alone as proving that gelatin can replace proteid to the extent of two-thirds the fasting nitrogen; but in conjunction with the experiments on Dog C it shows that with the coincident sparing effect of this quantity of gelatin and high (two-thirds of the total) carbohydrate calories it is possible for the body to reduce its own proteid metabolism, at least for a short period, to about one-third what it would be on the third day of a fasting period immediately preceding, without loss of nitrogen equilibrium. Equilibrium would unquestionably have been lost² on dropping the meat from the diet, if gelatin food had not been eaten and would not have been regained, if at all, for a great many days.

FINAL SUMMARY AND CONCLUSIONS.

1. With dogs on a diet containing one-fourth more than the fasting requirement of nitrogen, half of this in the form of cracker-meal

¹ Cf. FALTA: *Deutsches Archiv für klinische Medicin*, 1906, lxxxvi. p. 517.

² This has been shown directly in another experiment to be reported in my next paper.

and half in the form of casein, and containing considerably more than the requirement of potential energy, of which nearly one-half was supplied by fat, it was not possible to replace the casein nitrogen by gelatin nitrogen without increased loss of body proteid.

2. On a diet containing one-sixth more than the fasting requirement of nitrogen and 10 Cal. per kgm. more than the requirement of potential energy, of which nearly one-half was supplied by fat, it *was* possible to replace proteid N (beef-steak) by gelatin N to the extent of one-third without loss of body proteid. The loss with one-half gelatin N was extremely small.

3. With the same dog (as in 2) on a diet containing one-fourth more than the fasting requirement of nitrogen and 10 Cal. per kgm. more than the requirement of potential energy, of *which two-thirds were supplied by carbohydrate*, it was possible to replace proteid N by gelatin N to the extent of 58 per cent without loss of body proteid.

4. In an experiment on a man of 70 kgm. net weight, receiving a diet containing 10 per cent more than the fasting requirement of nitrogen and 51 Cal. per kgm. of potential energy, of which fully two-thirds were supplied by carbohydrates, it was possible to supply 63 per cent of the total nitrogen in the form of gelatin nitrogen for a period of two days, and still to maintain a daily retention of nitrogen amounting to 0.71 gm.

5. The sparing action of the high carbohydrate calories is an important factor in obtaining this high replacement.

6. The rate of decline in the fasting metabolism of proteid from the beginning to the end of an experiment, where there is under-nutrition as regards nitrogen, depends upon the proteid condition of the animal body at the beginning of the experiment, as well as upon the net loss of proteid during the experiment.

7. The power of the organism to utilize gelatin as a substitute for proteid in maintaining nitrogen equilibrium depends to some extent on the proteid condition of the body at the time of the experiment. The lower this proteid condition becomes the more strongly does the organism lay claim to gelatin as a means of protecting its living substance. Herein appears a biological adaptation of no small importance.

TABLE I.

DOG A.

Period: Source of nitrogen.	Date.	Weight in kgm.	Gm. N fed.	Gm. N in urine.	N in feces.	Total N excreted.	N dif.
Fasting	Nov. 12 ¹	13.0	3.09	0.10	3.19
	13	2.89	0.10	2.99
All gelatin N	14	3.06	4.81	0.22	5.03	-1.97
	15	3.06	4.96	0.22	5.18	-2.12
	16	3.06	4.93	0.22	5.15	-2.09
Half cracker-meal N, half casein N	17	2.80	3.01	0.36	3.37	-0.57
	18	2.80	2.95	0.36	3.31	-0.51
	19	2.80	2.89	0.36	3.25	-0.45
Half cracker-meal N, half gelatin N	20	3.02	3.10	0.30	3.40	-0.38
	21	3.02	3.38	0.30	3.68	-0.66
	22	3.02	3.44	0.30	3.74	-0.72
Fasting	23	11.1	1.73	0.10	1.84	Total loss in 9 days. 9.47 gm.
	24	1.89	0.10	1.99	
	25	1.91	0.10	2.01	

¹ Third fasting day.

TABLE II.

Dog B.

Period: Source of N.	Date.	Weight in kgm.	Gm. N in food.	N in urine.	N in feces.	Total N.	N dif.
Fasting	Feb. 4	9.8	1.96	0.09	2.05
	5	2.03	0.09	2.12
	6	2.21	0.09	2.30
All gelatin N and 63 Cal. per kgm. . . .	7	8.8	2.02	4.15	0.21	4.36	-2.34
	8	2.02	3.52	0.21	3.73	-1.71
	9	2.02	3.44	0.21	3.65	-1.63
Fasting	23 ¹	8.6	2.57	0.11	2.68
	24	2.30	0.11	2.41
	25	2.27	0.11	2.38
Half cracker-meal N, half casein N, and 74 Cal. per kgm. . .	26	8.2	2.26	3.40	0.14	3.54	-1.28
	27	2.26	2.73	0.14	2.87	-0.61
	28	2.26	2.82	0.14	2.96	-0.70
Fasting	29	7.9	1.86	0.04	1.90
	March 1	2.20	0.04	2.24
All cracker-meal N, and 114 Cal. per kgm.	2	7.6	2.25	2.69	0.10	2.79	-0.54
	3	2.25	2.78	0.10	2.88	-0.63
	4	2.25	2.58	0.10	2.68	-0.43
Fasting	5	7.4	1.67	0.08	1.75
	6	1.79	0.08	1.87
Half cracker-meal N, half gelatin N, and 82 Cal. per kgm. . .	7	7.3	2.26	2.74	0.11	2.85	-0.59
	8	2.26	2.90	0.11	3.01	-0.75
	9	2.26	2.70	0.11	2.81	-0.55

¹ Experiment interrupted on account of cystitis.

TABLE III.
DOG C. ONE FEEDING EACH DAY.

Period: Source of N.	Date.	Weight in kgm.	Gm. N fed.	N in urine.	N in feces.	Total N excreted.	N dif.
All-proteid N . . .	April. 24	9.9	3.32	0.12	3.44
	25	3.14	0.12	3.26
	26	2.72	0.12	2.84
	27	8.8	2.86	3.08	0.26	3.34	-0.48
	28	..	2.86	2.88	0.26	3.14	-0.28
	29	..	2.86	2.82	0.26	3.08	-0.22
One-fourth gelatin N .	30 May. 1	..	3.03	2.77	0.26	3.03	0.0
	2	8.9	2.96	2.38	0.24	2.62	+0.34
	3	..	2.96	2.70	0.24	2.94	+0.02
One-third gelatin N .	4	..	2.97	2.75	0.24	2.99	-0.02
	5	8.8	3.03	2.45	0.25	2.70	+0.33
	6	..	3.03	2.68	0.25	2.93	+0.10
One-half gelatin N . .	7	..	3.03	2.82	0.25	3.07	-0.04
	8	8.7	3.01	2.90	0.19	3.09	-0.08
	9	..	3.01	2.63	0.19	2.82	+0.19
	10	..	3.01	2.82	0.19	3.01	0.0
Two-thirds gelatin N .	11	..	3.01	2.92	0.19	3.11	-0.1
	12	..	3.01	2.88	0.19	3.07	-0.06
	13	8.7	3.04	2.84	0.18	3.02	+0.02
	14	..	3.04	2.84	0.18	3.02	+0.02
	15	..	3.04	3.13	0.18	3.31	-0.27
Two-thirds gelatin N, given in two feed- ings	16	..	3.04	3.11	0.18	3.29	-0.25
	17	..	3.04	3.21	0.18	3.39	-0.35
	18	8.6	3.04	3.21	0.18	3.39	-0.35
	19	..	3.04	2.98	0.24	3.23	-0.19
	20	8.6	1.62	0.08	1.70	Net loss in 23 days 1.48 gm.
	21	1.49	0.08	1.57	
	21	1.86	0.08	1.94	

TABLE IV.

DOG C. TWO FEEDINGS EACH DAY (9.30 A.M. AND 6.30 P.M.).

Period: Source of nitrogen.	Date.	Weight in kgm.	Gm. N fed.	N in urine.	N in feces.	Total N excreted.	N dif.
47 per cent, gelatin N (1.06 gm.).	May 21	1.86	0.08	1.94 ¹
	22	8.2	1.90	1.93	0.16	2.09	-0.19
	23	1.97	1.95	0.16	2.11	-0.14
	24	1.97	1.77	0.16	1.93	+0.04
	25	2.24	1.86	0.16	2.02	+0.20
	26	2.24	1.95	0.16	2.11	+0.13
63 per cent, gelatin N (1.40 gm.).	27	8.4	2.24	2.06	0.17	2.23	+0.01
	28	2.24 ²	0.17
	29	2.24	2.17	0.17	2.34	-0.10
	30	2.24	2.21	0.17	2.38	-0.14
Fasting.	31	8.4 ³	Net loss in 9 d. .19 gm. (?)
	June 1	1.30	0.08	1.38	
46 per cent, gelatin N (0.94 gm.).	2	1.72	0.08	1.80	-0.46
	3	8.0	2.06	2.28	0.24	2.52	-0.17
	4	2.06	1.99	0.24	2.23	-0.10
58 per cent, gelatin N (1.27 gm.).	5	2.06	1.92	0.24	2.16	-0.01
	6	8.2	2.20	1.93	0.28	2.21	+0.17
	7	2.20	1.75	0.28	2.03	+0.04
	8	2.20	1.88	0.28	2.16
Fasting.	9	8.2	1.48	0.09	1.57
	10	1.31	0.09	1.40
	11	1.53	0.09	1.62
	12	1.77	0.09	1.86
58 per cent, gelatin N (1.24 gm.).	13	7.7	2.14	2.56	0.28	2.84	-0.70 ⁴
	14	2.14	1.60	0.28	1.88	+0.26
	15	2.14	1.61	0.28	1.89	+0.25
	16	2.14	1.70	0.28	1.98	+0.16

¹ Third fast day (see Table III). ² Urine lost by accident.³ Not analyzed.⁴ Omitted in summary because gelatin not all available.

ON GLYCOSURIA AND THE ALIMENTARY EXCRETION OF CARBOHYDRATES.

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INTRODUCTION.

IN 1904 J. B. MacCallum¹ described a number of experiments on rabbits, in which he noted the very interesting fact that while these were excreting sugar in the urine they were at the same time excreting sugar into the stomach and the intestine. The experiments consisted in injecting various amounts of a one-sixth molecular sodium chloride solution into the ear veins of morphinized animals. The explanation of the phenomenon as given by MacCallum² reads: "It seems that the infusion of $\frac{m}{6}$ NaCl solution causes sugar to appear in the blood. On reaching a certain concentration it is secreted by the kidneys. If the kidneys are cut off from the circulation, the sugar is excreted by the intestine." The last sentence is later modified by MacCallum, and the phenomenon is shown to be "not entirely dependent on the absence of the kidneys" by an experiment in which he found sugar in the intestinal fluid when the kidneys were still functioning actively.

The four experiments detailed by MacCallum have more than a passing interest because of the conclusions which one is likely to draw from them. The intravenous injection of sodium chloride and other salt solutions into rabbits when properly performed is followed by a glycosuria.³ The cause of this glycosuria resides in part in the effect of this salt upon the central nervous system whereby a hyperglycæmia

¹ MACCALLUM, J. B.: University of California publications, *Physiology*, 1904, i, p. 130.

² MACCALLUM: *Loc. cit.*, p. 132.

³ See FISCHER, MARTIN H.: University of California publications, *Physiology*, 1903, i, p. 77, and 1904, i, p. 87; PFLÜGER'S *Archiv für die gesammte Physiologie*, 1904, cvi, p. 80, and 1904, cix, p. 1.

is produced, in part (according to Underhill's observations) in its effect upon the kidneys whereby their permeability to sugar is increased. If the presence of sugar in the intestine is explained by the sentences quoted above, then we ought to expect that every condition which is associated with a hyperglycæmia should show the presence of sugar in the intestinal secretions. We would in consequence expect to find sugar in the intestine of rabbits after a diabetic puncture or after the injection of sugar solutions. Yet, as the experiments about to be described show, this does not occur.

Examination of MacCallum's protocols reveals two facts: first, he never injected enough one-sixth molecular sodium chloride solution in the unit of time (400 c.c. in three hours; 180 c.c. in two and one-half hours; 470 c.c. in three hours; 497 c. c. in three hours) to guarantee the appearance of sugar in the urine; and, second, he gave his animals morphine in sufficient amounts to bring about a glycosuria through this substance alone. These considerations led us to the tentative conclusion that MacCallum's observations are to be explained by saying that he rendered his rabbits diabetic through the use of morphine (perhaps aided in part through sodium chloride injections), and that diabetic (hyperglycæmic) rabbits excrete sugar into their intestine if sodium chloride solutions are injected intravenously. This conclusion we found supported through the experiments which follow.

METHODS.

The special details bearing upon each experiment are given in the protocols. Belgian hares were used throughout. The rabbits weighed from 1000 to 1800 gm., had been fed carrots for a week or more before being used in the experiments, and were ordinarily employed directly after being removed from their hutches. Unless otherwise noted, the animals were well nourished, and were not starved before being used in the experiments. Wherever a previous period of starvation seemed necessary to reduce a possible experimental source of error, it is so noted in the protocols.

Unless so stated, the rabbits received no anæsthetic, and operations were unnecessary from the nature of the experiments. We are in danger of being criticised for not having employed isolated and washed loops of intestine, but we consider the disturbing elements that would have been introduced in this way as greater than those present in the experiments as we performed them.

To inject the salt, the sugar, or the salt and sugar solutions employed in the various experiments, the injection apparatus illustrated in Fig. 1 was used. *C* is an ordinary antitoxine syringe. *V* represents the handle of an adjustable valve. *A* and *B* are two soft rubber tubes, one of which (*A*) dips into a beaker containing the solution which is to be injected, while the other (*B*) ends in the hypodermic needle *N* which can be inserted into the blood vessel of a rabbit. By properly regulating the positions of the valve, *V*, and by raising and lowering the piston of the syringe, any liquid can be rapidly drawn into the cylinder *C* through *A*, to be ejected at any desired rate through *B*.

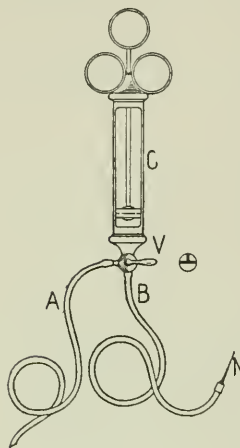


FIGURE 1.

The presence and amount of sugar in the various body fluids was determined through titration with a freshly prepared standard Fehling's solution. While this method is open to objections, it yields sufficiently accurate results to warrant the conclusions drawn from the experiments which follow.

The urine was obtained by passing a soft rubber catheter, — an easy matter in a male rabbit. To secure the gastro-intestinal contents, the rabbit was killed by a blow on the head, the abdomen was opened, and the small intestine, stomach, and large intestine were successively removed as rapidly as possible. With the help of distilled water the contents of these viscera were then washed into beakers, and, after *gentle* mixing, filtered and subjected to analysis. As in these experiments the alimentary tract always contained more or less food, care was taken to no more than *rinse* the gastro-intestinal contents, in order to avoid as largely as possible rupture of the still undigested vegetable cells and the escape of the sugar contained in some of them.

EXPERIMENTS.

For purposes of control it was necessary to see how much sugar (more accurately, reducing substances regarded as sugar) is found in the alimentary tract of a rabbit immediately after being removed from its hutch, or within a few hours thereafter.

Our analyses of the contents of the gastro-intestinal tract in seven rabbits led us to the following conclusions:

Under normal circumstances the *small intestine* of the rabbit contains no sugar, even when an animal is killed shortly after having consumed several hundred grams of carrots and carrot tops or cabbage. This statement is based upon the fact that no reduction is obtained in the intestinal wash water even after prolonged boiling with a Fehling's solution. That traces of sugar could be found with more delicate analytical methods is not disputed, but this is a question not important for the argument which follows.

No sugar is found under ordinary conditions in the contents of the *large intestine*. In one animal a trace of yellow appeared in the bottom of the test tube containing Fehling's solution when allowed to stand two hours after prolonged boiling had failed to bring down a precipitate.

The *stomach* ordinarily contains sugar. It may be absent, but ordinarily seems to be present in proportion to the amount and character of the food found in the stomach on autopsy. When carrot tops or cabbage have been consumed, very little or no sugar may be found. When carrot pulp fills the stomach, the presence of sugar is unquestionable. In one analysis we found as much as 0.348 gm.

These statements receive further support from the experiments about to be described, which are not included in the list of analyses given above.

The first two experiments are introduced here to show that small doses of morphine are sufficient to bring about an intense glycosuria

EXPERIMENT 1.

Well-nourished Belgian hare. Urine obtained by catheterization.

Time.	Urine in c.c. excreted during last interval of time.	Remarks.
4.45	None.	Animal tied down. Catheterized. 5 c.c. of a 1 % morphine hydrochlorate solution are injected under the skin of the abdominal wall.
5.00	Few drops. No sugar.
5.20	Few drops. No sugar.
5.45	1 No sugar.
5.55	Few drops. No sugar.
6.15	1 No sugar.
8.30	8	The animal, which has been very restless throughout the experiment, is liberated and returned to its hutch. 5 % sugar present.

On the following morning the urine found in the bladder contains a large trace of sugar. The bladder is washed out with distilled water. At 5.00 in the afternoon 5 c.c. of urine obtained by catheterization are found to contain no sugar.

EXPERIMENT 2.

Medium-sized, fairly well-nourished Belgian hare. Urine obtained by catheterization.

Time.	Urine in c.c. excreted during last interval of time.	Remarks.	
1.45	2	0.18 gm. morphine sulphate injected beneath skin of abdomen.	No sugar in the urine.
2.00	1	No sugar.
2.30	Few drops	Trace of sugar.
2.45-4.15	35	The animal shows marked tetanus-like spasms. A slight touch or a breath of air suffices to initiate them. The animal is killed.	Much sugar.

The total *urine* is found to contain *1.05 gm. of sugar.*

The contents of the *small intestine* are made up to 150 c.c., which are found to contain *no sugar.*

The *stomach* contents show *no sugar.*

The peritoneal fluid shows a large trace of sugar.

in rabbits. In the second experiment an analysis of the gastrointestinal contents is appended to show that rabbits rendered diabetic through morphine injections do not excrete sugar into their intestine.

We know, from such analyses as those of Seegen,¹ that the concentration of sugar in the blood is increased after the administration of morphine, and we are in consequence allowed to conclude that the appearance of sugar in the urine of such animals is dependent upon the hyperglycæmia. As we found no sugar in the intestine after the administration of morphine, we wished to see whether this was not true in every case of hyperglycæmia. It is known, since Claude Bernard's classical studies, that puncture of the floor of the fourth ventricle is followed by a hyperglycæmia and a glycosuria. We made an analysis, in consequence, of the intestinal contents of four rabbits that had been rendered glycosuric through Claude Bernard's diabetic puncture. None of these showed the presence of sugar in the small intestine. The sugar present in the stomach of one of the animals was not above the amount that could be attributed to the carrot pulp present. The results of two of these four experiments are detailed in Experiments 3 and 4. The diabetic puncture was made according to the simple method outlined by Krause.²

¹ SEEGEN, J.: Diabetes mellitus, 3te Aufl., Berlin, 1893, p. 95.

² KRAUSE, W.: Anatomie des Kaninchens, 2te Aufl., Leipzig, 1884, p. 287.

EXPERIMENT 3.

Large, well-nourished male Belgian hare. Cabbage and carrots have been fed for ten days past. Urine obtained by catheterization.

Time.	Urine in c.c. excreted during last interval of time.	Remarks.
3.35	40	Animal tied down and catheterized. No anæsthetic. Urine yellow and slightly turbid.
3.45	Diabetic puncture made. Head of animal thrown back. No sugar.
4.05	2	No sugar in urine.
4.25	$\frac{1}{2}$	The urine is allowed to accumulate. Sugar present in urine.
5.15	Animal killed by blow on the head.

The *urine* with the wash water from the bladder amounts to 30 c.c., which are found to contain 0.066 gm. *dextrose*.

The small intestine is filled with fluid. Its contents are washed into a beaker. 200 c.c. of fluid are obtained. *No sugar* is present.

EXPERIMENT 4.

Fairly well-nourished, medium-sized male Belgian hare. Food for the past ten days has consisted of carrots and cabbage. Urine obtained by catheterization.

Time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
2.30	45	Animal tied down. No anæsthetic. Urine clear, yellow.
2.40	Diabetic puncture made. Head of animal thrown back. No sugar.
3.00	3 Trace (?) of sugar in the urine.
3.20	1	The urine is allowed to accumulate. Sugar abundantly present in urine.
4.40	Animal killed by blow on the head.

The bladder is washed out with distilled water, and this wash water is added to the accumulated *urine*. The mixture amounts to 70 c.c., which on analysis is found to contain 0.203 gm. *dextrose*.

The *small intestine* is removed as rapidly as possible, and its contents are washed into a beaker. 300 c.c. of fluid are obtained. *No sugar* is present.

Fearing that in none of our experiments we had succeeded in getting a sufficiently high concentration of sugar in the blood to allow it to pass into the intestine, we resorted to intravenous injections of *dextrose*, and finally, out of experimental curiosity, to intravenous injections of cane-sugar. But even when injected in the

large amounts detailed in Experiments 5 and 6, neither of these sugars passes out of the blood into the intestine.

EXPERIMENT 5.

Medium-sized male Belgian hare. Urine obtained by catheterization. Injection fluid: one-fourth molecular dextrose solution.

Time.	Amount of solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
2.20	6	Animal tied to animal board and catheterized. No anæsthetic.
2.21	Injection of one-fourth molecular dextrose solution into lateral vein of ear begun.
2.30	1	
2.40	1
2.50	10	Trace of sugar in urine.
3.00	2	Much sugar present.
3.10	3	
3.20	124.8	8	The animal is loosed from the animal board and killed by a blow on the head.

Analysis shows the total *urine* (not counting the few drops used for qualitative tests) to contain *0.62 gm. dextrose*.

The contents of the *small intestine* are made up to 140 c.c. *No sugar* is present.

The *stomach* is filled with carrot tops and a little carrot pulp. The contents are made up to 100 c.c. *No sugar* is found. The fecal matter of the *cæcum and large intestine* are washed into a beaker. *No sugar* is found.

EXPERIMENT 6.

Medium-sized, poorly nourished Belgian hare. Urine obtained by catheterization. Injection fluid: one-fourth molecular cane sugar solution.

Time.	Amount of solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
3.55	6	Animal tied to animal board and catheterized. Injection of one-fourth molecular cane sugar solution begun.
4.00	1	Urine is getting paler. After diluting, and boiling with H_2SO_4 , neutralizing with KOH, to an alkaline reaction and boiling with Fehling's solution, a <i>trace of sugar</i> is found.
4.10	Except for the few drops needed for analysis, the urine is allowed to collect in a beaker.

EXPERIMENT 6 (*continued*).

4.25	31.2	
4.26 The bladder is washed out with distilled water and the wash water is added to the urine.

The total urine collected since 4.00 amounts to 33 c.c. A part of the urine is boiled with H_2SO_4 , and after neutralizing is titrated with Fehling's solution. Calculation shows 0.6944 gm. of "invert-sugar" to be present.

The contents of the *small intestine* are made up to 200 c.c. Direct treatment with Fehling's solution shows *no sugar*. After boiling with sulphuric acid, neutralizing and again boiling with Fehling's solution, *no reduction* is obtained.

The *gastric contents*, consisting of carrots and carrot tops, are made up to 250 c.c. Neither, when directly treated nor after preliminary boiling with H_2SO_4 , is a reduction of Fehling's solution obtained. After standing twenty-four hours a trace of red is found at the bottom of one of the tubes. The few drops of *peritoneal fluid* which can be obtained *reduce a Fehling's solution* strongly after a preliminary boiling with H_2SO_4 .

The foregoing experiments have shown that while sugar is abundantly present in the urine after administration of morphine, after a diabetic puncture or after the injection of dextrose or cane-sugar intravenously none appears in the small intestine under these circumstances.

In the experiments which follow, it will now be shown that this occurs in all these instances when, in addition to carrying out any of the procedures outlined above, a sodium chloride solution is injected intravenously.

The already quoted experiments of MacCallum may be introduced here to illustrate how sugar is excreted into the gastro-intestinal tract when a sodium chloride solution is injected into rabbits that have been rendered diabetic through administration of morphine.

Experiment 7 shows how a rabbit rendered glycosuric through a diabetic puncture will excrete sugar into the small intestine, provided it receives, for some time before and after the puncture, an intravenous injection of a one-eighth molecular sodium chloride solution. We chose this concentration of sodium chloride because it is known that by itself it will not bring about a glycosuria.¹ Sodium chloride solution above this concentration will, when injected in sufficient amounts, bring about a glycosuria. As it takes some time before the sodium chloride so affects the intestine as to allow sugar to escape through it into the lumen of the gut, we injected the sodium chloride solution intravenously for some time before making the diabetic puncture.

¹ FISCHER, MARTIN H.: University of California publications, Physiology, 1904, i, p. 95; PFLÜGER'S Archiv für die gesammte Physiologie, 1904, cvi, p. 81, and 1904, cix, p. 7.

EXPERIMENT 7.

Medium-sized, fairly well-nourished Belgian hare. Fed barley and carrots for some days past. Urine obtained by catheterization. Injection fluid: a one-eighth molecular sodium chloride solution. During the course of the injection a diabetic puncture is made.

Time.	Amount of solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
6.33	5	Animal tied down and catheterized.
6.35	Injection with one eighth molecular sodium chloride solution begun.
6.45	3
7.00	15
7.10	9
7.25	24
7.35	11
7.45	24
7.55	22
8.05	Diabetic puncture made.
8.10	No urine.	
9.10	478.4	Few drops.	The animal is killed.

Autopsy reveals a marked ascites. The *ascitic fluid* contains a large trace of *sugar*.

The contents of the *small intestine* are made up to 120 c.c. These are found to contain 0.075 gm. *sugar*.

The *stomach* contents show *no sugar*.

We have the records of two further experiments in which we *failed* to find sugar in the gastro-intestinal tract when we injected a one-eighth molecular sodium chloride solution intravenously in addition to making a diabetic puncture. We attribute our failure to the fact that the sodium chloride was not injected in sufficient amounts to affect the intestine. When sodium chloride solutions having a concentration higher than one-eighth molecular are injected intravenously, an excretion of sugar into the intestine is obtained the more readily the higher the concentration of salt. A one-fourth molecular sodium chloride solution works very well, but such a concentration can by itself bring about a glycosuria, and so cannot be used in conjunction with a diabetic puncture when one wishes to be certain that a glycosuria has been produced by the latter means.

Experiments 8 and 9 show that the addition of sodium chloride to a pure dextrose solution causes the dextrose to be eliminated not only through the kidneys but also through the alimentary tract. These

EXPERIMENT 8.

Medium-sized, poorly nourished, male Belgian hare. Urine obtained by catheterization. Injection fluids: At first a one-eighth molecular sodium chloride solution, then a mixture of equal parts of a one-half molecular dextrose solution with a one-fourth molecular sodium chloride solution (= a one-fourth molecular dextrose solution in a one-eighth molecular sodium chloride solution).

Time.	Amount of solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
3.22½	55	Rabbit tied to animal board and catheterized. No anæsthetics.
3.23	Injection of one-eighth molecular sodium chloride solution into lateral vein of ear begun.
3.50	57.2	10	<i>Injection fluid changed!!</i> Injection with a mixture of equal parts of a one-half molecular dextrose solution and a one-fourth molecular sodium chloride solution begun.
3.52½	By this time the rubber tubing (B) leading to the animal's ear has become filled with the dextrose solution and sugar is beginning to enter the animal.
3.02½	18 Large trace of sugar in the urine.
3.20	64 Much sugar in the urine.
3.25	72.8	25	The animal is freed from the holder and killed by a blow on the head.

The total remaining *urine* (100 c.c.) contains 1.5 gm. *dextrose*.

The juice in the *small intestine* is made up to 150 c.c. These contain 0.1125 gm. *dextrose*.

The contents of the *stomach*, consisting chiefly of carrot tops with a little carrot pulp, contain *no sugar*.

The contents of the *large intestine* show a *trace of sugar*.

Five c.c. of *peritoneal fluid*, representing all that could be recovered in an uncontaminated state from the peritoneal cavity, turn 3 c.c. of a standard Fehling's solution *bright red*.

experiments show at the same time that most of the alimentary elimination of the carbohydrate occurs through the small intestine. The large intestine seems to eliminate a little sugar. While we do not feel justified in drawing any definite conclusions from the analyses of the gastric contents in our animals (owing to the food always present under the conditions of our experiments) we would point out the number of times we failed to find sugar in this viscus when the small

EXPERIMENT 9.

Medium-sized Belgian hare. Urine obtained by catheterization. Injection fluids: At first a one-eighth molecular sodium chloride solution, then a mixture of equal parts of a one-half molecular dextrose solution and a one-fourth molecular sodium chloride solution (= a one-fourth molecular dextrose solution in a one-eighth molecular sodium chloride solution).

Time.	Amount of solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
3.15	5	Animal tied down and catheterized. No anæsthetic. Injection of one-eighth molecular sodium chloride solution begun. No sugar in the urine.
3.25	4 No sugar.
3.35	3 No sugar.
3.42½	28.6	<i>Injection fluid changed!!</i> Injection with a mixture of equal parts of a one-half molecular dextrose solution and a one-fourth molecular sodium chloride solution begun.
3.45	6	By this time the rubber tubing (B) leading to the animal's ear has become filled with the dextrose solution and sugar is beginning to enter the animal. No sugar.
3.55	8	Sugar is present in the urine.
4.30	49.4	The rabbit is killed by a blow on the head.

The total *urine* (72 c.c.) collected since 3.45 contains 1.08 gm. *dextrose*. The contents of the *small intestine* are made up to 220 c.c. These contain 0.0825 gm. *dextrose*.

The *stomach* is partially filled with carrots and carrot tops. The wash water contains *no sugar*.

The contents of the *large intestine* are made up to 325 c.c. A trace of *sugar* is present.

intestine showed it very clearly. We would also call attention to the presence of sugar in the peritoneal fluids of these animals. The normal peritoneal fluid of the rabbit does not reduce a Fehling's solution. In Experiment 6 sugar was found in the peritoneal fluid when cane sugar alone was injected intravenously. The presence of sodium chloride in the injection mixture does not therefore seem to be necessary in order to make the sugar escape into the peritoneal cavity, but possibly only an increased concentration of the sugar in the blood.

While Experiment 6 showed that cane sugar does not appear in the gastro-intestinal tract when injected intravenously, Experiment 10 may be taken to evidence that this happens as soon as sodium chloride is injected along with the cane sugar into the blood. The analyses

EXPERIMENT 10.

Medium-sized, poorly nourished male Belgian hare. Urine obtained by catheterization.

Injection fluids: at first a one-eighth molecular sodium chloride solution; later a mixture of equal parts of a one-fourth molecular sodium chloride and a one-half molecular cane sugar solution (one-fourth molecular cane sugar solution in a one-eighth molecular sodium chloride solution).

Time.	Amount of solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.	Remarks.
1.15	4	Animal tied down and catheterized. Injection of one-eighth molecular sodium chloride solution begun.
1.30	1	The urine is boiled with sulphuric acid, neutralized, and tested with Fehling's solution.
1.42½	57.2	Few drops	<i>Injection fluid changed!!</i> Injection with a mixture of equal parts of a one-fourth molecular sodium chloride and a one-half molecular cane sugar solution begun.
1.45	2 drops	By this time the tubing (B) leading to the animal's ear has become filled with the new solution and sugar is beginning to enter the animal.
1.50	15.6	2 drops	After treatment with sulphuric acid, etc., sugar is present in the urine.
2.00	Frequent defæcations are noted. While no fæcal masses were expelled for an hour before the animal was tied down, some fifty have accumulated since then.
2.15	52.0	The animal is killed.

The urine which has accumulated since 1.50 plus the wash water obtained from irrigation of the bladder amounts to 96 c.c. After boiling for 20 minutes with sulphuric acid, neutralizing and titrating with Fehling's solution, these are found to contain 0.5175 gm. "invert-sugar."

The wash water from the small intestine amounts to 100 c.c., which after treatment with sulphuric acid, etc., yields on analysis something over 0.4125 gm.

The stomach contents, consisting of carrot tops and some carrot pulp, are made up to 250 c.c. While immediate treatment of the filtrate with a Fehling's solution gives no reduction, 1.0375 gm. of "invert-sugar" are discovered after the filtrate has been boiled for twenty minutes with sulphuric acid.

The ascitic fluid is made up to 40 c.c. Immediate treatment with Fehling's solution gives no reaction. After being boiled with sulphuric acid 0.071 gm. of "invert-sugar" are found.

The contents of the cæcum are made up to 200 c.c. No reduction of a Fehling's solution is obtained either before or after boiling the cæcal contents with sulphuric acid.

would seem to indicate that a very large part of the cane sugar is excreted into the stomach, but we are inclined to believe that the high figure obtained is in part due to the formation of reducing substances from other bodies found in the gastric filtrate beside the cane sugar.

In conclusion we wish to point out that a pure sodium chloride solution, when of the proper concentration and when injected intravenously in sufficient amounts, can by itself lead to an excretion of sugar by both the kidneys and the intestine. In such experiments we deal with two effects of the sodium chloride solution, — first, its power of bringing about a hyperglycæmia; and, second, its power of so affecting the intestine as to allow the sugar to escape into the lumen of the gut. While a one-eighth molecular sodium chloride solution when injected in sufficient amounts and for a sufficiently long time will act upon the intestine (as shown by the above-described experiments), such a solution is by itself unable to bring about a glycosuria. Not until 75–100 c.c. of an (at least) one-sixth molecular sodium chloride solution are infused intravenously during each fifteen minutes can we be sure of getting a glycosuria in rabbits. It is not strange, therefore, that we found a pure sodium chloride solution by itself to lead to an excretion of sugar in the urine and in the intestinal fluid (in much larger quantities than have been described above) only when employed in the amounts and concentrations which experiment has shown to be necessary for the production of the glycosuria.¹

CONCLUSIONS.

1. Rabbits which are made to excrete sugar in the urine through a diabetic puncture, the hypodermic injection of morphine, or through intravenous injections of dextrose or sucrose solutions, do not at the same time excrete sugar into their gastro-intestinal tracts. Simple hyperglycæmia does not in consequence seem able of itself to bring about an elimination of sugar through the alimentary tract. When present in excessive amounts in the blood, both dextrose and sucrose pass into the peritoneal cavity.

2. Sugar is excreted by the small intestine in all the experiments enumerated when a sodium chloride solution is injected intravenously at the same time. Through the sodium chloride solutions we believe

¹ FISCHER, MARTIN H.: University of California publications, *Physiology*, 1904, i, p. 95; PFLÜGER'S *Archiv für die gesammte Physiologie*, 1904, cvi, p. 81, and 1904, cix, p. 7.

the cells of the intestinal mucous membrane to be so altered as to become permeable to a substance to which they were once impermeable. The large intestine perhaps shares with the small intestine the power of excreting sugar under the described experimental conditions. Our experiments do not permit us to say definitely whether the stomach also assumes the functions of an excreting organ for sugar when intravenous injections of sodium chloride are given or not.

The experiments described in this paper show how the small intestine, which under ordinary circumstances acts only as an organ of absorption for dextrose or cane sugar, may through experimental means be converted into an organ of excretion for these same substances. Many other salts (such as the saline cathartics and diuretics) seem to behave in a way similar to sodium chloride, and what has been said of the two sugars no doubt holds for a large number of other chemical substances. Whether means similar to those outlined in this paper may not be utilized to increase or render possible the elimination of poisonous substances from the body by way of the alimentary tract is now being studied.

RESUSCITATION OF THE RESPIRATORY AND OTHER BULBAR NERVOUS MECHANISMS, WITH SPECIAL REFERENCE TO THE QUESTION OF THEIR AUTOMATICITY.¹

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INTRODUCTION.

IN a previous paper² it has been shown that, after total loss of function of the cerebral centres resulting from temporary ligation of the head arteries, there is a gradual return of function following restoration of the circulation, and that this return occurs in a fairly definite order. We have thought it well to make a more detailed study of the time relations of the resuscitation process in the most important of the bulbar centres, namely, the respiratory, vaso-motor, and cardio-inhibitory. We made a few incidental observations on some of the other centres, *e. g.*, the swallowing centre, but do not propose to consider these in the present paper. Since all reflexes, as well as the voluntary functions, of the anæmic region are temporarily abolished, we have taken advantage of the opportunity afforded of studying the relation between the return of such functions as the respiration

¹ A preliminary report on the "Automatism of the Respiratory Centre" was read before the Society for Experimental Biology and Medicine at New York on March 20, 1907 (Proceedings, April 15, 1907).

² STEWART, GUTHRIE, BURNS, and PIKE: *Journal of experimental medicine*, 1906, viii, p. 289.

or cardiac inhibition and the opening up of the reflex pathways to or through their centres.

If we consider (in terms of the neurone hypothesis) the anatomical arrangement of the afferent pathways, the centre and the efferent pathways, it is clear that all afferent paths must have at least one synapse within the anæmic region, while this is not necessarily the case for the efferent paths. We may take the respiratory centre as an example. The afferent fibres in the vagus terminate in the bulb, and impulses passing along them to the respiratory centre must cross at least one synapse there. The efferent fibres, however, presumably the axones of perikarya in the bulb, may be assumed to run to the spinal nuclei of the principal respiratory muscles (diaphragm, intercostals, levatores costarum, and muscles of the abdominal wall) before forming synapses. On the efferent path to the chief muscles the only synapses which lie in the anæmic region will be those at the level of the phrenic nuclei. These are toward the lower edge of the anæmic region and probably suffer, therefore, only a partial deprivation of blood. In the case of the cardio-inhibitory nervous mechanism the efferent fibres coming from the nucleus ambiguus and passing downward in the vagus are still more clearly devoid of synapses within the anæmic area, while the afferent paths which can affect the cardio-inhibitory centre (including the depressor fibres) are plainly interrupted by synapses in this region. Doubtless the same general difference holds between the afferent and efferent paths of the bulbar vaso-motor centre.

Whatever view we take of the anatomical nature of the synapse, there are many facts which indicate that, when exposed to adverse influences, it proves a weak link in the nervous chain. We might suppose, then, that, at a certain stage in the occlusion and at a certain stage in the resuscitation, the afferent pathways to the bulbar centre may be interrupted while the efferent paths are pervious. We have obtained evidence that this is the case. If this evidence is good, it obviously affords a means of testing the much debated question of the automatism of these centres, and especially of the respiratory centre.

The literature of the resuscitation of the bulbar centres has been pretty fully cited in our first paper. The recent literature on the activity of the respiratory centre has been given by Boruttau,¹ Prevost

¹ BORUTTAU: *Ergebnisse der Physiologie*, 1904, p. 89.

and Stern,¹ Kuiper,² and Nikolaides.³ Kuiper more particularly deals with the automaticity of the respiratory centre. We will not, therefore, enter into any review here. Special papers will be cited in connection with the various topics upon which they bear particularly.

TECHNIQUE.

The animal having been anæsthetized with ether, the cerebral arteries are temporarily ligated, as we have previously described. A modification of this technique consisted in division of the pectoral muscles on the left side so as to lay bare the brachial plexus and the subclavian artery of that side. This artery is then isolated from the surrounding tissues, and a ligature passed about it central to the origin of the left vertebral artery. The bundle of nerves constituting the brachial plexus is doubly ligated, and the nerve trunks divided between the ligatures. The blood-pressure tracing was taken from the left carotid artery, as a rule. The central end of one vagus nerve (usually the right), or of the depressor (nearly always the right) when rabbits were used, and the central end of the left brachial plexus were stimulated by induced currents through shielded electrodes. In the cat the vagus was sometimes separated from the sympathetic. Sometimes the vago-sympathetic trunk was stimulated. Control experiments were made to eliminate the possible error due to excitation of the efferent vaso-motor fibres in the cervical sympathetic, although the effect produced by these on the general blood pressure is slight. In a certain number of experiments the central end of the sciatic was excited. Respiratory tracings were taken by a tambour connected through one limb of a T-piece with the tracheal cannula. The open limb of the T-piece was attached to a short rubber tube communicating with the air, and regulated by a screw clamp. All the nerves were stimulated before occlusion, as a rule, in order to have a basis for comparison of the effects produced by excitation at different times during the experiment. In a few observations the aorta was first ligated just distal to the origin of the left subclavian artery, and the cerebral vessels occluded later. By this means we endeavored to eliminate the possible effects of the spinal centres upon the phe-

¹ PREVOST and STERN: *Archives internationales de physiologie*, 1906, iv, p. 285.

² KUIPER: *Archiv für die gesammte Physiologie*, 1907, cxvii, p. 1.

³ NIKOLAIDES: *Archiv für Physiologie*, 1907, p. 68.

nomena studied. Artificial respiration was maintained during the period of inactivity of the respiratory centre by the apparatus described in the former paper.

The tests applied to determine whether or not the afferent pathways are open depend upon the effect produced by stimulation of an afferent nerve. If the impulses set up by electrical stimulation are reaching a particular centre at a given time, it is reasonable to suppose that some effect will be produced, comparable in some degree at least to that produced when we know that the centre is functioning normally. And in the absence of such a response on the part of the centre, it is permissible, we believe, to assume that the afferent impulses in question are not reaching the given centre. Evidence in favor of this assumption is quoted later on.

In the present series observations on thirty-five cats and rabbits are included.

THE EXPERIMENTAL RESULTS AND CONCLUSIONS.

(1) **The respiratory nervous mechanism.** (a) *The respiratory mechanism during occlusion.* — The march of events as regards the respiratory mechanism has already been dealt with in some detail in our first paper. In good experiments in which occlusion was complete according to our tests, the respiration ceased in from ten seconds to two minutes after the ligatures on the head arteries were tightened. As a rule, there occurred a pause of thirty seconds to three minutes during which no respiratory movements of any kind were seen. Also, as a rule, there occurred a terminal or secondary series of strong gasps lasting until two to six or eight minutes after the beginning of occlusion. After the cessation of these secondary gasps respiratory movements were entirely absent until the recovery of the centre following restoration of the circulation to the head.

Stimulation of the brachial or vago-sympathetic nerve at an early stage in the occlusion produces, in general, a greater effect on respiration than was produced before occlusion with the same strength of stimulation. As a rule, the effect at this period is qualitatively the same as before occlusion, whether that effect be inhibitory or augmentor. But some exceptions occur. As an instance, in one experiment stimulation of the brachial nerve before occlusion caused a marked increase in rate and depth of respiration, while thirty seconds after occlusion, it caused inhibition of respiration in the expiratory phase.

Later on in the occlusion, stimulation of either the vagus or the brachial nerves is totally ineffective, and this at a time when the secondary gasps are still going on, or even in the pause which succeeds the first disappearance of respiration. The length of time, varying with the individual animals and the completeness of the occlusion, during which some effect can be produced by stimulation of these nerves, does not generally exceed one minute.

The exaggerated effect of stimulation of afferent nerves in the first part of the occlusion may be due either to increased excitability of the respiratory centre under the influence of anæmia, or, what is perhaps a condition not to be physiologically separated from increase of excitability, increased conductivity across the synapses of the afferent path permitting the impulses to pass to the perikarya of the efferent path more easily and in greater strength. An increase of conductivity at the synapses might perhaps be produced by some such change of the membrane (if a membrane exists here) as is produced by asphyxia in the envelope of red blood corpuscles, leading to an increase in their permeability to water.

An increase in the blood supply of the respiratory centre, such as occurs during the period of high blood pressure following ligation of the aorta distal to the left subclavian, usually diminishes the effect on the respiration produced by stimulation of the brachial or vagus, as compared with the normal effect. Here the respiratory centre may be less excitable or the afferent paths less permeable to the excitation than normal.

(b) *The respiratory mechanism during resuscitation.* — It has been stated in our previous paper that the respiration returned in from thirty seconds to eighty-one minutes after release of the head arteries, when the occlusion had lasted five to sixty-one minutes. In the present series, in which the occlusions were all relatively short, the time of return of respiration is shown in the following table (Table I), as well as the time at which stimulation of the afferent nerves first proved effective.

The manner of the return of respiration and its initial rhythm after restoration of the circulation has been treated in considerable detail in the former paper. We pointed out there that the respiratory movements continued for a certain period during cerebral anæmia, apparently independently of the state of oxygenation of the blood in the rest of the systemic circulation, or of the integrity of the vagi. We have since observed that if at the moment when respira-

TABLE I.

Showing the length of the occlusion period, the time after release of the return of the respiration, and the time at which stimulation of the afferent nerves first produces an effect on respiration, blood pressure, and heart rate.

Date of exp.	Period of occlusion.	Time of first respiratory gasp.	Time after release, when afferent nerves become effective on			Blood pressure at time when nerves first affect it.
			Respiration.	Blood pressure.	Heart rate	
	min. sec.	min. sec.	min. sec.	min. sec.	min. sec.	mm. of Hg.
Feb. 20	12 00	6 00	7 00	7 00		52
" 22	17 00	8 20	17 50			
" 23	(1) 8 00	6 00	15 00			
" "	(2) 7 00	3 30	12 00			
" "	(3) 6 00	7 00	18 00			
" 25 (2)	12 30	14 00	27 00			
" 26 (2)	10 00	7 30	20 00			
" 27	18 30	13 00	22 00	5 00	26 30	78
March 5 (3)	(1) 4 45	1 15	2 30		24 00	
" "	(2) 6 00	4 00	8 30			
" 7	(1) 7 15	1 45	7 45	7 45	30 45	140
" "	(2) 8 30	24 15	42 45	18 30	42 45	104
" 13	(1) 7 00	3 45	7 30	6 40	80
" "	(2) 7 00	10 00	6 00	64
" "	(3) 7 00 ²	8 00	77 00	40
" 18	7 00	3 30	15 30	5 00	26 30	144
" 19	(1) 15 15	5 15	5 35	120
" "	(2) 10 00 ²	9 45	60
" 20	15 00	11 00	37 00	90
" 29 (1)	7 45	1 00	5 00	
" 29 (2)	9 00 ²	2 00		
April 2	(1) 5 00	2 00	0 20	87
" "	(2) 6 00	4 00	4 45	50
" 15	(1) 6 45	1 30	1 30	12 00	46
" "	(2) 15 00	5 30	3 00	68
" 20	(1) 19 30 ¹	1 00	1 15	30
" "	(2) 5 55	9 15	7 45	26 00	38
" "	(3) 5 00	9 00	21 30	30
" 22	13 00	6 13	7 30		
" 23	36 00 ¹ ²	6 30		
" 24	8 20	7 40	2 05		60
" 30	(1) 16 10	5 35	120
" "	(2) 11 00	11 20	7 05	24 00	

Numbers in parentheses in the date column indicate the number of the experiment done on that day; in the occlusion column they indicate the number of the occlusion in that experiment.

¹ Imperfect occlusion.

² No return of function.

tion has returned, or during a period after its return, which varies with the time of occlusion, the vagi are divided, the respiratory movements go on without the least change, and the elimination of all afferent paths posterior to the first or second thoracic segments by ligation of the aorta just distal to the origin of the left subclavian artery does not alter the time of return of the respiration, or the strength of the initial contractions of such respiratory muscles as remain supplied with blood. The same is true when the brain is divided above the level of the respiratory centre just posterior to or through the posterior corpora quadrigemina.

A period in resuscitation can readily be found during which the respiratory centre is discharging rhythmically and effectively, while, to all tests that may be applied, the afferent paths to it are still interrupted. For example, during this period stimulation of the central end of the vagus or of the brachial plexus produces no effect upon respiration, although a marked effect has been obtained before occlusion and may be obtained later in the resuscitation period. Again, as has been mentioned, section of both vagi or of the higher paths at this time does not affect respiration in any way, which proves that normal impulses from the lungs or from the upper parts of the brain are not reaching the centre. Asphyxia produces the usual increase in respiratory movements, showing that the centre is capable of being affected directly, and of responding by increased discharge along the afferent paths.

(c) *Bearing of the results on the question of respiratory automatism.* — All these facts constitute a new proof of the automatism of the respiratory centre. For if the afferent fibres of the vagus which come into such intimate anatomical relation with the respiratory centre and exert such a preponderating physiological effect upon it, are still blocked, it is reasonable to suppose that no other bulbo-spinal afferent paths are open. Paths from the higher parts of the brain are still less likely to be open, since resuscitation of the brain is more tardy than that of the bulb and cord.

It might be objected to our argument, as has been done by a friend in a private communication, that it is possible that the "normal" reflex stimuli for the respiratory centres, if such exist, are stronger than the stimuli set up by artificial (electrical) excitation of the brachial, sciatic, or vagus nerves. But we have much evidence that the opposite is the case, and it might very well be argued that stimulation of the sciatic, in experiments where the respiratory centre has

been paralyzed by magnesium salts, for example, is effective before natural spontaneous respiration has returned, because it is stronger than the hypothetical normal stimulation. We conceive the normal discharge of the respiratory centre as an automatic process, but the threshold of the "internal" excitation can be raised or lowered by afferent impulses as well as by influences acting directly upon the centre.

We do not maintain that our observations afford a formally crucial test, but we believe that when one studies the regular "march" of the restoration of conduction during the resuscitation, as we have done in relation to other bulbar centres as well as the respiratory centre, the evidence will appear very strong that the respiratory centre is capable of discharging effective rhythmical impulses at a time when, to tests which succeed in demonstrating open afferent paths elsewhere, the afferent paths to it are still blocked. On the other hand, if we search at this moment for indications that "natural" stimuli are affecting the centre through afferent nerves, we fail to find them.

A second objection is that the negative result of stimulation of the vagus, brachial, or sciatic might be due to an exact balance of excitatory and inhibitory impulses, or to a preponderance of inhibitory effect when two sets of antagonistic fibres are stimulated. But we have always stimulated the nerves during the various periods with the same strength of stimulus. And when we obtain the same results before occlusion, early in occlusion, and again after resuscitation has proceeded to a certain point, showing that no such exact balance nor any such preponderance of inhibition exists at these times, it seems a forced assumption that the negative results in the early part of resuscitation are due to the establishment in some way or other of that balance or preponderance. This assumption will be considered further in a subsequent section.

If it be true, as we suppose, that when respiration first returns in resuscitation the respiratory centre is still physiologically isolated both from the lower afferent paths and from the paths which connect it with the higher parts of the central nervous system, the question arises why we do not obtain the peculiar spasmodic type of respiration seen by Marckwald,¹ when both vagi were divided after elimination of the higher paths, *e. g.* by paraffin emboli. We can only say that we have never seen, in our experiments after complete occlusion,

¹ MARCKWALD: *Zeitschrift für Biologie*, 1887, xxiii, p. 149; *Ibid.*, 1890, xxvi, p. 260.

any such spasmodic respiration, even when both vagi have been divided previous to occlusion. In fact, as has been mentioned, division of both vagi causes no change in the time of return of the respiration in resuscitation, nor in its initial rhythm when it does return. In incomplete occlusions, however, in which respiration does not disappear for a relatively long time, we have rather frequently observed spasmodic respiration at a certain stage in occlusion. When in these cases respiration first returns during resuscitation, it is of the ordinary type, — slow, deep, and regular, but as resuscitation proceeds, it becomes spasmodic. The same is true in the resuscitation in the great majority of cases, even after perfect occlusion (Fig. 1). This character, in its turn, disappears with the further progress of resuscitation, and the respiration becomes normal. We suggest, therefore that in Marckwald's experiments it was not the mere removal of the influence of the higher paths which produced the spasmodic character of the respiration, but some form of irritation of these paths or their centres possibly connected with incomplete occlusion of their blood supply. Even where section of the upper paths is made by the knife, the phenomena may be due to irritation, although the removal of the cerebral lobes alone does not cause it. For example, in one experiment on a dog the entire cerebrum above the tentorium was removed after ligation of both carotid arteries. The operation caused slight slowing of the respiration. On division of the vagi, the respiratory rate became extremely slow (three per minute), and the gasps were very deep. Spasmodic or convulsive respiratory movements were not observed. This condition lasted about thirty-five minutes, at which time the dog was killed.

It must, of course, be remembered that in our experiments not only were the higher paths physiologically eliminated, but the bulbar respiratory centre itself was subjected to a period of complete anæmia. It may be, therefore, that at a time when it first began to discharge itself effectively in resuscitation it had not been resuscitated to the point at which the sustained inspiratory discharges seen by Marckwald and others were possible to it, and that by the time a sufficient degree of resuscitation had occurred to render these discharges possible, the influence of the vagi (in experiments in which they were not divided) or even of the higher paths had been restored.

The rate of respiration in our cats at the time when it first returns is about the same as the rate, according to Nikolaides,¹ in dogs after

¹ NIKOLAIDES: *Loc. cit.*

section of the higher paths and both vagi, namely, about four a minute.

This is less than the rate usually seen in dogs after double vagotomy alone (5 to 8 a minute in more than 50 per cent, 9 to 12 a minute in 30 per cent of a series of about thirty dogs observed by one of us¹). Sometimes in such dogs the rate may be as low as 4 a minute. The constancy of the initial rate of respiration in resuscitation, although not perhaps of itself a conclusive argument for the automa-

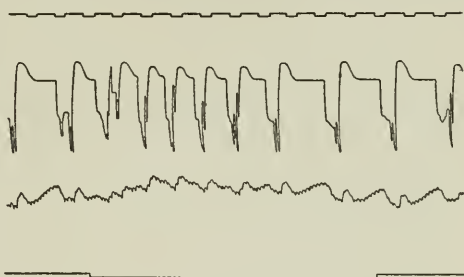


FIGURE 1.— One half the original size. Showing the spasmodic inspiration twenty minutes after the release of the head arteries, and twelve and one-half minutes after the first gasp. Down stroke indicates inspiration. The vagus was stimulated during the period indicated by the signal. Note the acceleration of respiration. Time trace at top in seconds. Respiration immediately below. Blood pressure below respiration = 140 mm.

tism of the respiratory centre, seems more likely to be dependent upon internal stimuli, perhaps related to the nutritional condition of the centre, than upon the arrival of reflex impulses. For it is improbable that in a large series of experiments even on animals of the same species, afferent impressions conducted to the centre along paths of different length and different degrees of anatomical complexity, should be integrated at a particular period in the resuscitation to a practically constant sum of excitation, measured and expressed by the practically constant rhythm and strength of the initial respiratory discharges. The sudden and strong gasp which announces the return of function of the respiratory mechanism is suggestive of the liberation of an energy which has for some time been accumulating in the centre and at last overflows. If it were discharged by afferent impulses, we should expect a more gradual onset, since the response to artificial stimulation of afferent nerves when it first appears is relatively feeble and increases in successive stimulations. This is true of the vaso-motor as well as of the respiratory mechanism.

The general agreement of the initial respiratory rate in resuscitation with that seen after section of the vagi and the higher paths

¹ STEWART: *Science*, 1905, xxi, p. 889.

with the knife is a strong support to our view that at the time when respiration returns, the centre is physiologically isolated from afferent impulses. The relatively slight effect produced on the rate of respiration in dogs after double vagotomy even by circumstances which in normal animals produce a very marked increase (for example, the psychical excitation and muscular exercise involved in chasing a mouse) is an illustration of the relatively great, although of course imperfect, isolation which an operation of such slight experimental difficulty as division of the two vagi may produce. How much more perfect must the isolation be where in addition to section of the vagi cerebral anæmia is produced!

After a longer or shorter interval, depending on the length of the occlusion period and other conditions which we could not always control, the afferent nerves again became capable of influencing the respiration when stimulated. In general, the power of the vagus to influence the respiration is restored somewhat earlier than that of the brachial, possibly because the brachial path runs for a greater distance in the previously anæmic area. The usual result of stimulation of the vagus, when its influence on respiration has first returned, is an acceleration of respiratory movements, and this without regard to the effect produced by it either before occlusion (most commonly inhibition in inspiration) or later in resuscitation. As an instance, in the experiment of February 25, the effect of the vagus before occlusion was partial inhibition in expiration. The occlusion lasted twelve and one-half minutes. Twenty-nine minutes after release of the head vessels, and fifteen minutes after the first gasp, the vagus produced marked acceleration of the respiration; forty-three minutes after release, it caused acceleration and weakening of the movements of the diaphragm, which remained partially relaxed, as shown by direct inspection through a wound in the abdomen; fifty-four minutes after release, marked inhibition in expiration, with complete stoppage of the diaphragm in relaxation.

We found numerous instances of the same kind. This is worthy of note because it affords evidence that the reason why no effect of the vagus on respiration can be detected until some time after the return of respiratory movements is not due to its exerting an inhibitory influence on the respiratory centre. Further evidence is obtained by comparing the time of return of the action of the brachial, which is rarely inhibitory, with that of the vagus. Frequently the interval elapsing between the return of the influence of the two

nerves is small, and occasionally the brachial may perhaps be active even a little before the vagus.

Confirmatory evidence that the first excitatory effect of the vagus in resuscitation is really its first effect exists in the work of Prevost and Stern,¹ who state that "les centres d'arrêt de la respiration" are less resistant to asphyxia than the "centres d'excitation."

(d) *The action of the spinal respiratory centres in cerebral anæmia.*—The various conditions under which activity of the spinal respiratory centres has been observed are reviewed by Prevost and Stern. We have occasionally observed respiratory movements due to these centres during the inactivity of the bulbar centre. In one experiment on a young cat the rate of the movements was nine per minute, and involved the lower ribs and abdominal muscles. No movements of the neck muscles occurred, and the fore limbs were limp and motionless. The upper cervical region of the cord had been rendered anæmic by occlusion of the head arteries. These movements began six minutes after occlusion and continued until four and one-half minutes after release of the head arteries,—eleven and one-half minutes in all. Then there occurred a pause of seven minutes during which no respiratory movements of any kind were seen. Immediately afterward, the ordinary gasps observed in resuscitation occurred, and soon increased in number to twenty-six per minute.

Stimulation of the central end of the sciatic or brachial nerves, at a time when there were no spontaneous respiratory movements, sometimes produced contraction of the muscles of the lower ribs and abdomen. We do not regard these movements as due to the bulbar respiratory centre, but as analogous to those movements of the intercostal muscles obtained by Chauveau² in the horse and ass on rhythmical stimulation of the central end of an intercostal nerve after section of the cord at the atlo-axial level.

(e) *Stimulation of the phrenic nerve by the action current of the heart.*—It is well known that under certain conditions the phrenic nerve, and especially the left, may be stimulated at each beat of the heart so as to cause a contraction of the diaphragm synchronous with the cardiac contraction. It has been supposed that section of the phrenic nerve and opening the thorax were particularly favorable, if not essential, conditions for the appearance of this phenomenon. Langendorff,³ however, observed it frequently, without opening the

¹ PREVOST and STERN: *Loc. cit.*

² CHAUVEAU: *Memoires de la Société de Biologie*, 1891, 9th series, iii, p. 155.

³ LANGENDORFF: *Archiv für die gesammte Physiologie*, 1903, xciii, p. 277.

thorax or sectioning the nerve, when a large quantity of blood was taken from the animal, or when it was allowed to bleed to death. He agrees with Schiff¹ that the cause of the phenomenon is the excitation of the phrenic nerve by the action current of the heart, but does not come to any definite conclusion as to how the circumstances under which the contractions appear are favorable for the excitation of the nerve. He suggests two explanations, (*a*) that after hemorrhage the empty heart affords a worse short circuit for the action current; (*b*) that shifting of the position of the empty heart takes place. We have frequently observed contractions of the left side of the diaphragm synchronous with the heart beat beginning at a certain stage in the occlusion, and continuing either throughout the remainder of the period of anæmia, or for several minutes, ceasing some time before the end of occlusion and perhaps beginning again some minutes after release. The heart was well filled with blood at the time these contractions were occurring, although doubtless the endocardiac pressure was lower than normal. It does not appear, therefore, how a less favorable short circuit through an empty heart, or a more favorable position of the heart, could be brought about at this time. In any case the condition and position of the heart remain the same for a considerable period in resuscitation, yet contraction of the diaphragm occurs only within a fairly definite interval. It seems more probable to us that the contractions are due to a change in the irritability of the nerve trunk. The cause of this change may be (1) the anæmia of the trunk, since the superior phrenic artery arises from the internal mammary, and would therefore at its origin be within the anæmic area, or (2) the lack of nervous impulses coming down from the respiratory centre. Since the movements of the diaphragm may sometimes cease before the end of occlusion and begin again after release, we are inclined to the opinion that the anæmia first causes an increase in the excitability of the nerve trunk and afterward a total loss of excitability. In the resuscitation there would be first an increase of excitability, and later on a decrease, as the nerve approached more nearly to its normal condition. We have not observed stimulation of the nerve in all our experiments. It may be that in some cases the anastomosis with the inferior phrenic artery is free enough to furnish a quantity of blood sufficient for the needs of the nerve in the lower part of its course. Or there may be

¹ SCHIFF: Archives des sciences physiques et naturelles, 1877, lix, p. 375; Gesammelte Beiträge, 1894, i, p. 752.

other reasons why the threshold does not sink low enough to permit stimulation. The influence of section of the phrenic (without anæmia) is very naturally explained, in accordance with Schiff,¹ as a consequence of the increase of excitability which propagates itself from the section towards the periphery, as the nerve dies, according to the Ritter-Valli law.

(2) **The vaso-motor nervous mechanism.** (a) *The vaso-motor mechanism in occlusion.* — The blood-pressure curve in occlusion has been described in the former paper. It quickly rises to a certain height, then falls sometimes even below the normal pressure before occlusion, rises again to the maximum pressure attained during the anæmic period, then falls, rather rapidly at first but more slowly later, to a level which is maintained, with only a slight decline, throughout the period of occlusion. The cause of these inequalities in the blood-pressure curve was there briefly discussed and will be considered more in detail in the section on the cardio-inhibitory mechanism.

The suddenness of the rise in pressure in occlusion is not due materially to the mechanical effect of cutting off a portion of the vascular path. For in previously reported experiments ligation of the head arteries after section of the spinal cord in the mid-cervical region gave only a slight rise, and the initial fall on release, when the spinal cord is intact, is also slight.

The stimulation of the vaso-motor centre associated with occlusion is much more prompt than that which follows asphyxia produced by interference with the respiration without any operative interference with the circulation. In one experiment in a cat, in which occlusion produced a rise of pressure from 140 to 268 mm., asphyxia alone caused no rise whatever, and in several other experiments the asphyxial rise was small compared with that produced by occlusion. The explanation may be that in asphyxia the changes in the vaso-motor centre which lead to exhaustion may sometimes proceed so rapidly, or its excitability to the altered condition of the blood may be initially so small or may so quickly decline, that the point at which strong excitation usually occurs is missed. In occlusion, on the other hand, the vaso-motor centre very quickly becomes anæmic, so that the stimulus rises almost at once to its maximum strength before the excitability of the centre has had time to decline.

Afferent nerves produce, in general, the same qualitative effects on the blood pressure during occlusion as before it, but there are occasional exceptions. Stimulation of the brachial, early in the occlusion,

¹ SCHIFF: *Loc. cit.*

causes a greater rise for the same strength of stimulus and stimulation of the vagus a greater fall of pressure than before occlusion. This is the case even when stimulation occurs at such a period that the inhibitory effect on the heart is not increased, or when the inhibitory effect has been replaced by acceleration. In one experiment excitation of the vagus before occlusion caused a fall of blood pressure from 82 mm. to 73 mm., and a diminution in pulse rate from 200 to 175 a minute. After the (imperfect) occlusion had lasted eleven and one half minutes, stimulation of the same nerve with the same strength of stimulus caused a fall of pressure from 148 mm. to 102 mm., while the pulse rate increased from 155 to 175.

Later on in the occlusion, the afferent nerves cease to affect the blood pressure, often even before the culmination of the second rise, and always during the final fall.

(b) *The vaso-motor mechanism in resuscitation.*—On release of the occluded arteries the blood pressure, which, in all good occlusions lasting from seven to ten minutes, has fallen to a fairly constant level of 20 mm. to 100 mm., falls a little more, owing to the opening of the head arteries. Then for a period depending partly upon the length of the occlusion, partly upon other conditions, it remains practically constant. During this time whatever tone the vessels possess must be maintained either by the spinal vaso-motor centres or by peripheral mechanisms. This period of constant low pressure is succeeded by a period during which the blood pressure rises more gradually at first, then more rapidly. After short occlusions the rise is more abrupt than after longer ones. After occlusions of moderate length, not exceeding ten to fifteen minutes, the blood pressure may rise to a height greater than that before occlusion. Sooner or later, however, it sinks, generally rather slowly, to a level usually a little below that before occlusion. After a second or third occlusion the minimum blood pressure before release is generally reached much more rapidly than after the first, and the rise of pressure in resuscitation is far more gradual and less pronounced.

The effect of stimulation of afferent nerves on blood pressure in resuscitation.—Stimulation of all the afferent nerves tested (vagus, depressor, brachial, and sciatic) is ineffective for a certain period in resuscitation, the length of the period depending partly on the duration of the occlusion. In general, in cats, the vagus becomes effective somewhat earlier than the brachial, and it is worthy of remark that, although the vagus (in the cat and rabbit) has invariably

produced a fall of blood pressure in our experiments before occlusion, the first effect produced by it in resuscitation is a rise of pressure not followed by any depressor or cardio-inhibitory action. The same is true of the depressor in the rabbit. As a rule, the maximum rise of pressure produced by the vagus or depressor in resuscitation, although

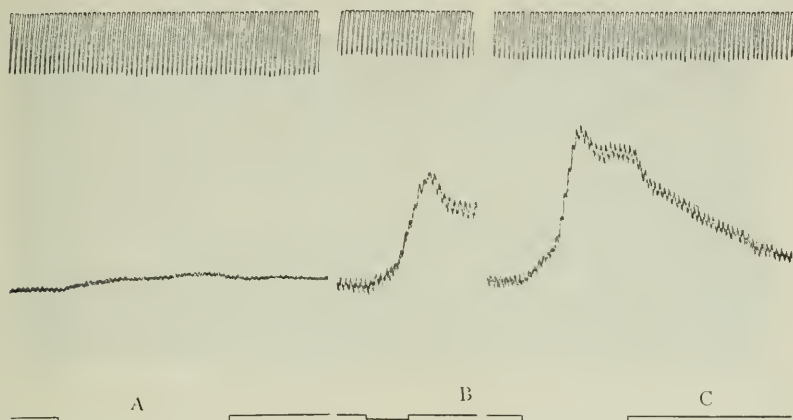


FIGURE 2.—Two thirds the original size. Showing the effect of successive excitations, *A*, *B*, and *C*, indicated by the signals of the vagus at intervals of sixty-five, seventy-three, and seventy-nine minutes respectively, after a third occlusion of seven minutes in a cat. Note the increasing pressor effect of the vagus with practically constant blood pressure. Artificial respiration at top, 30 per minute.

distinct, is not very great (10 mm. to 30 mm.). Not infrequently however, it is considerably more, and in one experiment stimulation of the vagus caused a rise of 42 mm., 60 mm., and 70 mm., in three successive observations; and during asphyxia induced in the resuscitation period, a rise of 126 mm. (Fig. 2). Later on, in resuscitation the pressor effect of the vagus (or of the depressor nerve) is replaced by the depressor effect. It is interesting that the rabbit's vagus gives the same result as the cat's vagus before occlusion and during resuscitation, notwithstanding the existence in the rabbit of a separate depressor nerve.

The reappearance of the fall of blood pressure produced by the vagus or depressor in resuscitation does not depend primarily upon the pressure before stimulation. For instance, in the experiment of April 15 (rabbit) with the blood pressure at 112 mm., the depressor caused no effect. In the next observation, with the pressure at 113 mm., it caused a slight fall; in the next, with the pressure at 115 mm., it

caused a good fall of pressure to much below that at which previous stimulations were ineffective. It is impossible to suppose that these stimulations were ineffective because there was no vaso-motor tone present which was removable by the depressor. The natural conclusion is that the stimulation was ineffective because the conductivity of the afferent depressor path was not yet restored.

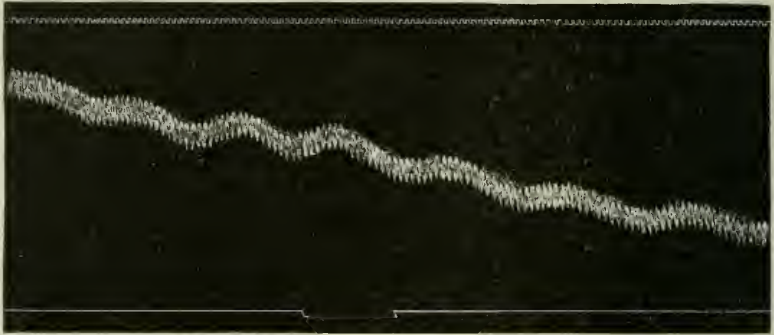


FIGURE 3. — Two thirds the original size. Showing Traube waves in the blood-pressure curve during the final fall in occlusion. Vagus stimulation (ineffective) at signal. Blood pressure fell from 166 mm. to 108 mm. during period shown in the tracing. Time trace at top in seconds.

After a second occlusion in the same experiment, stimulation of the depressor with a blood pressure of 140 mm. caused, instead of a fall, a rise of 13 mm. Several minutes later, with a pressure of 126 mm., a slight fall (to 117 mm.) was caused, and greater falls in succeeding stimulations.

It has generally been stated that the depressor nerve never, under any circumstances, causes a pressor effect. This statement must be modified in the light of our results. The easiest way of explaining them is to assume the existence in the rabbit's depressor of two sets of afferent fibres which can affect the vaso-motor centre (in addition, of course, to the afferent fibres which act on the cardio-inhibitory centre). The pressor fibres or their synapses may be supposed to be more resistant to anæmia and more easily resuscitated than the depressors. The number of pressor fibres in the depressor nerve may be assumed to be smaller, or their excitability lower, in comparison with the depressor fibres, so that under ordinary circumstances stimulation of the nerve always causes a fall of blood pressure. The same explanation would apply to the vagus.

It may be, of course, that in the first period of resuscitation the

vaso-motor centre is in such a condition that it responds differently to impulses reaching it along the depressor fibres than when in its normal condition or later on in resuscitation. But there seems to be no advantage in framing a hypothesis of this sort merely to save the current conception that the depressor is a nerve with fibres of but one anatomical description, since we must, in any case, cease to conceive of it as a nerve whose fibres always produce the same physiological effect.

Stimulation of the brachial causes, practically always, the same qualitative effect before occlusion, during occlusion, and in resuscitation, namely, a rise of blood pressure. The difference between the results in the case of the brachial (or sciatic) and of the vagus we conceive to be due to the presence in the brachial of an excess of pressor fibres. Since, by hypothesis, these are more resistant than the depressor fibres, our procedure does not afford an opportunity for establishing a preponderance of the depressors at any stage of the resuscitation.

A difference in the qualitative effect of stimulation of the sciatic in occlusion and resuscitation was seen in one experiment. Stimulation in occlusion caused a fall of pressure (from 210 mm. to 184 mm.). In the first period of resuscitation the effect was a rise of pressure (from 52 mm. to 61 mm., 60 mm. to 82 mm., and 102 mm. to 120 mm., in successive stimulations). The next stimulation caused a rise from 150 to 156 mm., succeeded by a fall to 144 mm. Later on, a fall of pressure from 154 mm. to 124 mm. was obtained without any preliminary rise. Clearly the pressor fibres were the first to be resuscitated in this instance.

It might be asked whether stimulation of the brachial or vagus might not produce an effect on blood pressure through the lower spinal vaso-motor centres at a time when the bulbar centre and the cervical vaso-motor centres in the previously anæmic region were still unable to respond. We have often observed that impulses may be conducted through an area of the central nervous system at a stage when resuscitation has not proceeded to the point at which the centres in that area become responsive to reflex stimulation. For example, stimulation of the fore limb or of the brachial nerve may cause movements of the hind limbs (in resuscitation after anæmia of the head end) at a time when no reflex movements of the fore limbs are elicited. The possibility that some of the vaso-motor effects observed on stimulation of the vagus or brachial in the first period of

resuscitation might be due to conduction of the impulses tailwards to the lower spinal vaso-motor centres was eliminated by ligating the aorta in a considerable number of control experiments just distal to the origin of the head arteries, thus rendering the region below the first or second rib permanently anæmic. The results were quite similar to those obtained when the lower parts were not rendered anæmic.

(c) *Bearing of results on the question of the automaticity of the vaso-motor centre.* — Do these experiments throw any light on the question whether vaso-motor tone is automatic or reflex? We have tried to test this question by determining whether the bulbar vaso-motor centre regains its tone before, after, or simultaneously with the return of effective stimulation of afferent nerves. In the great majority of our observations the afferent nerves begin to produce an effect on blood pressure while it is still low, but not until there has been some increase of pressure above the constant level established at the beginning of resuscitation. This is especially the case after long or repeated occlusions, — conditions under which blood pressure remains during resuscitation for a considerable period at a relatively low level (see protocol of second occlusion in experiment of April 15, p. 351). After single short occlusion the blood pressure rises more rapidly, and may have increased considerably before the first effective stimulation of afferent nerves occurs. For example, in one experiment, the mean pressure before an occlusion of fifteen and one-fourth minutes was 150 mm. ; at release, 80 mm. ; and 124 mm. before stimulation of the brachial first became effective, five and three-fourth minutes after release.

Such facts seem to show that the bulbar vaso-motor centre may have some power of maintaining even a considerable degree of vaso-motor tone at a time in resuscitation when the afferent paths are still blocked. This supports the idea that there is an automatic element in the activity of the centre in the wide sense of the word which implies that it is a centre which can discharge itself in the absence of reflex impulses. And this is the picture which is presented to us in experiments where the centre has not been subjected to too severe treatment by long occlusion. On the other hand, we may see the opposite condition of affairs, where as yet but little bulbar vaso-motor tone has returned, while stimulation of afferent nerves produces an effect — sometimes a great one. For instance, in the experiment from which Fig. 2 is taken, after the third occlusion the

blood pressure after release was 60 mm., and the return of the effect of stimulation of afferent nerves was not heralded by any increase in pressure. Here, to our tests, the reflex vaso-motor paths were open. Since the vaso-motor centre at this stage is easily influenced by artificial reflex stimulation, it may be asked why the natural reflex stimuli, if such exist, do not support a certain vaso-motor tone. The answer may be that at this time the hypothetical normal stimuli are either not being originated or, because of their relative weakness, are not reaching the vaso-motor centre. There can be little doubt that artificial electrical stimulation of such nerves as the brachial and sciatic produces at least as great an effect on that centre as can possibly be produced under the most favorable circumstances by any "natural" reflex stimuli, since we so easily obtain maximal blood pressures in this way. It might very well be that the normal stimuli, were so much weaker as to be ineffective at this particular stage of resuscitation. On the hypothesis of automatism, however, the absence or the feebleness of the vaso-motor tone may be explained as due to the incapacity of the centre, after the severe strain of a long period or repeated shorter periods of anæmia, to discharge itself automatically or in response to stimuli acting directly upon it, *e. g.*, the changes in blood pressure, rather than to the absence of normal reflex stimuli.

That the vaso-motor centre can be stimulated by changes in the quality or pressure of the blood, at a time when the bulbar vaso-motor tone has not returned in the resuscitation period, is shown by the fact that asphyxia may cause a rise of pressure. This is seen independently of the excitation of the lower spinal centres in experiments in which these have been previously eliminated by permanent ligation of the aorta beyond the origin of the head arteries. The excitation of the centre in this case might possibly be looked upon as a reflex through afferent nerves whose peripheral endings were excited by asphyxia. But a similar asphyxial rise has been noted in cases where artificial stimulation of afferent nerves was producing no effect on the blood pressure. In some of these cases a considerable degree of vaso-motor tone had returned; in others, little or none.

To sum up, the experiments seem to show that a portion, at least, of the tone of the vaso-motor centre is not of reflex origin. We do not deny that there may be a reflex element in it as well, but our observations throw less light upon this factor than upon the other.

That there is an element in the vaso-motor tone which is absent, or at least less important, in the case of the respiratory centre is indicated by the much greater abruptness of the return of respiration.

We do not propose to discuss the question of the actual nature of the automatic element in the tone of the centre. Its automatism may be nothing more or less than its response to variations in the pressure or the composition of the blood. Various writers have held the view that when the blood-pressure tended to sink, the vaso-motor centre was stimulated, and that when it tended to rise the centre was inhibited. The change of blood pressure in the bulb may act as a mechanical stimulus or it may affect the nutrition of the centre, whose automatism may, after all, be finally dependent upon internal nutritive changes. The great stimulation of the centre in sudden anæmia due to occlusion is, of course, in favor of this. In asphyxia without occlusion, we might expect that sometimes the two factors, the tendency of the blood pressure to rise owing to interference with the nutrition of the centre, and the tendency of the high pressure to cause inhibition of the centre, would balance each other. In occlusion the pressure in the bulb falls very suddenly, causing perhaps a mechanical stimulation of the centre in addition to that produced by interference with its nutrition.

As regards the relative time of resuscitation of the respiratory and the vaso-motor mechanisms, it may be mentioned that reflex vaso-motor effects, both pressor and depressor, may be got from nerves connected with the previously anæmic area considerably earlier in resuscitation than reflex effects on respiration.

This agrees with the fact that the vaso-motor centre remains active in occlusion longer than the respiratory centre. Even at a time, during the second fall of pressure, when it is totally unaffected by stimulation of afferent nerves, evidence in the form of Traube waves (Fig. 3), that the vaso-motor centre is still discharging itself, is a further indication that an element which we may term automatic exists in the vaso-motor tone. At this time all respiratory movements have long since disappeared, and it would seem very improbable that the rhythmical changes in the vaso-motor centre are dependent on discharges from a respiratory centre which has long ceased to discharge along the efferent respiratory paths.

(3) **The cardio-inhibitory nervous mechanism.** (a) *The cardio-inhibitory mechanism before occlusion.* — Stimulation of the central end of

the cat's vagus has given, without any exceptions, a reflex fall of blood pressure at this time, with inhibition of the heart. Generally the fall of pressure was not extreme with the strength of current used, and complete standstill of the heart was not observed. But in one instance we saw complete and permanent stoppage of the heart and respiration, while the blood pressure rapidly fell to the base line when the right vagus was ligatured and divided below the ligature. The animal was not deeply anæsthetized at this moment. The heart was readily started by direct massage. Reflex inhibition was easily elicited by electrical excitation of the central end of the vagus during the rest of the experiment, but the heart was not stopped completely. This is the only instance of complete reflex standstill of the heart of which we were certain in more than 100 experiments, although we had observed extreme slowing in one or two other cases. In dogs anæsthetized with morphia and ether, or ether alone, stimulation of the central end of one vagus, the other being intact, often fails, in our experience, to cause any trace of inhibition. We ought, therefore, to be cautious in assuming that the importance of cardiac inhibition as a factor in surgical shock or in death during the administration of anæsthetics is equally great in different species of animals; and experiments on a particular kind of animal, for example, Friedenthal's¹ observations on rabbits ought not to be transferred without care to man.

(b) *The cardio-inhibitory mechanism in occlusion.*—Some of the phenomena observed during occlusion of the head arteries have been dealt with in our previous paper. The provisional explanation there given of the first fall of blood pressure during occlusion has been confirmed by numerous additional experiments, all of which go to show that it is due to strong excitation of the cardio-inhibitory centre during anæmia. Both the fall of pressure and the slowing of the heart are, in general, greater than are obtained by electrical stimulation of the vagus before occlusion. At the beginning of the second rise of pressure the inhibition begins to fail and the heart becomes increasingly rapid, often beating more than 200 to the minute (cats) at the crest of the second rise. The second rise of pressure is due (1) to vaso-motor tone and (2) to active acceleration of the heart after the failure of the cardio-inhibitory mechanism.

There is little reason to suspect any failure of vaso-motor tone during the first fall of pressure, since the curve does not show, except

¹ FRIEDENTHAL: *Archiv für Physiologie*, 1901, p. 31.

in rare instances, any but the final fall of pressure after section of both vagi.

The extremely high pulse rate observed during the second rise cannot be due merely to failure of cardio-inhibitory tone from anæmia of the centre. Bernstein and others¹ have shown that when both the vagi and the nervi accelerantes are divided, the heart either does not change at all in rate with changes in blood pressure, or has a somewhat slower rate at high pressures. The only agency, therefore, which is capable of producing a high pulse rate with high blood pressure, is active acceleration of central origin. The conduction pathways for these accelerator impulses have not yet been completely isolated, but there seems little reason to doubt that part of them at least pass downward in the vagus, while some certainly pass to the heart through the accelerantes, since acceleration of the heart during cerebral anæmia after section of both vagi has been observed by Mayer² and by us.

As a provisional explanation of the inhibition sometimes observed prior to the acceleration during the high blood pressure in occlusion after section of both vagi, we suggest that it may be due to the local governing mechanism in the heart.³

The second rapid fall of blood pressure during occlusion is due to the failure of cardiac acceleration associated with failure of vaso-motor tone, chiefly to the latter.

Stimulation of afferent nerves, in general, produces the same qualitative effect on the cardio-regulative mechanism during the occlusion as before it. The increased susceptibility to reflex stimulation in the first part of the occlusion observed for the respiratory and the vaso-motor mechanisms is seen also in the case of the cardio-inhibitory. For example, stimulation of the depressor in the rabbit before occlusion caused a change of pulse rate from 150 to 130 a minute; after nine minutes (imperfect) occlusion, a change from 150 to 120 a minute. After a certain period in the occlusion stimulation of the depressor or vagus produces no effect on pulse rate, although a fall of blood pressure can still be obtained at this point.

Verworn⁴ has analyzed the factors which act on the cardio-inhibi-

¹ For literature see HERLITZKA, *Archiv für die gesammte Physiologie*, 1905, cvii, p. 557; GUTHRIE and PIKE: *This journal*, 1907, xviii, p. 14.

² MAYER: *Sitzungsberichte der kaiserliche Akademie der Wissenschaften zu Wien*, 1879, lxxix, 3 Abth., p. 87.

³ GUTHRIE and PIKE: *Loc. cit.*, p. 28.

⁴ VERWORN: *Archiv für Physiologie*, 1903, p. 65.

tory centre when respiration is hindered. According to him, the increase of blood pressure increases the excitability of the centre, while the deficiency of oxygen has a similar effect. In our experiments increase of blood pressure, of course, plays no part, since the pressure falls to zero, on occlusion. The increase of excitability must, therefore, depend on changes produced in the metabolism of the centre by the anæmia, including doubtless the changes caused by lack of oxygen.

(c) *The cardio-inhibitory mechanism in resuscitation.* — Of the three mechanisms particularly studied this is the last to regain its tone in resuscitation, and also the last to regain the power of being acted upon by artificial stimulation of afferent nerves. For example, in the experiment of April 15 in a rabbit after an occlusion (the second) of fifteen minutes the first respiration occurred in five and a half minutes after release. In the first three minutes after release the blood pressure had risen from 57 mm. to 68 mm. At this time stimulation of the depressor caused a rise of pressure to 82 mm.; thirty-one minutes after release slowing of the heart was first obtained by stimulation of the depressor, the rate before stimulation being 120 and during stimulation 62. Thirty-two and a half minutes after release the right vagus was cut, no change being produced in the pulse rate. Forty-three minutes after release the pulse rate was 127; two minutes later 128, and section of the left vagus left it practically unaltered. We give a portion of the protocol of this experiment.

Experiment, April 15. — Rabbit. Tracheotomy. Ether. Central end of right depressor nerve was prepared for stimulation, but not separated from cervical sympathetic. Vagi intact. Central end of sciatic nerve also prepared for stimulation. Blood pressure trace from cannula in left carotid. There were two occlusions, the first of six and three-fourths minutes being followed after an interval of twenty-eight and one-fourth minutes by a second occlusion of fifteen minutes, beginning at 5.08 P. M.

5.08 P. M. Occluded head arteries. Pulse rate before occlusion, 189 in the minute.

5.08.20 P. M. Stimulated depressor. Pulse before stimulation, 162; during stimulation, 124. Slight fall of pressure. Twenty seconds after stimulation, pulse 120 in the minute.

Subsequent stimulation of the depressor produced no effect.

5.23 P. M. Released head arteries. Pulse, 136. Pressure, 57 mm.

5.24 P. M. Stimulated depressor. Slight rise of pressure. Pulse, 136.

5.26 P. M. Stimulated depressor. Rise of pressure, 68 to 82 mm. Pulse before stimulation, 142; during stimulation, 150.

5.28.30 P. M. Stimulated depressor. Rise of pressure from 98 to 110 mm. Pulse, 124 before stimulation; 124 during stimulation.

Respiratory gasps are just beginning.

5.30.30 P. M. Stimulated central end of sciatic. Rise of pressure from 128 mm. to 150 mm. Pulse, 122 before stimulation; 121 during stimulation.

5.31.40 P. M. Stimulated depressor. Rise of pressure from 140 to 153 mm. Pulse, 129 before and during stimulation. No corneal reflex has yet returned.

5.35 P. M. Stimulated sciatic. Fair rise of pressure with no change in pulse rate.

5.38 30 P. M. Stimulated depressor. Fall of pressure from 126 to 117 mm. Pulse, 126 before, and 122 during, stimulation.

5.41 P. M. Stimulated depressor. Fall of pressure from 117 to 102 mm. Pulse, 131 before, and 126 during, stimulation.

5.45 P. M. Stopped artificial respiration.

5.46 P. M. Stimulated depressor. Apparently a rise of pressure after a long latent period, succeeded by a fall with slight inhibition, but possibly this is simply a respiratory wave.

5.47.30 P. M. Stimulated depressor. Fall of pressure.

5.50 P. M. Stimulated depressor. Fall of pressure from 97 to 92 mm. Pulse, 107 before, and 108 during, stimulation.

5.54 P. M. Stimulated depressor. Fall of pressure from 98 to 56 mm. Pulse before stimulation 120; during, 62.

5.55.30 P. M. Cut right vagus.

5.56.45 P. M. Stimulated depressor. Fall of pressure from 100 to 92 mm. Pulse 97 before, and 93 during, stimulation.

6.06 P. M. Stimulated depressor. Fall of pressure from 94 to 53 mm. Pulse, 127 before stimulation, 117 in first part of, and 60 at close of, stimulation. Latent period of inhibition is longer than when reflex occurred through vagus of same side.

6.08 P. M. Cut left vagus. Pulse before division, 127; after division 120.

Rabbit died from hemorrhage due to accidental rupture of one of the large arteries.

Almost invariably the first reflex cardio-inhibitory effect is obtained before there is any return of cardio-inhibitory tone. This is illustrated in the experiment just quoted. In the experiment from which Fig. 3 was taken, in a cat, after a (second) occlusion of eleven minutes the pulse rate immediately after release was 186; fourteen and one-third minutes after release it was 129. At this time stimulation of the central end of the vagus caused a slight rise of blood pressure,

but no change in heart rate. Twenty-four minutes after release the pulse rate was 147; stimulation of the central end of the vagus at this time caused a fall of blood pressure from 90 mm. to 74 mm. and a diminution in pulse rate to 132.

Of course, in determining whether cardio-inhibitory tone has returned or not, we cannot trust to observations on the pulse rate alone, as in prolonged experiments changes may occur in this, not only when both vagi have been cut, but also when all extrinsic cardiac nerves have been eliminated by anæmia. For instance, when the occlusion lasts more than a very few minutes, there is a gradual progressive fall in the pulse rate at a time when all the bulbar mechanisms have ceased to function, and this diminution in pulse rate is accompanied by a gradual fall of blood pressure. It would lead, of course, to totally erroneous results to conclude that, because the heart was beating at this time or in the first part of resuscitation at a decidedly slower rate than before occlusion, a strong inhibitory tone was present. The slowing of the heart in this case is simply an expression of the deterioration of the cardiac muscle, or the intrinsic cardiac ganglia.

(d) *Resuscitation of the crossed afferent paths to the cardio-inhibitory centre.*— In the previous paper it was pointed out that, during the period of recovery, a crossed spinal reflex path required a longer time for its resuscitation to the point at which it regained its conductivity than a path confined to the same side of the cord as the afferent segment of the arc. For example, a reflex movement of the forelimb could be obtained on the side stimulated earlier than a crossed reflex involving the opposite forelimb. The same is true of the cardio-inhibitory reflexes. The experiment of April 15 (p. 351) illustrates this. Fine ligatures were slipped under each vagus, but not tightened. In the resuscitation the central end of the right depressor was stimulated from time to time, until at last it began to produce reflex inhibition of the heart. As is always the case, stimulation of this nerve caused vaso-motor effects much sooner than cardio-inhibitory, and the first excitation which was effective in producing inhibition caused a preliminary depressor effect without any considerable alteration in the heart rate. The change from a purely depressor action to a combined depressor and cardio-inhibitory action was abrupt (Fig. 4). The inhibition was very marked, although no inhibition whatever had been produced four minutes earlier. The right vagus was now cut, and stimulation of the right depressor repeated (Fig. 5). It gave, as

before, a fall of blood pressure, but no cardiac inhibition whatever, showing that at this time the afferent impulses, while able to affect the part of the cardio-inhibitory centre on the right side, were unable

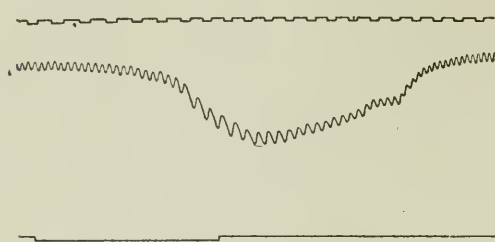


FIGURE 4.—About one half the original size. Showing the first marked cardio-inhibitory and depressor effect, after release of the head arteries from stimulation of the central end of the right depressor, both vagi being intact. Duration of stimulation marked by signal. Note the long latent period before inhibition results. Time trace at top in seconds.

to cross the middle line and to act through the still intact vagus. This condition of affairs continued for twelve minutes after the first reflex inhibitory effect had been obtained, or ten and one-half minutes after section of the right vagus. Then again, suddenly, stimulation of the right depressor became effective in causing marked inhibition

of the heart (Fig. 6). This was, of course, produced through the opposite vagus. In other experiments it was seen that, after section of the second vagus, the fall of blood pressure was produced by the depressor as before, while there was no cardio-inhibitory effect, the resuscitated mechanisms thus exhibiting the same behavior as the normal. This experiment furnishes evidence that the cardio-inhibitory centre can act as a functionally as well as an anatomically bilateral centre; that is, that each lateral half of it can discharge inhibitory impulses along its own vagus in the absence of physiological connection with the other half.

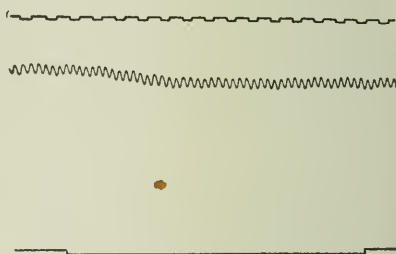


FIGURE 5.—One half the original size. Same experiment as in Fig 4. Effect of excitation of central end of right depressor after division of right vagus. Duration of stimulation shown by signal.

It may be mentioned that the mechanical excitation of the right vagus when it was cut caused an inhibitory effect (Fig. 7) which was practically a replica in all its details of the inhibition previously caused by electrical stimulation of the depressor. This seems worthy of note, because we have often observed that there is a certain "figure" which an inhibitory or a vaso-motor effect is apt to assume

in one and the same animal, even when elicited under such varying conditions as are afforded during the pre-occlusion, the occlusion, and the resuscitation periods. One often gets the impression that the general typical response is stamped with a certain idiosyncrasy, — the sign manual, so to speak, of the individual animal or mechanism.

The long latent period elapsing before stimulation of the depressor or vagus produces cardiac inhibition, or even a fall in blood pressure, which is so constantly seen in the resuscitation at the time when stimulation is first becoming effective, is strikingly shown in this experiment. After section of the right vagus the depressor

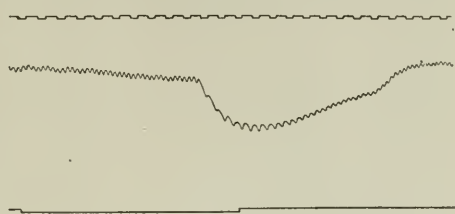


FIGURE 6. — About one third the original size. Same experiment as in Fig. 4. Shows the first marked cardiac inhibition on excitation of the central end of the right depressor after division of the right vagus. Duration of stimulation shown by signal. Note the longer latent period before cardiac inhibition occurs.

effect followed after a latent period of seven seconds, and the inhibitory effect after seventeen seconds (Fig. 6).

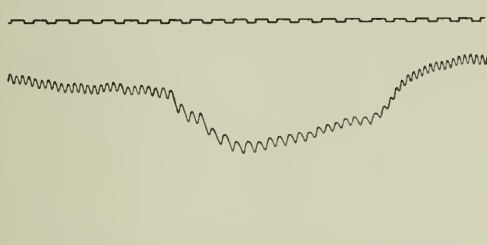


FIGURE 7. — About one half the original size. Same experiment as Fig. 4. Showing the effects of mechanical stimulation following division of the right vagus.

We have looked for evidence that the commissural path between the halves of the respiratory centre is also resuscitated at a later period than either half,

but we have not found it possible to apply a crucial test. It might be supposed that when the vagus first begins to affect the respiration, it should cause a change in the action only of the respiratory muscles on the corresponding side if the connection across the middle line between the two parts of the respiratory centre was still non-conducting. Now, we have never seen this. Wherever an effect is produced, it involves equally the muscles on the two sides. But it has been shown by Porter¹ and by Nikolaides² that each half

¹ PORTER: *Journal of physiology*, 1895, xvii, p. 455; *Centralblatt für Physiologie*, 1894, viii, p. 258.

² Nikolaides: *Loc. cit.*

of the respiratory centre is connected with the muscles on both sides, the impulses crossing in the cord below the medulla oblongata; in the case of the phrenics at the level of the phrenic nuclei (Porter).

(e) *The behavior of the associated action of the respiratory and cardio-inhibitory centres* which causes the normal increase in pulse rate towards the end of inspiration, especially when the breathing is deep and slow, is an interesting point to study in resuscitation. We find that this action may be present at a time when stimulation of afferent nerves, *e. g.*, the depressor, is as yet ineffective in producing cardiac inhibition. In such cases we may assume that the path from the respiratory to the cardio-inhibitory centre has been restored, while as yet the afferent paths to the cardio-inhibitory centre are blocked.

In many of our experiments we did not observe the return of this associated action, but in these cases neither the cardio-inhibitory tone nor the reflex effect of afferent nerves on the cardio-inhibitory centre was restored, the period of occlusion being perhaps too long, or the blood pressure after resuscitation too low, or the experiment not being followed for a sufficient time.

We have already stated that, during occlusion, respiration as a rule ceases definitively either about the same time that the cardio-inhibitory tone disappears or a little later. We have, however, observed in imperfect occlusions in a few instances the persistence of the tone of the cardio-inhibitory centre, and also of the power of afferent nerves to influence it for some time after respiration had ceased and could no longer be excited by reflex stimulation. We do not assert that the relative power of resistance of the various centres in animals of different species, or even in individuals of the same species, is always the same. We have stated the broad differences. Nor do we attempt to decide whether the power of resistance of the centres varies because of anatomical differences, such as the number or histological nature of the synapses, or because of physiological differences.

(f) *Bearing of the results on the question of the nature of cardio-inhibitory tone.* — We have already pointed out that our results afford new evidence of the existence of an automatic factor, that is, a factor not depending on reflex impulses in the discharge of the respiratory centre, and also, although it may not be so prominent here, in the tone of the vaso-motor centre. In the case of the cardio-inhibitory centre the evidence is not so clear, because the return of cardio-inhibitory tone is so tardy. Upon the whole, however, our observations favor the view that the reflex factor is relatively more important for

the cardio-inhibitory than for the vaso-motor tone, and *a fortiori* more important than for the respiratory discharge.

The early return of respiration in resuscitation before the afferent paths are opened up, while the cardio-inhibitory tone returns relatively late, and not until some time after the restoration of the reflex paths to the cardio-inhibitory centre, indicates a difference in the nature of the natural excitation, whatever it may be, which supports the tonic discharge of the two mechanisms. The fact that in dogs after double vagotomy there is a tendency to recovery of cardio-regulative tone, although, as a whole, little or no tendency for the respiration to return to its normal rate (apart from those exceptional cases where the animals survived indefinitely)¹ is a further indication of a difference between the two centres, although the precise nature of the regulation of the heart rate after double vagotomy is in doubt.

The frequent return of a considerable degree of vaso-motor tone before the reflex paths to the vaso-motor centre have recovered their conductivity, and long before there is any restoration of cardio-inhibitory tone, points to a similar difference between the vaso-motor and the cardio-inhibitory mechanisms. For example, in the experiment of March 7 (second occlusion), where, as a preliminary procedure, the aorta was tied below the origin of the head arteries, practically no cardio-inhibitory tone had returned at a time when stimulation of the vagus caused good reflex inhibition of the heart, in addition to a depressor effect, and stimulation of the brachial caused a pressor effect. The blood pressure was well over 100 mm. (as high as 116 mm.), notwithstanding that the occlusion was a second one and the pressure only 28 mm. after release. The blood pressure had risen rapidly, and there could be no question that the vasomotor centre had recovered its tone. If the cardio-inhibitory tone depends upon the same factors as the vaso-motor tone, for example, on the mechanical or circulatory or nutritive changes in the centres, associated with changes of blood pressure, it is not easy to see why one should be absent and the other so distinctly present.

Since blood pressure generally returns only gradually to the normal in resuscitation, it might be supposed that the reason for the delay in the return of the cardio-inhibitory tone is that a certain blood pressure is necessary for its genesis. However, the last experiment quoted does not agree with this view, although, as a matter of fact, it is well known that a sudden rise of blood pressure, such as is produced

¹ STEWART: *Loc. cit.*

by ligation of the aorta beyond the origin of the head arteries will cause marked inhibition of the heart; and we have had the opportunity to verify this in the control experiments alluded to above. That intense stimulation of the cardio-inhibitory centre may occur when the blood pressure in the bulb is practically zero is demonstrated in every occlusion of the head arteries, as has already been mentioned. Here, of course, the excitation may be due to the abrupt change in the nutrition of the cardio-inhibitory centre, or to the accumulation of waste products, but the possibility is not excluded that the stimulation of afferent nerve endings by the high blood pressure in parts of the body where the circulation is still going on may play a part—perhaps the most important one. The fact that the cardio-inhibitory centre can be excited reflexly at a time when it has not regained tone is in favor of a reflex origin of its normal tone, since at this time a sustained reflex excitation may cause a continuous tonic discharge. Possibly the depressor may be the channel, or one channel, of this reflex excitation, the excitant being the changes in the pressure of the blood in the heart and aorta.

SUMMARY.

To sum up, we believe that our experiments indicate that, of the three mechanisms considered, the respiratory is the most purely automatic, and the least dependent upon reflex impulses for the origination of its discharge; the cardio-inhibitory mechanism is the most dependent upon such impulses, and the least automatic; the vaso-motor mechanism occupies an intermediate position. As regards the regulation of the discharge from all three, it is, of course, profoundly affected by reflex impulses.

The vaso-motor centre is, in general, capable of being affected by reflex stimulation at an earlier period in resuscitation than the respiratory centre, and the respiratory centre, in general, at an earlier period than the cardio-inhibitory centre.

The respiration returns at a time in resuscitation when it is still incapable of being influenced by stimulation of afferent nerves.

The vaso-motor centre may or may not have regained its tone at the time when it first becomes capable of being affected by reflex stimulation.

The cardio-inhibitory centre regains its power of being affected by reflex stimuli sooner than its tone.

The tone of the vaso-motor centre returns sooner than that of the cardio-inhibitory centre. Usually there is some return of vaso-motor tone before the respiration is restored.

The first reflex vaso-motor effects obtained by the stimulation of afferent nerves are pressor effects. This is true even of the vagus and the depressor.

Stimulation of the (left) phrenic nerve by the action current of the heart is often observed at a certain period in the occlusion and again at a certain period in the resuscitation. An important factor in the production of the phenomenon seems to be increased excitability of the nerve caused by anæmia.

ON THE MECHANISM BY WHICH WATER IS ELIMINATED FROM THE BLOOD IN THE ACTIVE SALIVARY GLANDS.

BY A. J. CARLSON, J. R. GREER, AND F. C. BECHT.

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I. INTRODUCTORY.

ASSUMING the correctness of the observations of Asher and his pupils, and of Barcroft and Bainbridge, to the effect that a greater quantity of lymph flows from the active than from the resting salivary glands, it would seem probable that new light could be thrown on some of the processes of salivary secretion by physical and chemical analysis of the lymph collected directly from the active gland. By comparing the osmotic pressure of the gland lymph and the serum, for example, it ought to be possible to demonstrate whether or not osmosis is the principle involved in the transfer of water from the blood to the tissue lymph spaces in the active gland. The present paper embodies the results of our experiments on this phase of the question.

That the saliva secreted by the submaxillary gland on stimulation of the chorda tympani nerve is the result of the action of nerve fibres directly on the gland cells is an established fact. Those who would doubt or deny the existence of secretory nerves to the salivary glands have only the principle of filtration to fall back on. Stimulation of the cranial secretory nerves produces vaso-dilatation in the glands. The dilatation of the arterioles increases the pressure in the capillaries, other factors being the same, and this would lead to a greater quantity of plasma filtering through the capillary walls into the lymph and intercellular spaces in the gland, assuming that mere filtration is a factor in lymph formation. But this would result, not in the flow of saliva, but in an increased output from the lymph channels leading from the gland, as the relatively thick walls of the secretory tubules would offer a greater resistance to the lymph filtering into the lumen

of the gland than the resistance (friction) offered to the flow of the lymph along the lymph channels. Defibrinated blood at body temperature may be passed through the living gland for hours, even at a greater pressure and rate than that during the stimulation of the chorda, yet not a drop of liquid appears in the salivary duct, or can be forced into the duct by compressing the gland. Furthermore, the maximum secretory pressure of the salivary gland, as well as the relation of the composition of the saliva to the rate of secretion, cannot be accounted for on the filtration principle. We do not, therefore, have to fall back on the evidence from the selective actions of atropin and other drugs to demonstrate the existence of the cranial secretory nerve fibres to the salivary gland cells.

The water and probably most of the salts of the saliva are taken out of the blood passing through the active gland. This is shown by the fact that the blood leaving the active gland contains a greater percentage of formed elements than the blood entering the gland, as well as by the fact that the total quantity of saliva that may be obtained from the gland by a single period of stimulation may greatly exceed the total volume of the gland itself. The water cannot, therefore, come from the gland cells; and since the valves prevent a back flow in the lymph channels, the water of the saliva must come from the blood passing through the gland. While the lymph bathing the secretory elements constitutes the immediate source of the water in the saliva, this lymph must in turn be replenished from the blood.

Now, what is the mechanism by which water (and salts) are transferred from the blood vessels into the tissue spaces in the active gland pari passu with the transfer of water from the lymph through the secretory cells into the saliva?

We may conceive of five possible mechanisms effecting this transfer :

1. The activity of the gland, except in the case of stimulation of the cervical sympathetic, is always accompanied by vaso-dilatation and increased flow of blood through the gland. Other things being equal, this increases the pressure in the capillaries, which in turn increases the formation of lymph, on the theory that lymph formation is a process of filtration. Even on the secretion theory of lymph formation, the greater pressure and rate of flow in the capillaries would in all probability lead to an increased formation of lymph, as these two factors would probably favor the secretory activity of the capillaries. If this is the mechanism involved, it is obvious that in

the case of vaso-dilatation in the gland in the absence of secretion, which may be brought about by the tying of the salivary duct, or by atropin, the augmented output of lymph from the gland should equal or exceed the quantity of saliva that would have been secreted in the same amount of time. But, according to Heidenhain, the vaso-dilatation in the gland following chorda stimulation after paralysis of salivary secretion by atropin is not accompanied by any increase in the output of lymph from the gland.

2. The active gland cells may produce and pass into the lymph some substance partaking of the nature of a "hormone," increasing the permeability or secretory activity of the capillary walls. On this principle we may or may not have an increased output of lymph from the active, as compared with that from the resting, gland. In case there is an increased lymph output from the active gland, this lymph may be of either higher or lower or the same osmotic pressure as the blood. If this principle is the one actually involved, we might expect to find this hormone in the lymph and blood from the active gland, and possibly a trace of it in the saliva.

3. The water and some of the solids may be removed from the blood in passing through the active gland by osmosis. This may be done in two ways: (1) the active gland cells may take water and salts from the lymph in the proportion found in the saliva, in this way concentrating the lymph and drawing water (and some salts) from the capillaries in virtue of the higher osmotic pressure of the lymph. This mechanism would stop all flow of the lymph from the gland for a time at the beginning, and in all probability during the whole of the activity, as the direction of the current would be towards the secretory cells, both from the blood capillaries and the lymph spaces.

(2) The active gland cells may liberate certain cleavage, or oxidative substances into the lymph in the tissue spaces, raising its osmotic pressure, which again would draw water from the blood stream into the lymph spaces. This mechanism would lead to an increased formation and flow of lymph from the active gland, as the continually accumulating osmotic products from the active cells would draw more water from the capillaries than that used by the gland cells in forming saliva. The hydrostatic pressure in the tissue spaces would be increased, and this in turn would lead to increased flow of lymph from the gland, *lymph of greater osmotic pressure than the blood*. In case the lymph flowing from the active gland has less osmotic press-

ure than the blood, osmosis cannot be the mechanism for the water transfer.

4. The transfer of some of the water and salts in the lymph through the gland cells into the saliva at the beginning of the glandular activity may, by the diminished tension and altered percentage composition, in other ways than by osmosis, increase the permeability or secretory activity of the capillary walls. This possibility might be in part put to the experiment, by continuous massage of the resting gland, removing the lymph as quickly as formed. If diminished hydrostatic tension in the lymph spaces leads to an increased transfer of water and salts from the capillaries into the lymph spaces, massage of the resting gland should produce an increased output of lymph from the gland.

5. The cranial secretory nerves may contain secretory fibres to the endothelial cells of the gland capillaries, which by central as well as peripheral co-ordinating mechanisms are thrown into activity synchronously with the secretory fibres to the gland cells.

II. EXPERIMENTAL METHODS.

For our purpose it is necessary to obtain the lymph directly from the salivary gland before it has passed through any lymph gland. The lymph may undergo changes in osmotic pressure in passing through a lymph gland. The results obtained by analysis of lymph from an active salivary gland after passing through a lymph gland would therefore be inconclusive.

In the dog the main lymph vessels leading from the submaxillary gland leave the gland at the hilus and enter the large retropharyngeal lymph gland situated at the level of bifurcation of the common carotid. This lymph gland also receives afferent lymph vessels from the submaxillary and the auricular lymph glands. The efferent vessel — sometimes two — from the retropharyngeal gland forms the main neck lymphatic. Several attempts were made to isolate and put a cannula into one or more of the lymphatics between the submaxillary gland and the retropharyngeal lymph gland for the purpose of collecting the lymph directly from the gland, but these attempts with one exception proved failures, despite the fact that the largest-sized dogs were used. The lymphatics can be isolated in the living animal, but they proved too small and delicate for the cannula. It would be possible to tie off all the afferent lymphatics to the retropharyngeal

gland, except those coming from the submaxillary gland, and collect the lymph from the large efferent lymphatics, but this method would be of no value, as submaxillary gland lymph would still have to pass through the lymph gland before being collected.

We then turned our attention to the parotid gland of the horse in hope of better success. This gland in the horse is of an enormous size, but not compact as the salivary glands of the dog. The horse parotid fills the space between the posterior border of the lower maxilla and the transverse process of the atlas, and extends from the base of the ear to the level of the bifurcation of the jugular and the glosso-facial veins. The ventral half of the gland is built around the jugular vein. The gland is easily exposed by removal of the skin and the thin parotido-auricularis muscle. Stenson's duct is of large calibre and readily isolated. The cranial secretory fibres are not readily isolated for stimulation, as they enter the gland on the inner surface near the border of the inferior maxilla. The nerve fibres ramify on the several branches of Stenson's duct in the gland, and may be stimulated at this part of their course.

The lymphatics leading from the parotid gland were first worked out by the aid of injection of India ink into the body of the gland. The numerous strong valves prevent injection backwards from the lymph glands towards the parotid. From four to six fairly large lymphatics pass from the parotid to enter the large group of guttural or pharyngeal lymph glands internal to it. These lymphatics leave the gland at diverse points, which vary from one specimen to another, but usually one or two of them come to the surface of the gland in the cavity formed by the jugular vein and pass posteriorly in this cavity to enter lymph glands at the level of the bifurcation of the jugular and the glosso-facial veins. Any one of the main lymphatics leaving the parotid gland is large and strong enough for a fair-sized cannula to be inserted and tied. The practical difficulty comes in the finding and isolation of them in the living animal. The lymphatic that usually emerges in the canal of the jugular vein is the most readily isolated, if care is taken to prevent all bleeding. Sometimes the vessel lies close to the jugular vein, at other times it is located deeper in the gland substance, but if bleeding is avoided the lymphatic may be recognized by the yellow color of the contents.

During the experiment the horse was kept on one side on an ordinary veterinary table under light chloroform anæsthesia.

The results of these experiments on the horse parotid seemed to

show that the activity of the gland does not increase the output of lymph from the gland, contrary to the results of Asher and Bainbridge on the dog's submaxillary, and of Moussu on the parotid of the horse. This led us to repeat Bainbridge's experiments on dogs in the following manner. The dogs were kept under light ether anæsthesia, no morphia being used. The cannula was placed in one of the neck lymphatics and adjusted so that the lymph would flow horizontally along the cannula and drop into a test tube or bottle. The rate of flow of the lymph was then determined by the number of drops per unit of time. The drops were recorded by means of an electro-magnetic signal on the kymograph. A cannula was inserted in the duct, and the chorda tympani exposed for stimulation on the side of which the lymph cannula was prepared. The drops of saliva were recorded on the drum in the same manner as the lymph. Added to this, the time signal and the signal in the circuit for the chorda stimulation, very accurate record of the lymph flow and the relation of the flow to the activity of the submaxillary gland was obtained. The following variations in the further procedure were used in some of the experiments. Experiments were run without any massage of head or neck. Massage was tried in the form of opening and closing the lower jaw by a mechanical contrivance that secured absolute uniformity in rate and amplitude of the movements. This method of massage was tried on the theory that the resting digastric muscle may obstruct the outflow of lymph from the gland as the efferent lymphatics pass in close proximity to the muscle. The movements of the lower jaw also exert some tension variation on the submaxillary and the lymph glands adjacent to it. This device was made use of in connection with recording the lymph flow from the neck lymphatics on both sides, the submaxillary gland on one side, only, being thrown into periodic activity by chorda stimulation. The rate of flow of lymph from the resting side affords a check on variations due to changes in the degree of anæsthesia, muscular tonus, etc., in the head region. In some experiments the submaxillary gland was massaged continually through the skin, while the submaxillary was thrown into periodic activity.

The difficulties attendant on the clotting of the lymph in the cannula might be avoided by the injection of hirudin into the blood, but as this procedure would have resulted in continuous bleeding from the operation wounds, it was not made use of. The lymph from the neck ducts of the dog does not clot very rapidly, and the small clot

that sometimes forms in the cannula is not firm enough to appreciably retard the flow, especially if as large-neck cannula as the ducts permit are employed. When visible clots were formed in the cannula, the experiment was interrupted and the clot removed.

The ordinary Beckmann apparatus was employed for the freezing-point determinations.

III. THE FORMATION OF LYMPH IN THE RESTING SALIVARY GLAND.

It is assumed by Asher, Barcroft, and Bainbridge that there is no appreciable output of lymph from the resting salivary glands. The question cannot be definitely settled for the dog until we have succeeded in isolating the lymphatics and measuring the lymph that comes directly from the gland. Observations on the lymph flow in the neck lymphatics are of no avail, as it is impossible to say whether or not the resting salivary glands contribute to the flow. In the case of dogs in good condition and under light ether anæsthesia, there is always a free flow of lymph from the neck ducts. If the anæsthetic is pushed till the blood pressure falls markedly, or in the case of dogs in poor condition exhibiting low blood pressure even under light ether anæsthesia, there is usually no free flow of lymph from the neck ducts, even though the neck and laryngeal respiratory muscles are as active as in the dogs that give the spontaneous flow. It would therefore seem that the lymph flowing without massage from the neck ducts cannot come exclusively from the laryngeal respiratory muscles. All the other structures are quiescent, and the inference is that some of the lymph comes from the resting organs in the head region, including the salivary glands. But, as just stated, this evidence is not conclusive, as the lymph may be produced in the lymph glands themselves.

In the horse the question can be put to the experimental test. In all the horses worked on the parotid gland was quiescent, except when the secretory nerves were stimulated, or pilocarpin was injected into the circulation. By quiescence of the gland we mean that there was no evidence of saliva being formed by the glands. The gland was, of course, living, and the respiratory and metabolic processes were undoubtedly going on in the gland, just as they go on in the resting muscle. But the gland was resting so far as regards its special functions of secreting saliva.

In seven out of the nine experiments (Table V) there was a spon-

taneous flow of lymph from the resting parotid gland. This lymph was not forced out of the gland by movements of the head or by artificial massage of the gland, as it flowed in the absence of both movements and massage, although these factors increased the flow. Under the conditions of the experiments the lymph flow from the parotid lymphatics was not rapid. In some of the experiments it was very slight indeed. Nor was the lymph under high pressure. But it must be remembered that the lymphatic under observation drained only a small portion of the gland. In general, the horses that yielded the greater spontaneous flow from the lymph ducts in the neck also yielded a greater flow from the parotid lymphatics, and *vice versa*. The specimens that yielded no spontaneous lymph from the resting parotid gave no spontaneous flow from the neck ducts. In these specimens a small quantity of lymph was forced from the resting gland by direct massage of the gland.

The specimens exhibiting the greatest spontaneous lymph, both from the neck ducts and the parotid ducts, had relatively high venous pressure, and those exhibiting scanty spontaneous flow of lymph from both sources had invariably a low venous pressure. The specimens that yielded a flow of lymph from the neck as well as the parotid on massage only had the lowest venous pressure of any of the animals worked on. The factors determining the spontaneous flow of lymph from the quiescent head region appear, therefore, to be the same as the factors determining the spontaneous flow of lymph from the resting parotid glands. From our observations it is clear that flow of lymph from the quiescent organs in the head region is greater the higher the venous pressure, and that below a certain degree of venous pressure the flow of lymph from the resting organ ceases.

This direct relation between venous pressure and lymph flow from the resting organs may on first sight seem to favor the filtration theory of lymph formation, but they certainly do not constitute a demonstration of it, as the pressure and rate of blood flow in the capillaries may be conditions for the secretory activity of the endothelial cells; or the factors producing a low blood pressure, such as deep anæsthesia, or general poor and weakened condition of the animal, may also depress the secretory activity of the capillaries directly.

It should be stated that these experiments were not performed on horses in prime condition, as the resources of our laboratory are insufficient for the necessary expense. The animals used were "killers," specimens assigned to the boneyard because of old age, disease of

the legs or hoofs, etc. These specimens are generally in poor condition. If horses in prime condition had been available, we would no doubt have obtained a greater spontaneous flow of lymph from the resting parotid glands. The anæsthetic probably also diminishes the lymph formation or flow from a resting organ, especially if lymph formation is a process similar to secretion. A permanent fistula in one of the parotid lymphatics would therefore probably show the greater flow of lymph from the resting gland than that observed in our experiments.

The fact that lymph is produced in the perfectly quiescent organ renders necessary a limitation of Asher's theory of lymph formation. Asher holds that lymph is a product of organ activity, and may therefore be designated as a secretion of the organ. This may be true in its entirety for some organs, but it is not true for the salivary glands, as lymph is formed in these glands in a resting condition. So far as these observations go, Asher's theory may still be true for the salivary glands to the extent that the activity of the glands may be accompanied by an increased output of lymph from the gland.

IV. THE ACTIVITY OF THE GLANDS DOES NOT INCREASE APPRECIABLY THE OUTPUT OF LYMPH FROM THE GLANDS.

1. *The literature.* — Our results both on dog and horse touching this point are in accordance with Heidenhain's original statement,¹ but contrary to the results of later observers. Heidenhain worked mainly on the possible formation of lymph in the submaxillary gland after injection of atropin, in order to determine whether the passage of water through the capillaries in the active gland is due to the dilation of the arterioles and the consequent increased pressure in the capillaries. Asher and Barbéra² publish two experiments on the rate of the lymph flow in the neck lymphatics of the dog with the salivary glands in resting and in active state. The animals were kept under morphia anæsthetic; the neck and head massaged; and the salivary glands thrown into activity reflexly by placing acidified filter paper in the dog's mouth. Their results (Table IV) indicate an augmented flow of lymph following the acid stimulation of the mouth. It is not clear from their paper in what way uniformity in the massage

¹ HEIDENHAIN: *Archiv für die gesammte Physiologie*, 1874, ix, p. 346; 1878, xvii, p. 22. HERMANN'S *Handbuch*, 1880, v, p. 73.

² ASHER and BARBÉRA: *Zeitschrift für Biologie*, 1898, xxxvi, p. 199.

of the neck was secured. For their observation to stand as a demonstration of an increased flow of lymph from the active gland, it must be shown that the acid in the mouth did not by direct absorption alter the permeability or secretory activity of the capillaries in adjoining structures, or reflexly alter the blood pressure or the tonus of the muscles of the head and neck.

Bainbridge¹ obtained the same results as Asher and Barbéra by different methods. The dogs were under morphia and A. C. E. mixture; the submaxillary gland thrown into activity by direct stimulation of the chorda or by pilocarpin, and the lymph flow determined for definite periods, the neck and gland being massaged at intervals. Bainbridge's conclusions, based on the experiments of four animals, are to the effect that the greater the flow of saliva the greater the augmentation of lymph from the neck duct. The stimulation of the cervical sympathetic gave, according to Bainbridge, as great an increase in the lymph flow as the stimulation of the chorda, although the former produced but a scanty secretion of saliva or at times no secretion at all. The augmentation of the lymph flow on chorda stimulation follows as promptly as the appearance of the saliva, and ceases with the stimulation unless the salivary secretion continues. Asher and Barbéra and Bainbridge agree in the statement that there is only a scanty flow of lymph from the neck ducts, or no flow at all in the absence of massage.

Bainbridge's conclusion that "stimulation of the cervical sympathetic leads to an increased flow of lymph from the submaxillary gland" certainly does not follow from the observations, because the cervical sympathetic produces vasomotor changes in many other of the structures that contribute to the neck lymph. The sympathetic may even send motor fibres to the muscular walls of the lymphatics themselves analogous to the innervation of the lacteals, as observed by Bert and Lappont, and Camus and Gley.²

The only inconstant factor in Bainbridge's experiments appears to be the massage, but the differences obtained in the rate of lymph from the neck duct in the case of the resting and the active submaxillary gland is so considerable that it is difficult to understand how it might be due to differences in the massage designed to be uniform.

Moussu³ reports briefly on three experiments on the parotid of the

¹ BAINBRIDGE: *Journal of physiology*, 1900, xxv, p. 79.

² CAMUS and GLEY: *Archives de physiologie*, 1894, ii, p. 454.

³ MOUSSU: *Comptes rendus de la société de biologie*, 1900, liiii, p. 288.

horse, and two experiments on the parotid of the ox, the lymph being collected from the common neck ducts. In the horse the gland was thrown into activity by pilocarpin, in the ox the secretory nerves were stimulated directly. Moussu did not find any marked difference in the output of the lymph from the neck lymphatics in the resting and in the active condition of the gland, and concludes that if the activity of the gland increases the output of lymph from it, the increase is so slight as to be negligible. The observations of Barcroft¹ on the loss of water of the blood in passing through the active submaxillary gland of the dog seem to point to an increased output of lymph by the active gland. Barcroft seems to have shown that more water leaves the active gland than appears in the saliva. If such is the case, the excess of water leaving the blood must go to swell the total amount of lymph from the gland. Barcroft's results are, however, not conclusive. With the method of erythrocyte count his results are negative in three out of the eight experiments; in one experiment (8) nearly twice the amount of water appeared in the saliva than seemed to have left the blood. By his potassium ferro-cyanide method his four experiments show, if anything, that a slightly greater amount of water appears in the saliva than that which leaves the blood. By the calorimetric method he obtains positive results in three out of four series of experiments, but even in these three series some of the separate observations came out negative; that is, the amount of saliva appears greater than the amount of water withdrawn from the blood. Of the eight separate tests in series III, three give negative results, one comes out positive, while the remaining four show that the same quantity of water that left the blood appears in the saliva. It seems to us that these are rather precarious data on which to base positive conclusions. Furthermore, no observations were made to determine whether water leaves the blood in passing through the resting gland. In case lymph is formed in the resting gland, and we have seen that such is the case of parotid of the horse, water must necessarily be withdrawn from the blood passing through the gland. Barcroft's observation cannot therefore be taken to show that the activity of the gland augments the output of lymph from it. Falloise² failed to obtain an augmented flow of lymph from the active liver and pancreas in the dog, the glandular activity being induced by the injection of secretin

¹ BARCROFT: *Journal of physiology*, 1900, xxv, p. 479.

² FALLOISE: *Bulletin de Académie de Médecine de Belgique*, 1903, xvi, p. 49, cited from *Centralblatt für Physiologie*, 1904, xvii, p. 194.

from which the depressor substance had been removed.¹ Falloise's observations have been contradicted by those of Bainbridge, and again confirmed by the recent extensive experiments of Wertheimer.² Asher³ considers that the observations of Falloise are completely overthrown by those of Bainbridge, but this does not seem to be the case in view of Wertheimer's results.

2. **The results on dogs.**—The results of our experiments on dogs are summarized in Table III, and two typical series are given in detail in Tables I and II. Contrary to the statements of Asher and Bainbridge, we never failed to obtain spontaneous flow of lymph from the neck lymphatics in our dog experiments, except when the anæsthetic was pushed and the blood pressure became feeble, or in the case of dogs in poor condition. After this later fact had become evident to us, we never used dogs except when in prime condition. Needless to say, we also selected the largest specimens available.

In our first four experiments no massage of the neck or head region was employed. The lymph flow from the neck ducts was therefore entirely spontaneous. The only variable factor that may enter in those experiments is the degree of anæsthesia. The lymph flow from one of the neck lymphatics only was recorded. It will be seen from Table III that in Experiments 1 and 3 the average lymph flow is slightly slower during than before the stimulation of the chorda on the same side, while in Experiments 2 and 4 we have the relation reversed. But the difference in either case is too slight to be of any significance, and the fact that the variation appears in both directions goes to show that it bears no relation to the state of activity or rest of the submaxillary gland.

In all the subsequent experiments some form of massage was resorted to in order to circumvent the possibility of the lymph formed in the submaxillary gland being prevented from reaching the neck ducts, when the head and neck region remained perfectly at rest except for the pulse and the respiration movement. The only feasible way that massage may be applied to the head and neck region with absolute uniformity is by opening and closing movements of the lower jaw, imitating the chewing movements of the animal. In Experiments 5 to 7 this massage was performed by hand to the uniform rate of a metronome, the lower jaw being tied

¹ BAINBRIDGE: *Journal of physiology*, 1904, xxxii, p. 1.

² WERTHEIMER: *Journal de physiologie et de pathologie*, 1906, viii, p. 804.

³ ASHER: *Biochemisches Centralblatt*, 1906, iv, p. 1.

to the fixed upper jaw so as to secure absolute uniformity of the amplitude of the excursions of the former. This form of massage increases the flow of lymph from the neck ducts. It necessarily produces some changes in the pressure on the submaxillary gland, situated as it is in the angle of the jaw. By the alterations in the tension of the digastric muscle it seems probable that the flow of lymph from the submaxillary gland into the neck ducts must be facilitated as the efferent lymph channels for the gland came in close contact with that muscle. But even this form of massage has the personal equation of the vigor of the movements, so that while the rate and amplitude of the movements remain the same the operator may alter the rate of lymph output at will by altering the force of the movement. This factor was removed in all the subsequent experiments (except 11 and 13) by procuring the movements of the lower jaw by a mechanical device.

In Experiments 6, 7, 8, 10, 11, 12, and 13 the additional check was introduced of also recording the lymph flow from the side of the head and neck region on which the submaxillary gland was at rest throughout the time of observation. We reasoned that this device would serve as a check on the variable factors of degree of anæsthesia, blood pressure, muscular tonus, and the rate and intensity of the respiratory movement.

In Experiments 11 and 13 the submaxillary gland was massaged continuously by kneading through the skin. As we have stated before, it is impossible to secure absolute uniformity in this form of massage, but it was employed in these experiments to meet the objection that in the absence of such massage the lymph formed in the active gland does not get into the neck lymphatics.

By examinations of Experiments 6, 7, 8, 10, 11, 12, and 13 it will be seen that the output of lymph from the side of the resting submaxillary gland exhibits as great variations as that from the side on which the gland was thrown into periodic activity by chorda stimulation. In Experiments 5 and 10 there is a slight increase in the output of lymph during the period of stimulation as compared to that before the stimulation; in the rest of the series the relation is reversed. Nor is there any increase in the lymph output during the period following the stimulation as compared with that preceding the stimulation. As noted by Asher, there is a gradual diminution of the output of lymph from the neck ducts during the course of our experiments. This diminution is apparent in some

of our experiments in the lower average rate of flow for the periods following the stimulation as compared to that before the stimulation.

It seems to us that this series of experiments comprises a sufficient number of animals to warrant a general deduction on this question for the dog. Moreover, checks have been introduced and variable factors eliminated as far as possible. The conclusion seems therefore justified that in the dog there is no appreciable increased output of lymph from the active as compared to that from the resting submaxillary gland. If there is any increased output at all in the active gland, it is too slight to be demonstrated by our present method.

3. **The results on the parotid of the horse.** — The horse parotid was thrown into activity both by the stimulation of the secretory nerves and by the injection of pilocarpin into the circulation. The secretory nerves were usually stimulated on their course along Stenson's duct. Such stimulation always results in the flow of saliva from the duct. But as it is not certain that the stimulation involves the secretory nerves to every part of the gland, and, further, since the lymphatic worked with drains only a part of the gland, we do not consider our results from electrical stimulation of the secretory nerves conclusive. These objections do not apply to the pilocarpin stimulation. But in the case of positive results with pilocarpin there might be another interpretation of the increased lymph output than that given by Asher and his school. The pilocarpin may stimulate the secretory activity of the capillary cells, and the increased lymph output thus brought about, rather than as a consequence of the activity of the salivary glands proper. However, as our results are negative, we need not consider this possibility at present.

Out of our nine experiments on horses in no case were we able to secure an appreciable increase or decrease in the output of lymph from the gland in the state of activity as compared to that in the state of rest. The subjects giving a scanty flow of lymph from the resting gland exhibited the same scanty flow when the gland was thrown into intense activity by pilocarpin; and in the case of the specimens that yielded a fairly active spontaneous flow from the resting gland the flow was not appreciably augmented when the gland was thrown into activity. Subjects yielding scarcely or practically no spontaneous flow from the resting gland did not yield any greater quantity on the pilocarpin injection. These statements apply both to the spontaneous flow as well as to the rate of the lymph on direct

massage of the gland, despite the necessary variability of the massage. Our results on the horse parotid thus point to the same conclusion as our data on the dog's submaxillary. No appreciable increase in the lymph output from the gland accompanies glandular activity.

We made no observations on the effect of pilocarpin injection on the rate of the lymph flow in the common neck lymphatics of the horse.

We cannot account for the discrepancy between our results and those of Asher and Bainbridge, except as indicated in connection with the statements of their results. In the case of our experiments on the parotid of the horse, it might be objected that in the necessary dissection some of the lymph spaces and smaller channels in the gland were ruptured so that the lymph formed escaped into the wound. That this is not the explanation of our negative results is shown by the fact that we actually obtained a flow of lymph from the parotid lymphatics, both in the active and the resting gland. As regards our work on the dog's submaxillary, the only possible objection that may be made to our conclusion is that the lymph formed in the gland in activity was prevented from reaching the neck lymphatics. If such was the case, what became of this lymph? On the basis of Barcroft's and Bainbridge's conclusion that one tenth of the water leaving the blood in the active gland does not enter the saliva secreted, in some of our experiments 15 to 20 c.c. of lymph must have been formed in the gland, a quantity greater than the total bulk of the gland. And yet the gland did not even show the beginning of œdema. We might take refuge in reabsorption of the lymph by the capillaries, if it did not force us to the absurd position that the water enters the tissue spaces from the blood, and the blood from the tissue spaces, through the same capillary cell at the same time.

V. THE OSMOTIC PRESSURE OF THE LYMPH FROM THE NECK LYMPHATICS.

I. **The literature.** — The observations of Hamburger¹ on the osmotic pressure of the lymph from the neck lymphatic of the horse, and those of Leathes² on the lymph from the thoracic duct of the

¹ HAMBURGER: Beiträge zur pathologische Anatomie, 1893, xiv, p. 446; Osmotischer Druck und Ionenlehre, 1904, ii, p. 36.

² LEATHES: Journal of physiology, 1895, xix, p. 1.

dog, seem to show that the osmotic pressure of lymph is greater than that of the serum of the same animal. This generalization has been made, and found place in the text-book literature. Hamburger did not use the cryoscopic method, but a method devised by himself, based on the percentage of water that must be added to serum and lymph to cause laking of the erythrocytes by hypotonicity. He con-

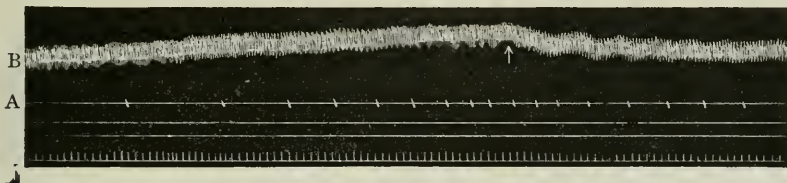


FIGURE 1.—One third the original size. Tracing of the blood pressure in the left carotid (*B*) simultaneous with recording the rate of the spontaneous flow of lymph from the right neck lymphatic (*A*). Dog. Light ether narcosis. ↑, animal put into deeper anæsthesia. Showing a greater output of lymph from the neck duct *pari passu* with the greater the blood pressure. Time, seconds.

cludes that the osmotic pressure of lymph is 13 per cent greater than that of serum. It does not appear from Hamburger's published results on how many horses these conclusions were based.

Leathes collected lymph from the thoracic duct of anæsthetized dogs, and by using the cryoscopic method found its osmotic pressure to be slightly higher than the serum (-0.005° C. to 0.01° C.); and this relation was maintained even when the osmotic pressure of the serum was altered artificially.

2. **The osmotic pressure of the neck lymph of the dog.**—In our experiments the cryoscopic method was the only one employed. The results tabulated in Table IV go to show that the osmotic pressure of the lymph from the neck lymphatics under the conditions of our experiments is usually less than that of the serum. Thus in all the experiments, save 8 and 9, the lymph has a lower osmotic pressure than the serum. In Experiments 8 and 9 the osmotic pressure of the two is practically the same, or that of the lymph is a little higher.

Experiments 8, 10, 11, 12, and 13 show that the osmotic pressure of the lymph from the neck ducts may be different at the beginning from that at the end of an experiment. As already stated, our animals were kept under light ether anæsthetic throughout the experiments. The variations in the osmotic pressure of the lymph cannot therefore be due to variations in the activity of the organs drained by the neck lymphatics.

In all the experiments (except 12 and 13) the right chorda tympani was stimulated at intervals to note the effect of the activity of the submaxillary gland on the lymph flow. But this factor accounts neither for the variations in the lymph on the same side during the course of one experiment nor for the differences exhibited by the lymph from the right and left sides of the same animal. Thus in Nos. 7 and 11

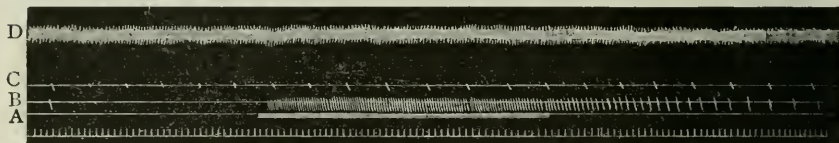


FIGURE 2.—About one fourth the original size. Dog. Light ether anæsthesia. *A*, stimulation of right chorda tympani. *B*, record of drops of saliva from the right Wharton's duct. *C*, record of spontaneous flow of lymph (drops) from the right neck lymphatic. *D*, record of blood pressure in the left carotid. Showing the uniform spontaneous flow of lymph from the neck ducts with high but steady blood pressure, and the failure of the activity of the submaxillary gland to influence this lymph output. Time, seconds.

the lymph from the left side, with all the salivary glands at rest, has a lower osmotic pressure than the two samples of lymph from the right side with the submaxillary gland thrown into periodic activity by chorda stimulation. In No. 8 the reverse is the case of the lymph first drawn. In subsequent samples the lymph from the right side becomes gradually more concentrated, while that from the left side remains practically the same. Again, in No. 10, first portion of the lymph from the left side, with all the salivary glands at rest, is more concentrated than the lymph from the right side with the submaxillary gland made active by chorda stimulation. The lymph from both sides collected towards the end of the experiment, however, is more dilute instead of more concentrated as in No. 8. It will be noted that the two samples of blood drawn at the beginning and at the end of the experiment exhibit the same relative difference in the osmotic pressure.

In the first six experiments the osmotic pressure of the lymph was compared with that of the serum at the end of the experiment. It occurred to us, however, that the concentration of the blood of the animal kept under anæsthetic for from two to four hours may vary during the experiment. From 75 c.c. to 150 c.c. of saliva were eliminated from the animal during the experiment. The water eliminated in respiration may or may not be compensated for by the solids elimi-

nated in the urine, and by the products of tissue metabolism. The submaxillary saliva may have less than half the osmotic pressure of the blood, and this difference may not be exactly counterbalanced by the osmotic factor of the end products of glandular activity reaching the blood stream through the capillaries or the lymph channels. The dilute neck lymph is eliminated. It is, moreover, probable that the co-ordinating mechanisms that serve to maintain a fairly constant

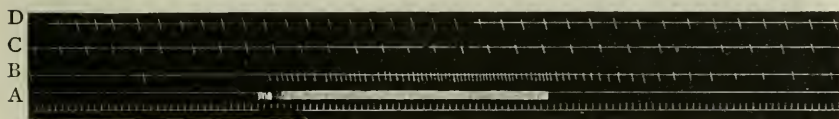


FIGURE 3. — One third the original size. Dog. Light ether anæsthesia. *A*, direct stimulation of the right chorda tympani. *B*, record of drops of saliva from the right Wharton's duct. *C*, records of drops of lymph from the left neck lymphatic. *D*, records of drops of lymph from the right neck lymphatic. Massage by opening and closing movements of lower jaw at the rate of thirty-six per minute by means of mechanical device securing absolute uniformity in rate, amplitude, and vigor of movement. Showing failure of the activity of the submaxillary gland to influence the output of lymph from the neck duct. Time, seconds.

concentration of the serum in the normal animal may partly break down in the anæsthetized animal. For that reason samples of blood were drawn both at the beginning and at the end of the experiments. In no case did the two samples of blood show the same osmotic pressure. In Nos. 7, 8, 9, and 13 the osmotic pressure of the blood at the end of the experiment was considerably greater than at the beginning, while in No. 10 relations are reversed, but the differences slight. These differences are too great to fall within the limits of experimental error. The cause of the variations must be left for further investigation.

In some of the experiments the osmotic pressure of the chorda saliva from the submaxillary gland was also determined for comparison. On the whole, it is evident from Table IV that the more concentrated saliva goes with the more concentrated serum, — a fact in support of recent observations of Japelli,¹ but this is not invariably the case, as will be seen by comparing Experiment 5 or 7 with Experiment 8.

It has been shown in the previous section that the active salivary gland does not contribute any more lymph to the neck lymphatic than does the resting gland. The further fact that all the lymph as

¹ JAPELLI: *Zeitschrift für Biologie*, 1906, xxx, p. 398.

collected by us passes through one or more lymph glands between the tissues and the collecting cannula makes these data of no value in the quest for the osmotic pressure of the lymph from the active salivary gland. The concentration of this lymph may be the same, or either greater or less than the serum, and the osmotic pressure of the composite lymph from the common neck duct still be that recorded

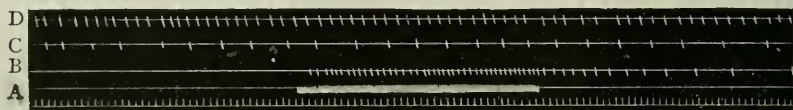


FIGURE 4.— One third the original size. Dog. Light ether anæsthesia. *A*, direct stimulation of the right chorda tympani. *B*, record of drops of saliva from the right Wharton's duct. *C*, record of drops of lymph from the left neck lymphatic. *D*, record of drops of lymph flowing from the right neck lymphatic. During the experiment the right submaxillary gland and side of neck massaged by direct and continuous kneading. Showing failure of activity of submaxillary gland to influence the flow of lymph from the neck lymphatic. Time, seconds.

in Table IV, as we do not know the concentration of the lymph from the other contributaries, nor the changes undergone by the lymph in the lymph glands.

Leathes found the osmotic pressure of the lymph from the thoracic duct of the dog to be slightly higher than that of the serum. We find that the lymph from the neck lymphatics of the anæsthetized dog has usually a lower osmotic pressure than the serum. There is no necessary contradiction between these conclusions, as the lymph from the viscera may be more concentrated than that from the head and neck. It is known from Starling's work that the lymph from the liver is much more concentrated than that from the lower extremities. Two experiments were made to test this possibility, and the results support the view just stated. By referring to Table IV, Nos. 12 and 13, it will be seen that thoracic lymph is in both cases considerably more concentrated in the elements that make up the osmotic pressure factor than is the neck lymph. In No. 12 the freezing-point of the thoracic lymph is the same, in No. 13 considerably lower than that of the serum, which is in line with Leathes' results.

3. **The osmotic pressure of the lymph from the neck lymphatics of the horse.** — The osmotic pressure of the lymph from the neck lymphatics of the horse under chloroform anæsthetic is a variable factor (Table V.). It may be considerably less than that of the serum (Nos. 1, 5, 9), slightly higher than the serum (Nos. 6, 7, 8), or practically the same as the

serum (Nos. 2, 3, 4). These results do not bear out Hamburger's conclusion that the lymph from the neck of the horse has 13 per cent higher osmotic pressure than the serum, although Hamburger's statement is true for individual cases, and may in fact be uniformly true for the horse when not under the anæsthetic.

In Experiments 1, 2, 3, samples of blood were taken at the end of the experiment only. But as great quantities of water were eliminated from the blood in the secretion following the injection of pilocarpin, it seemed possible that the concentration of the blood may vary during the experiment. Subsequently samples of serum were taken both at the beginning and at the end of the experiment. In four of the experiments (6, 7, 8, 9) the serum drawn at the end is slightly more concentrated than that drawn at the beginning; in the other cases (4, 5) there is no appreciable difference. The same has already been noted in the experiments on dogs. The usual result is an increase in the concentration of the blood during the progress of the experiment. The mechanism of this change remains to be worked out.

In some of the experiments the osmotic pressure of the serum is greater than that of the neck lymph by more than one atmosphere (Table IV, No. 1; Table V, Nos. 1, 5, 9). In several other cases in the dog the difference amounts almost to one atmosphere (Table IV, Nos. 2, 3, 4, 6, 11). That is to say, the force by which the serum would tend to draw water from this lymph through the capillary walls is many times greater than the force of the hydrostatic pressure within the blood capillaries by which serum may filter through the capillary walls into the lymph spaces. We are here face to face with the old problem encountered in the secretion of urine, only the relations are reversed. The kidney elements eliminate a secretion of vastly higher osmotic pressure than the serum, while here the capillaries apparently eliminate a secretion so much more dilute than the serum that a greater amount of energy is required than that which can possibly come from the pressure of the blood in the capillaries.

The criticism may be passed on some of these data that in Experiments 1 to 6 in Table IV, and in Experiment 1 in Table V, the comparison is made with serum drawn at the end of the experiments only; and since under the conditions of our experiments the serum drawn at the end of experiments usually showed a greater osmotic pressure than that drawn at the beginning, our figures probably show a greater difference in favor of the serum than actually existed at the time of

formation of the lymph. This criticism is valid, but it does not apply to Experiments 7 and 11, Table IV, nor to Experiments 5 and 9, Table V, in which cases samples of serum were drawn both at the beginning and the end of experiments, so that the mean osmotic pressure can be obtained in case the two samples show any difference.

In Experiments No. 5 and 9, Table V, the osmotic pressure of the serum exceeded that of the neck lymph by 960 and 1,348 mm. Hg., respectively. Admitting that the hydrostatic pressure in the blood capillaries in the head region is as high as 100 mm. Hg., we still have a force equal to the pressure, more than one atmosphere preventing lymph of this character leaving the capillaries. And yet this lymph is formed and flows spontaneously from the neck ducts. Obviously, both filtration and osmosis fail to account for the formation of this lymph.

There is, however, one possibility of bringing this fact in line with the filtration theory. This lymph may, namely, have been diluted by the tissue cells absorbing the constituents that go to make up the osmotic factor.¹ This does not seem probable to us, but the idea may be put to the experimental test by determining the particular elements that account for the lymph deficiency. If the difference is due mainly to the inorganic constituents, the explanation becomes highly improbable, as these could not be absorbed nor retained to such an extent by the tissue cells.

VI. THE OSMOTIC PRESSURE OF THE LYMPH FROM THE PAROTID GLAND OF THE HORSE.

For the comparison of the osmotic pressure of the serum with that of the lymph from the parotid gland we encountered the difficulty of securing enough lymph from the active gland for the determinations. As has been stated, some lymph usually flows from the resting as well as from the active gland, but we usually had to resort to massage of the gland in order to obtain the required amount. But even with the aid of massage we were unable in four out of the nine experiments to secure a sufficient quantity. If the lymph could have been collected from all lymph ducts leading from the gland, we should not have encountered this difficulty.

We have for this reason records from five horses only, and as we

¹ Suggested by our colleague Prof. G. N. STEWART.

do not wish to base final conclusions on so meagre data, we regard this section in the way of a preliminary report.

By referring to Table V it will be seen that the osmotic pressure of the parotid lymph is not a constant factor. It may be slightly higher than that of the serum (2, 4), and again considerably lower (1, 3, 7). The causes of these variations require further study.

These results go to show that osmotic pressure is in all probability not the mechanism by which water is eliminated from the blood capillaries of the active gland. The factor is not an increased osmotic tension in the gland cells themselves, for in that case the lymph obtained directly from the active gland by massage or spontaneous flow must invariably have a higher osmotic pressure than the serum, even if no more lymph flows from the active than from the passive gland. Nor is the mechanism an increased osmotic pressure of lymph in the tissue spaces due to the products of the active gland cells discharged into this fluid, for in that case the lymph from the gland must also have a higher osmotic pressure than the serum. While an osmotic pressure of the gland lymph higher than that of the serum would not necessarily prove that the mechanism of elimination of water from the capillaries is osmosis, the reverse relation demonstrates that this cannot be the factor.

The elimination of osmosis as a possible mechanism effecting the transfer of water from the blood capillaries to the lymph in the tissue spaces leaves the "hormone" mechanism and the secretory nerves in possession of the field. Nerve-endings have been described on the blood capillaries in various parts of the body. Sihler¹ has described them particularly in the case of the submaxillary gland. These nerves on the endothelium of the blood capillaries may be secretory in function and involved in the formation of lymph, but we have no clear evidence bearing on this point. The "hormone" mechanism appears to us the most probable one, our work being now directed to secure data proving or disproving it.

SUMMARY.

1. There is a spontaneous flow of lymph from the quiescent parotid gland of the horse, under chloroform anæsthesia, and probably also from the resting salivary glands of the dog.
2. In the case of dogs under light ether anæsthesia there is a spon-

¹ SIHLER: *Journal of experimental medicine*, 1901, v, p. 493.

taneous flow of lymph from the neck lymphatics, even when all the salivary glands are in a state of rest, and activity of the submaxillary gland caused by stimulation of the chorda tympani does not appreciably affect this lymph flow. Nor is such influence discernible on massage of the head and neck region. Activity of the parotid gland of the horse, caused by stimulation of the secretory nerves, or by pilocarpin, does not alter appreciably the lymph flow from the gland.

3. The osmotic pressure of the lymph from the active parotid gland may be higher, the same, or lower than that of the serum. The fact that it may be lower than that of the serum eliminates osmosis as the mechanism by which water is removed from the blood capillaries in the active gland. The mechanism effecting this transfer is probably a "hormone," which, produced by the active gland cells and passed into the lymph of the tissue spaces, increases the secretory activity of the capillaries.

4. The osmotic pressure of the neck lymph in the dog is usually lower than that of the serum. The osmotic pressure of the lymph from the thoracic duct is probably generally higher than that of the neck lymph.

5. The osmotic pressure of lymph from the neck lymphatics, both in the dog and the horse under anæsthesia, may be so much lower than that of the serum that the force tending to draw water from the lymph into the capillaries greatly overbalances the highest possible hydrostatic pressure in the capillaries. In such cases neither filtration nor osmosis suffices to account for the lymph formation or the lymph flow.

6. The osmotic pressure of the lymph from the head and neck region is very variable, and does not seem to bear any constant relation to that of the blood. It may vary on the two sides of the neck in the same animal. It may vary during the course of an experiment, the usual variation being in the direction of increased osmotic pressure the longer the animal is kept under anæsthesia. In this variability in osmotic concentration the neck lymph resembles the products of secretory activity of the salivary, the gastric, and the pancreatic gland.

TABLE I.

Detail of Experiment No. 6, Table III. Dog under light ether anæsthesia. Collecting cannula in both right and left neck lymphatics. Cannula in the right Wharton's duct, and the chorda tympani isolated on the right side. Salivary gland on left side quiescent throughout the experiment. Continuous massage by opening and closing movements of the lower jaw at the rate of 36 per minute. The upper jaw being fixed, and the amplitude of the excursions of the lower jaw being rendered uniform by tying the latter to the former so as to allow maximum movements. The periods of stimulation refer to the stimulation of the right chorda tympani.

No. of observation.	Periods of observation.	Time of observation in minutes.	Drops of lymph per min.		Drops of saliva per minute.
			L. lymph.	R. lymph.	
I	Before stimulation	4.00	1.55	1.75	0
	During stimulation	6.90	1.00	1.77	36
	After stimulation	8.90	1.46	2.00	10
II	Before stimulation	8.90	2.00	0
	During stimulation	1.45	1.55	33
	After stimulation	9.60	2.00	5
III	Before stimulation	4.87	2.46	1.93	0
	During stimulation	1.30	2.84	1.40	32
	After stimulation	6.60	2.53	1.53	15
IV	Before stimulation	8.60	2.59	1.32	0
	During stimulation	0.85	2.47	1.00	26
	After stimulation	8.10	2.22	1.06	6
V	Before stimulation	6.80	2.64	1.20	0
	During stimulation	3.00	2.46	1.10	29
	After stimulation	4.70	2.40	1.27	8
VI	Before stimulation	4.15	2.16	1.75	0
	During stimulation	2.80	1.90	1.60	24
	After stimulation	3.20	1.87	1.87	8
VII	Before stimulation	3.10	1.61	1.55	0
	During stimulation	2.46	1.62	1.21	19
	After stimulation	5.36	1.87	1.87	4
VIII	Before stimulation	3.60	1.40	1.80	0
	During stimulation	1.50	2.33	2.56	13
	After stimulation	3.06	2.71	2.22	3
IX	Before stimulation	2.80	2.32	1.35	0
	During stimulation	1.90	1.84	1.15	12
	After stimulation	4.94	1.61	1.53	2
X	Before stimulation	4.94	1.61	1.53	0
	During stimulation	1.48	1.38	1.35	13
	After stimulation	3.04	1.60	1.60	3
Average	Before stimulation	5.17	1.88	1.61	0
	During stimulation	1.76	1.92	1.57	23
	After stimulation	5.75	2.03	1.77	7

TABLE II.

Detail of Experiment No. 12, Table III. Dog under light ether anæsthesia. Chorda tympani isolated and stimulated on the right side. Saliva collected from the right Wharton's duct. Salivary gland on the left side quiescent during the experiment. Continuous massage by movements of the lower jaw by means of a mechanical device securing absolute uniformity in rate and amplitude.

No. of observation.	Period of observation.	Time of observation in minutes.	Drops of lymph per minute.		Drops of saliva per minute.
			L. Lymph.	R. Lymph.	
I	Before stimulation	3.25	2.64	4.43	0
	During stimulation	2.34	2.57	5.00	20
	After stimulation	6.90	3.90	5.02	15
II	Before stimulation	6.90	3.90	5.02	0
	During stimulation	2.60	3.40	4.61	16
	After stimulation	7.30	3.28	4.11	3
III	Before stimulation	7.30	3.28	4.11	0
	During stimulation	3.75	2.85	3.60	16
	After stimulation	10.02	2.42	2.91	2
IV	Before stimulation	10.02	2.42	2.91	0
	During stimulation	2.85	2.24	2.80	12
	After stimulation	2.70	2.29	2.90	7
V	Before stimulation	5.60	2.14	2.32	0
	During stimulation	3.70	2.02	2.54	13
	After stimulation	4.80	1.71	2.00	14
Average	Before stimulation	6.65	2.88	3.71	0
	During stimulation	3.05	2.57	3.57	15
	After stimulation	6.40	2.59	3.51	6

TABLE III.

Summaries of experiments on the influence of the activity of the submaxillary gland on the flow of lymph from the neck lymphatics. In Experiment 7 chorda on the left side stimulated. In all the rest of the experiments the salivary gland on the left side remained quiescent throughout, the chorda tympani was stimulated on the right side, and the saliva collected from the right Wharton's duct.

No. of exp.	No. of separate observations.	Period of observation.	Average time of observation in minutes.	Drops of lymph per minute.		Drops of saliva per minute.	Remarks.
				Left lymph.	Right lymph.		
I	1	Before stim.	4.05	0.864	0 copious scanty	No massage.
		During stim.	5.34	0.600		
		After stim.	6.33	0.730		
II	6	Before stim.	4.62	1.103	0	No massage.
		During stim.	2.62	1.130	27	
		After stim.	3.37	1.125	6	
III	7	Before stim.	5.57	2.950	0	No massage.
		During stim.	1.94	2.350	40	
		After stim.	4.80	2.300	4	
IV	13	Before stim.	3.40	3.450	0	No massage.
		During stim.	1.68	3.590	32	
		After stim.	3.45	3.300	6	
V	4	Before stim.	3.55	1.020	0	Continuous movement of lower jaw by hand.
		During stim.	2.12	1.030	15	
		After stim.	3.70	1.100	10	
VI	10	Before stim.	5.17	1.880	1.616	0	Same as Experiment No. V.
		During stim.	1.76	1.926	1.572	23	
		After stim.	5.75	2.033	1.775	7	
VII	2	Before stim.	3.91	3.130	2.790	0	Left chorda stimulated, mechanical massage of lower jaw.
		During stim.	2.61	2.750	3.090	21	
		After stim.	6.16	2.760	3.140	5	
VIII	9	Before stim.	6.35	1.070	0.850	0	Mechanical massage of lower jaw.
		During stim.	3.76	1.180	0.810	26	
		After stim.	5.50	1.140	0.800	2	
IX	5	Before stim.	8.09	1.030	0	Mechanical massage of lower jaw.
		During stim.	3.45	1.010	20	
		After stim.	8.34	0.960	2	
X	3	Before stim.	11.90	1.120	1.350	0	Mechanical massage of lower jaw.
		During stim.	2.11	1.260	1.480	24	
		After stim.	4.60	1.500	1.280	8	
XI	3	Before stim.	4.50	2.220	5.130	0	Continuous massage (direct), submaxillary gland and neck, right side.
		During stim.	4.00	1.900	4.600	12	
		After stim.	4.40	1.960	4.360	2	
XII	5	Before stim.	6.65	2.880	3.710	0	Mechanical massage of lower jaw.
		During stim.	3.05	2.570	3.570	15	
		After stim.	6.40	2.590	3.510	16	
XIII	3	Before stim.	5.10	2.590	5.900	0	Continuous kneading of submaxillary gland and neck, right side.
		During stim.	3.50	2.500	5.800	13	
		After stim.	5.00	2.350	5.140	1	

TABLE IV.

Comparison between the osmotic pressure of serum and of lymph from the neck lymphatics of the dog. Experiments 1-11 are the series recorded in Table III, the chorda tympani of the right side being stimulated periodically. In Experiments 11-13 the salivary glands remained quiescent on both sides. In Experiments 1-6 the serum sample was taken at the end (II) of the experiment; in 7-13 samples were taken at the beginning and at the end. When more than one set of figures are given for the neck lymph, they represent the freezing-point of samples collected during successive periods of the experiment.

No. of experiment.	Depression of the freezing-point in degrees centigrade.				
	Submaxillary saliva.	Lymph from thor. duct.	Neck lymph left side.	Neck lymph right side.	Serum.
I	-0.555	-0.670	-0.920 (II)
II	-0.703	-0.777 (II)
III	-0.531	-0.633	-0.694 (II)
IV	-0.512	-0.639	-0.714 (II)
V	-0.328	-0.590	-0.653 (II)
VI	-0.572	-0.681	-0.738 (II)
VII	-0.604	-0.640 (I) -0.640 (II)	-0.686 (I) -0.720 (II)
VIII	-0.532	-0.642 (I) -0.647 (II) -0.645 (III)	-0.627 (I) -0.642 (II) -0.650 (III)	-0.606 (I) -0.645 (II)
IX	-0.339	-0.609	-0.630	-0.569 (I) -0.643 (II)
X	-0.639 (I) -0.621 (II)	-0.630 (I) -0.626 (II)	-0.659 (I) -0.640 (II)
XI	-0.688 (I) -0.690 (II) -0.688 (III)	-0.686 (I) -0.697 (II) -0.707 (III)	-0.765 (I) -0.766 (II)
XII	-0.662	-0.639 (I) -0.656 (II)	-0.662 (II)
XIII	-0.672 -0.678	-0.626 (I) -0.638 (II)	-0.626 (I) -0.656 (II)

TABLE V.

Comparison between the osmotic pressure of serum, lymph from the neck lymphatics, and lymph from the parotid gland of the horse. In Experiments 4-8 samples of serum were taken both at the beginning (I), and at the end (II) of the experiment. Parotid gland stimulated through the secretory nerves and by the injection of philocarpin into the circulation.

No. of experiment.	Depression of the freezing-point in degrees centigrade.			
	Parotid saliva.	Parotid lymph	Neck lymph.	Serum.
I, March 27	-0.290	-0.560	-0.560	-0.758 (II)
II, March 30	-0.323	-0.631	-0.609	-0.618 (II)
III, April 6	-0.613	-0.609	-0.620 (II)
IV, April 16	-0.300	-0.630	-0.580	{ -0.589 (I) -0.585 (II)
V, April 20	-0.290	-0.440	{ -0.548 (I) -0.542 (II)
VI, May 4	-0.326	-0.604	{ -0.582 (I) -0.592 (II)
VII, May 4	-0.431	-0.527	-0.565	{ -0.534 (I) -0.545 (II)
VIII, May 11	-0.584	{ -0.575 (I) -0.581 (II)
IX, May 11	-0.398	{ -0.540 (I) -0.551 (II)

STUDIES IN EXPERIMENTAL GLYCOSURIA.—I. ON
THE EXISTENCE OF AFFERENT AND EFFERENT
NERVE FIBRES, CONTROLLING THE AMOUNT OF
SUGAR IN THE BLOOD.

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BY the discoveries of pancreatic and phloridizin diabetes, our general knowledge of carbohydrate metabolism has, within recent years, been greatly advanced. On the other hand, those forms of glycosuria which are supposedly related to puncture diabetes — such as the glycosuria following stimulation of the central end of sensory nerves, the glycosuria following asphyxia and hemorrhage, the various forms of drug glycosurias, etc. — have been largely neglected, and our knowledge of the mechanism of their production, and therefore our knowledge of the relation of the nervous system to carbohydrate metabolism, stands to-day just about where it stood over thirty years ago when Claude Bernard published his “*Leçons sur le Diabète*” (in 1877).

It was believed by Bernard and his followers that there is a centre situated in the medulla, closely related to the vaso-motor centre, from which impulses pass to the liver, regulating its production of sugar from glycogen, and therefore the percentage of sugar in the blood. It was further thought by Bernard and Schiff that this control was effected, not through secreting nerve fibres, but indirectly by vaso-motor influences. It was thought, for example, that when the centre in the medulla is punctured, dilatation of the hepatic vessels occurred, and thus brought more blood to the liver, thereby increasing the activity of the glycogenolytic ferment.

The discovery by Heidenhain in 1872 of secretory fibres in the chorda tympani occurred some years before Bernard's last course of lectures on diabetes, but yet does not seem to have prompted him to modify his views that glycogenolysis is the result of dilation in the

hepatic blood vessels. Writers since Bernard's time, however, have frequently assumed that the fibres in question are secretory.¹ The discovery of such fibres to many other glands has been mainly responsible for this view, but, as we shall see later, the experimental evidence of the existence of such fibres to the liver is almost entirely lacking. The recent work of Bayliss and Starling, and others, on chemical excitants of glandular activity (hormones), opens up another possibility as to how the production of sugar by the liver may be controlled. Indeed, when we consider the relative distribution of nervous and "chemical" glandular mechanisms in the digestive tract, we shall see that the former exist most marked at its beginning, *e. g.*, in the mouth and stomach (partly), and the latter lower down, *e. g.*, in the pancreas and intestine. We would naturally assume that the mechanism of sugar production by the liver is more closely allied to that of the pancreas than it is to that of the salivary glands. The glycogenic function of the liver is a metabolic function concerned in the control of the supply of sugar to the organism. This sugar is required for combustion, and it would be more natural to suppose that the calls of the tissues for more or less sugar would be communicated to the liver by chemical changes in the blood rather than by reflex nervous impulses through the medulla. This influence of the blood on the hepatic cells may be merely of the nature of a balanced action; or it may be that some specific chemical body controls the function, being produced by the tissues when these require more sugar and carried by the blood to the liver, where it acts on the glycogen to produce sugar.

Two years ago, Dolly and I² published some observations which we had made on rabbits, with reference to the influence of nicotin on the amount of sugar contained in the urine as a result of puncture of the floor of the fourth ventricle of the medulla. We found that such injections greatly diminished the glycosuria. Assuming that the glycosuria indicated an excessive production of sugar by the liver as a result of the stimulation of a glycogenolytic centre, we suggested that the nicotin had produced a block in the sympathetic ganglia through which such fibres take their course to the liver. In attempting a fur-

¹ PFLÜGER: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 11: VON WITTICH, art. "Diabetes Mellitus" in HERMANN'S *Handbuch der Physiologie*, 1881, ii, p. 382.

² MACLEOD and DOLLY: *Proceedings of the Physiological Society, Journal of physiology*, 1906, xxxii, p. lxii.

ther investigation of this problem we were soon convinced that the whole question of the existence of such fibres is in no settled condition, and consequently would offer an interesting field of research. The following paper records certain of the results of this investigation, and it will be followed from time to time by others which are at present not completed.

The present communication refers to the evidence, for or against a nervous control, of the glycogenolytic function of the liver. I shall not meanwhile include any observations on *piqûre* itself, but shall consider the following two questions: Is the glycosuria which follows stimulation of the central end of the vagus nerve the result of afferent stimulation of the so-called diabetic centre in the medulla? Can efferent "glycogenolytic" fibres be demonstrated, either in the cervical spinal cord or in the greater splanchnic nerves?

Well-fed dogs have been employed for the investigation. In all the experiments the animals were anæsthetized with pure ether, and kept under the anæsthetic throughout the experiment. It is true that during and following ether anæsthesia the urine usually acquires considerable reducing power,¹ amounting sometimes to 2 per cent (Pavy titration), but an examination of the blood does not, in these cases, indicate sufficient hyperglycæmia to account for it. Seelig² states that intravenous infusion of oxygen prevents this, so-called, ether glycosuria. I have not found, however, that it can be prevented by causing the animal to respire large quantities of oxygen.

What this ether glycosuria may be due to, is at present a mystery. It would seem to owe its cause either to some change in the permeability of the renal filter towards the blood sugars; or, if we assume that the sugar exists in the blood partly in a combined and partly in a free state, and that any excess of sugar existing in the latter state is immediately filtered off in the urine, then we may suppose that the ether acts on the sugar combinations in the blood, increasing the amount of free sugar. It is as yet, however, not definitely known if the reducing property acquired by the urine in ether anæsthesia is entirely due to dextrose, or whether it may not in part be due to other reducing substances.

¹ HAWK P. B.: Proceedings of the American Physiological Society, sixteenth annual meeting, American journal of physiology, 1904, x, p. xxxvii.

² SEELIG, A.: Archiv für experimentelle Pathologie und Pharmakologie, 1905, lii, p. 481.

Partly on account of this influence of ether on the urine, and partly for reasons which will be set forth later, it was decided that the only satisfactory way in which to determine the amount of sugar produced by the liver would be to examine the amount of sugar in the blood.

Ether anæsthesia does indeed cause a very slight rise in the percentage of blood sugars, but it is so slight as to be negligible; especially since it occurs as a constant condition in all our experiments. Underhill, in investigating the influence of ether on the blood sugars, has come to the same conclusions.¹ It may be stated here that when oxygen was freely administered to the anæsthetized animal by the method described below, I was unable to find the blood sugars even slightly increased in amount in two experiments. In one experiment the blood contained 0.089 per cent sugar after two hours' anæsthesia and 0.092 per cent after four and a half hours. In the other experiment the blood contained 0.101 per cent sugar after three hours' anæsthesia. It is further interesting to note that in both these observations the urine secreted about one and a half hours after starting the experiment contained about 1 per cent of reducing substance.

The determination of the amount of sugar in the blood was conducted by a modification of the method of Waymouth Reid.² This modification consisted in collecting, drying, and weighing the cuprous oxide direct, instead of reducing it to metallic copper, as Reid recommends. After evaporating the filtrate and precipitate washings to small bulk, and neutralizing, we filtered and made the resulting solution up to 100 c.c. This was then mixed with 30 c.c. each of the copper sulphate solution and Rochelle salt solution of the Allihn-Fehling reagent, and the further estimation conducted as directed by Pflüger.³ In calculating the amount of sugar we employed the tables published by Pflüger. Gooch crucibles with asbestos mats were employed to collect the precipitates.

This method has recently been employed by Vosburg and Richards⁴ and by Underhill.⁵ We have frequently tested its accuracy by duplicate analyses and by other tests which will be published later by Mr. J. M. Harsh, who has assisted in this part of the research. In certain cases we have also applied Volhard's titration.

¹ UNDERHILL: The journal of biological chemistry, 1905, i, p. 115.

² REID, E. W.: Journal of physiology, 1896, xx, p. 316.

³ PFLÜGER, E.: Archiv für die gesammte Physiologie, 1903, xxvii, p. 100.

⁴ VOSBURG and RICHARDS: This journal, 1903, ix, p. 38.

⁵ UNDERHILL: *Loc. cit.*

STIMULATION OF THE CENTRAL END OF THE VAGI.

That glycosuria follows stimulation of the central end of the vagus nerve in the neck was discovered in 1855 by Claude Bernard.¹ The first experiments in this connection were performed on well-fed dogs, and in one of these, after the glycosuria had become well marked, the animal was pithed, and the blood, from various parts of the vascular system, and the pericardial fluid, were found to contain large amounts of sugar. As a result of the stimulation the urine sometimes, but not always, became alkaline, and the respiratory movements were arrested (especially when the right nerve was used). Bernard further found that by merely cutting the vagi in the neck the sugar production (by the liver) was arrested, and argued from this that constant afferent impulses must pass up the nerve to stimulate some centre in the medulla. He thought that the afferent impulses come from the lungs. Eckhard,² experimenting mainly on rabbits, confirmed Bernard's discovery, and showed that the glycosuria thus induced was of a transitory character. In one experiment recorded by this worker the rabbit was observed for several days; the nerve was daily stimulated, each period of stimulation being followed by a glycosuria which disappeared in several hours.

E. Külz³ has also confirmed these observations, finding further that stimulation of the vagus after its passage through the diaphragm has the same effect as stimulation of it in the neck. This observation disproves Claude Bernard's assertion that it is only the fibres from the lungs which act on the diabetic centre.

Laffont,⁴ in repeating the experiments, pointed out that the glycosuria might be due to the disturbance of respiration produced by stimulation of the central end of the vagus.

Stimulation of the central end of the cardiac depressor nerve in rabbits is likewise followed by glycosuria.⁵

¹ BERNARD, CLAUDE: *Leçons de physiologie expérimentale* (Paris), 1854-1855, i, pp. 333 *et seq.*

² ECKHARD, C.: *Beiträge zur Anatomie und Physiologie*, 1879, viii, p. 77.

³ KÜLZ, E.: *Archiv für die gesammte Physiologie*, 1881, xxiv, p. 97.

⁴ LAFFONT: Vide E. KÜLZ' article on p. 101 (footnote).

⁵ FILEHNE: Vide PFLÜGER'S article, *loc. cit.*, p. 317. LAFFONT: *Loc. cit.* KÜLZ, E.: *Loc. cit.* ECKHARD, C.: Vide KÜLZ' article, *loc. cit.* DOLLY and MACLEOD: *Recent advances in physiology and biochemistry* (New York), 1906, pp. 342 *et seq.*

Külz¹ found that stimulation of the cephalic end of the cut cervical sympathetic in rabbits was occasionally followed by glycosuria. This inconstant result may have been due to an accompanying irritation of the vagus.

In none of these researches is any account given of the amount of sugar in the urine, and, with the exception of Claude Bernard's observations noted above, I cannot find any records of the amount of sugar in the blood. Dolly and I have published several observations on the percentage of sugar in the urine after stimulation of the vagus and cardiac depressor nerves in well-fed dogs and rabbits. The amount found was never very high, 1.7 per cent, but this is probably due to the fact that the estimations were made in samples of urine collected immediately after any reduction was noted, and hence containing a gradually increasing, but not a maximal amount of sugar.

It is commonly believed that the glycosuria produced by stimulation of the central end of the vagus is due to a reflex stimulation of the so-called diabetic centre in the medulla, and, indeed, the experiment is put forward as one of the strongest proofs of the existence of such a centre. No regard has, however, been taken of another effect of such stimulation, namely, that on the respiratory centre usually causing, with ordinary faradic stimulation, standstill for some time of the respiratory movements. This effect on the respiratory movements is at first distinct, but usually becomes less marked after repeated stimulation; it is frequently more marked with one vagus than with the other, and sometimes it is not noticed when either nerve is stimulated. Now it is well known that interference with the respiratory movements (partial asphyxia) may cause glycosuria, so that, without taking precautions against this interference, it is scarcely justifiable to conclude that the glycosuria following stimulation of the central end of the vagus results from stimulation of the diabetic centre. It might as well be a result of the dyspnoea.

How, exactly, interference with respiration produces glycosuria seems to be an unsettled question. Araki,² in a very careful research, showed that when animals are made to breathe in a confined space it is the deficiency in oxygen, and not the accumulation of CO₂, which causes the glycosuria. It could not be the accumulation of CO₂, for this was taken up by means of caustic potash, and yet the percentage of sugar in the urine sometimes rose as high as 3.4.

¹ KÜLZ: *Loc. cit.*, p. 111.

² ARAKI: *Zeitschrift für Physiologische Chemie*, 1891, xv, p. 333.

Araki, following Zuntz, further concluded that the glycosuria which follows the inhalation of carbon monoxide gas is likewise due to the displacement of oxygen in the blood. On the other hand, Edie¹ claims that asphyxial glycosuria is entirely the result of an excess of CO₂ in the respired air irrespective of the amount of oxygen which it contains, at least down to 6 per cent.

The influence of dyspnœa on the sugar content of the blood has been recently well shown by Underhill.² The dyspnœa, in Underhill's experiments, was brought about either by constricting the tracheal cannula, or by cutting out the respiratory centre by injecting a mixture of oil and melted paraffin through the arteria vertebralis. Underhill further found that by causing an animal to respire oxygen gas he could prevent the hyperglycæmia which is otherwise produced by poisoning with piperidin. Such drugs as piperidin produce dyspnœa.

To eliminate, as far as possible, any dyspnœa following stimulation of the central end of the vagus, the method adopted in the present research has been to supply an abundance of oxygen directly into the lungs by introducing a gum elastic catheter into the trachea (*i. e.*, inserted through the walls of the respiration tube) and allowing washed oxygen from a cylinder to pass in a continuous stream down it. Hirsch³ has recently shown that, when an excess of pure oxygen is introduced into the lungs in this way, a curarized animal can be kept alive without artificial respiratory movements.

In some cases we have also practised artificial respiration with the bellows, so as to be certain that the CO₂ was removed from the lungs.

Tables I and II give the results of the observations in this connection, and they do not call for much discussion. In the absence of any precautions against dyspnœa (Table II), stimulation of the central end of the vagus nerves causes hyperglycæmia and marked glycosuria, almost without exception. On the other hand, if such precautions are taken (Table I), there is no greater increase in the sugar content of the blood than is frequently noted as a result of the anæsthetic (ether) alone. The only experiments which seem an exception to this generalization are those numbered II, 13 on Table I,

¹ EDIE, E. S.: The biochemical journal, 1906, i, p. 455.

² UNDERHILL: The journal of biological chemistry, 1905, i, p. 124.

³ HIRSCH: Ref. Biophysikalisches Centralblatt, 1905, p. 614.

and II, 38 and I, 7 on Table II. In the first of these the blood pressure at the start was unusually high (210 mm.), and previous to stimulating the vagus no sample of blood was examined for sugar, so

TABLE I.

STIMULATION OF CENTRAL END OF VAGUS NERVE IN ANÆSTHETIZED DOGS RECEIVING O₂ BY HIRSCH METHOD.

No. of exp.	Per cent sugar in blood.		Sugar in urine.	Remarks.
	Before stimulation.	After stimulation.		
II, 9	{	{ 30 min. 0.176 75 " 0.188 110 " 0.178	Present	
II, 11	{	{ 57 min. 0.163 77 " 0.070 107 " 0.090	Present	
II, 12	85 min. 0.080	{ Question regarding estimations. Blood pressure very high (210 mm.).
II, 13	{	{ 20 min. 0.264 65 " 0.297 95 " 0.155	Bladder urine sugar-free. Abundant	
II, 22	0.136	60 min. 0.142	0.5 per cent	
II, 28	0.159	160 min. 0.173	2.5 per cent	
II, 29	0.147	180 min. 0.182	2.0 per cent	
II, 37	{	{ 60 min. 0.153 120 " 0.157 150 " 0.157 190 " 0.166	In 140 min after start of stimulation urine began to reduce. From then to end of expt. the urine collected contained 0.6 per cent sugar.	
Average	0.147	96 min. = 0.170		

that unfortunately we do not know what may have been its normal sugar content. In the case of II, 38, Table II, stimulation of the vagus did not affect the respirations. In II, 7, so far as can be seen from the records, there was no evident reason for the unusual result.

In practically every one of the experiments detailed in Table I it will be seen that, although there were no hyperglycæmia, glycosuria was present. In one of these (II, 28), indeed, the amount of reducing substance rose to 2.5¹ per cent, and yet the sugar content

¹ This figure represents only the reducing power of the urine, and not, therefore, necessarily its sugar content. The urine of a dog may contain reducing substances in considerable amounts other than dextrose.

of the blood was only 0.173 per cent. It is far from true that the amount of sugar in the urine is an index of its amount in the blood. Not only is glycosuria frequently present without any decided hyperglycæmia, but, as was well shown in Underhill's observations, there may be quite a marked hyperglycæmia without an immediate glycosuria. The striking hyperglycæmia almost invariably induced by

TABLE II.

STIMULATION OF CENTRAL END OF VAGUS NERVE IN ANÆSTHETIZED DOGS NOT RECEIVING O₂.

No. of exp.	Per cent sugar in blood.		Sugar in urine.	Remarks.
	Before stimulation.	After stimulation.		
II, 30	{ ...	75 min. 0.273	Small amount	{ Asphyxia just prior to removal of sample. For 55 min. prior to second sample O ₂ was given.
	{ ...	110 min. 0.313	1.7 per cent	
II, 31	{ 0.186	55 min. 0.171	7.1 per cent 8 per cent	
	{ ...	95 " 0.231		
	{ ...	145 " 0.246		
	{ ...	0.230		
I, 37	...	40 min. 0.218	Abundant	{ Asphyxia, artificial respiration recovery.
I, 34	...	35 min. 0.235	Abundant	
I, 6	0.146	60 min. 0.201	2.9 per cent	
I, 7	0.175	70 min. 0.187	Abundant	Diuresis.
I, 18	{ ...	16 min. 0.234	Abundant	Diuresis.
	{ ...	40 min. 0.270	5.0 per cent	{ O ₂ given for 20 min. before the sample.
Average	0.169	65 min. 0.222		
II, 38	{ ...	145 min. = 0.164	...	{ Specially fed dog. Breathing soon after beginning of experiment came not to be influenced by stimulation of vagi.
	{ ...	0.157	Absent	

stimulation of the central end of the vagus when no precautions against dyspnœa are taken shows us clearly that, from any such experiments, no proof of afferent fibres to the diabetic centre can be claimed.

From the results we may conclude that in dogs anæsthetized with ether, and in which precautions are taken against dyspnœa, stimulation of the central end of the vagi causes no distinct hyperglycæmia.

STIMULATION OF THE SPINAL CORD.

The shortest path from the medulla to the liver, when the vagi are cut, is *via* the cervical spinal cord and greater splanchnic nerves, the fibres leaving the cord from the sixth thoracic to the second lumbar nerve roots inclusive.¹

It is along this path that vaso-motor and visceromotor fibres pass to the upper abdominal viscera, and the efferent impulses from the diabetic centre to the liver must presumably take the same course. Claude Bernard² and his contemporaries believed that they had demonstrated the existence of such fibres in the cervical portion of the cord, but below this region these workers did not arrive at any very definite conclusion as to what course the fibres followed between the cervical cord and splanchnic nerves.

The experimental evidence offered of the existence of glycogenolytic fibres in the cervical portion of the cord was of two kinds. Eckhard³ found that after a section in this portion of the cord, puncture of the medulla in rabbits is no longer followed by glycosuria. He states that when the cord is cut between the tenth and eleventh vertebra (3-4 dorsal) piqûre is without effect, and that sections lower down, *i. e.*, at thirteenth vertebra, sometimes also prevent the glycosuria. Pavy⁴ states that irritation of the cord at the brachial swelling produces glycosuria.

Bernard also found that after section of the cord between the sixth and seventh cervical vertebra, the liver, when removed some time after (next day), did not yield any sugar when extracted with

¹ LANGLEY: SCHAFER'S Physiology, 1900, ii, p. 644.

² BERNARD, CLAUDE: *Leçons cours d'hiver*, 1854-1855, p. 338; *Leçons sur le Diabète*, 1877, p. 371. It is difficult to obtain evidence from CLAUDE BERNARD'S "Leçons" as to how far he conducted experiments bearing on this question. On page 338 of his "Leçons" delivered in 1854-1855, he points out that the course of the fibres must be as above described, but in that place he does not offer any experiments in proof of this conclusion. He states that the matter will be discussed later. The only further reference that I can find, however, is in "*Leçons sur le Diabète*," delivered in 1877, p. 388, and in that place the matter is referred to only briefly. I have not consulted "*Leçons sur le System Nerveuse*."

³ ECKHARD: *Loc. cit.*

⁴ PAVY: *Diabetes Mellitus*. Vide PFLÜGER, *loc. cit.*, p. 388.

boiling water. The phenomenon was not noted when the cord was cut elsewhere.

By making a section of the posterior columns of the cervical or upper dorsal spinal cord, Schiff found glycosuria to be produced, and, as a general conclusion of his work in this connection, he states that diabetes can be produced by irritation of the nerve centres from the brain stem down the spinal cord to where the nerve roots to the viscera leave. "Diabetes ist in den Nervencentren zu erzeugen vom Hirnschenkel aus bis zur Stelle, wo die Wurzeln der Eingeweidenerven aus dem Marke treten."¹

So far as I am aware these include all the observations on which evidence of the existence of the fibres in the cervical cord is based.

Regarding the path of exit of these fibres from the cord, Eckhard² found that section of the inferior cervical and the first and second dorsal sympathetic ganglia, or of the corresponding rami communicantes, but not of the interganglionic fibres, was followed by glycosuria. Marc Laffont³ stated that after tearing out the first three dorsal nerve roots, piqûre no longer produced glycosuria. From these results it was thought that the fibres left the cord by the upper dorsal nerves.

These researches were, of course, conducted prior to the work of Langley, and others, on the course of the fibres connecting the spinal cord with the sympathetic chain. As a result of this work, there remains little doubt that no fibres to the vessels of the abdominal viscera leave the cord higher than the third thoracic root, and from so high a level only in small numbers.

The above evidence can scarcely be taken as conclusive proof of the existence of efferent glycogenolytic fibres to the liver. In those experiments in which the spinal cord was cut and piqûre then found to be ineffective, the profound spinal shock below the section and the fall of blood pressure might account for the failure of the piqûre; and in those experiments in which the spinal cord was stimulated, the resulting tetanus of the intercostal muscles must have interfered so greatly with breathing as to have produced more or less dyspnœa which itself produces glycosuria.

¹ SCHIFF, MORITZ: *Gesammelte Beiträge zur Physiologie*, von A. HERZEN und E. LEVIER (Lausanne), 1898, iv, p. 343. The work from which the above quotation is taken was published in 1859.

² ECKHARD, C.: *Beiträge*, 1879, viii, p. 87.

³ LAFFONT, MARC: *Vide PFLÜGER, loc. cit.*, p. 388.

Moreover, the urine alone was examined in these cases. As pointed out above, the reducing power of the urine is not necessarily an index of the amount of sugar in the blood, and hence it gives us a very doubtful criterion of the rate of sugar production by the liver.

TABLE III.
STIMULATION OF SPINAL CORD IN THORACIC OR LUMBAR REGIONS.

No. of exp.	Per cent sugar in blood.		Urine.	Remarks.
	Before stimulating.	After stimulating.		
I, 33	0.157	34 min. 0.157	Absent	{ B. P. 90 + mm. Upper lumbar.
I, 35	0.148	Absent	
	0.196 { 30 min. after above			
I, 38	0.187 0.120	10 min. 0.149 20 " 0.150 Stimulation left off 10 min. after 0.150 25 " " 0.161	Not examined	{ Cord cut and lower end stimulated between 8th and 9th dorsal root. B. P. rose during stimulation.
I, 39	0.151	{ 17 min. 0.172 35 " 0.158 Stimulation left off 25 min. after 0.160	Not ex.	{ Cord cut and lower end stimulated between 1st and 2d lumbar root. No effect on B. P.
I, 41	0.139	{ 17 min. 0.170 50 " 0.154	Not ex.	{ Cord cut and lower end stimulated between 3d and 4th lumbar root. Upper end of cord cocaineized.
I, 42	0.207	{ 15 min. 0.204 33 " 0.176 Stimulation left off 17 min. 0.168	Small amt (bladder urine)	{ Cord cut and lower end stimulated between 7th and 8th dorsal root. Upper end of cord cocaineized.
Average	0.163	0.163		

In the present communication nothing will be reported regarding the influence of section of the spinal cord or puncture diabetes. The results which will be presented refer to the effect of stimulation of the spinal cord at various levels on the amount of sugar in the blood, with or without precautions against dyspnœa. The results of these observations are shown in Tables III, IV, and V.

When the cord is cut and the lower end stimulated below the seventh dorsal root (Table III), there is no change in the amount of

sugar in the blood. Assuming that the fibres concerned should leave the cord between the fourth dorsal and second lumbar (Langley), we would expect some increase in blood sugar in the experiments marked I, 38, I, 39, and I, 42, in which, however, it will be seen no

TABLE IV.

STIMULATION OF SPINAL CORD IN CERVICAL REGION WITHOUT PRECAUTIONS TO PREVENT DYSPNŒA.

No. of exp.	Per cent sugar in blood.		Level (roots).	Remarks.
	Before stimulating.	After stimulating.		
II, I	45 min. 0.266	6-7 (Cut)	{ Blood pressure averaged 60 mm. Hg. after cord cut. Rise in B. P. on stimulating. not marked.
II, II	0.153 0.180 ¹	30 min. 0.309 60 " 0.274 ²	6-7	{ ¹ After exposing cord. ² Known loss from cracked beaker. Average B. P. 90 min.
II, IV	0.2352 ³	{ 27 min. 0.254 55 " 0.267	5	{ ³ After exposing cord. Average B. P. 90 min.
II, 16	3 min. 0.196 30 " 0.170 O ₂ and artif. resp. 30 min. after 0.137	{ B. P. 65-70 min. Just prior to second blood <i>energetic artif. resp.</i> had to be practised.
II, 40	110 min. = 0.278	{ Breathing much lessened by stimulation. Rectal temp. 98.2 F. Good blood pressure. Cord uncut.
II, 39	0.175	{ 5 min. = 0.188 50 " = 0.215	{ Immediately after applying electrodes the B. P. suddenly fell from 112 to 30-40 mm. Hg, at which it remained despite stimulation of cord (spinal shock). It was necessary to use respiratory bellows freely.
Average	{ 0.232 (including II, 16) 0.256 (excluding II, 16)		

such increase exists. The blood sugar remains practically unchanged, just as it does when the cord is stimulated below the second lumbar root (I, 41).

Stimulation of the cord in the cervical region, on the other hand, produces a very marked increase in the amount of blood sugar when the cord is merely exposed and stimulated with the induced current (Table IV). Such stimulation, however, interferes with respiration, and it was noticed (No. II, 16), when this interference was so marked as to make artificial respiration necessary, that the increase in blood sugar did not occur. The increase in blood sugar is also not very

marked in No. II, 39, where also, on account of marked shock, artificial respiration had to be practised.

Very different results followed stimulation of the cervical cord when, from the outset of the experiment, precautions, as described above (p. 394), were taken against dyspnœa.

TABLE V.

STIMULATION OF SPINAL CORD IN LOWER CERVICAL REGION IN ANÆSTHETIZED DOGS WITH PRECAUTIONS AGAINST DYSPNŒA.

No. of exp.	Per cent sugar in blood.		Level (roots).	Remarks.
	Before stimulating.	After stimulating.		
II, 5	0.153	{ 25 min. 0.148 { 55 " 0.162	4 and 5 (Cord not cut)	{ Great care taken not to injure cord during exposure. { B. P. rose enormously on stimulating, viz., to 190 mm. { ¹ B. P. only 40 mm. and heart very slow. { ² For 20 min. previous to taking this blood sample artif. resp. as well as O ₂ .
II, 6	0.127	{ 35 min. 0.220 ¹ { 55 min. 0.167 ²	5 and 6 (Cut)	
II, 7	0.103	30 min. 0.103 60 " 0.124 70 " 0.121	6 and 7 (Not cut)	Initial blood pressure 160 mm.
II, 18	30 min. 0.188 60 " { 0.183 { 0.184	6 and 7 (Not cut)	Good blood pressure throughout.
II	10 min. 0.138 26 " 0.140 35 " 0.101 63 " 0.098	Artif. resp. throughout with O ₂ before last sample.
Average	0.127	40 min. 0.149		

The results of these experiments are given in Table V. In only one of the experiments (*viz.*, II, 6) was any increase in blood sugars noted, in which case the blood pressure was very low and the heart so feeble that, despite the administration of oxygen, the blood may not have been adequately arterialized.

From the results we may, therefore, conclude that *when every precaution is taken against dyspnœa, stimulation of the spinal cord at any level fails to produce hyperglycæmia; or, in other words, to betray the existence of efferent glycogenolytic fibres.*

STIMULATION OF THE GREATER SPLANCHNIC NERVES.

By whatever path the glycogenolytic fibres, if they exist, leave the cord, they must ultimately run to the liver in the greater splanchnic nerves. The evidence that such fibres may exist in these nerves is that after section of the nerves piqûre no longer produces glycosuria (C. Eckhard, etc.).¹

On the other hand, Eckhard found that when the splanchnic nerve is itself stimulated (after being cut?) no glycosuria is produced. This negative result of Eckhard's has been considered by some to show that the glycogenolytic fibres, as they exist in this nerve, must be of a somewhat different nature from their forerunners in the spinal cord, in which location their stimulation, so these writers thought, does produce glycosuria (Pflüger).² Such a supposition is, however, entirely unwarranted, since Cavazzani has conclusively shown that by stimulation of the coeliac plexus there is a great increase in the percentage of sugar in the blood of the hepatic vein, and a corresponding diminution in hepatic glycogen. On discontinuing the stimulation, according to Cavazzani,³ the sugar content of the hepatic vein blood again falls to normal.

It is interesting to note in this connection that, after section of the splanchnics, certain drugs, such as morphine and sodium chloride solution, which normally produce glycosuria, no longer have this action,⁴ whereas carbon monoxide still produces glycosuria after these nerves are cut. Such facts would seem to show that in drug diabetes at least two distinct mechanisms of sugar production exist, one acting through the splanchnics, the other independent of them.

This conflicting evidence regarding the presence of glycogenolytic fibres in the splanchnic nerves is, in itself, a betrayal of how little we know of the control of sugar production by the liver. According to the one school of investigators (Eckhard, Pflüger, etc.), it is claimed that no such fibres are demonstrable here, though they are so in the cervical spinal cord. According to the other school (the Cavazzani brothers), such fibres are easily demonstrable in the greater splanchnic nerves.

¹ ECKHARD, C.: *Beiträge*, 1869, iv, p. 4.

² PFLÜGER: *Loc. cit.*

³ CAVAZZANI: *Archiv für die gesammte Physiologie*, 1894, lvii, p. 181; CAVAZZANI (frères): *Archives italiennes de biologie*, 1893, xix, p. 270.

⁴ ECKHARD: *Loc. cit.*, 1879, viii, p. 77. KÜLZ, E.: *ECKHARD'S Beiträge*, 1872, vi, p. 17.

In the first experiments which we performed in solution of this question, the greater splanchnic nerves on both sides were cut, and the peripheral end of one stimulated.¹ The effect of this stimulation on the amount of sugar in the blood is shown in Table VI.

TABLE VI.
STIMULATION OF PERIPHERAL END OF CUT SPLANCHNIC NERVES.

No. of exp.	Per cent sugar in blood.		Urine.	Remarks.
	Before stimulating.	After stimulating.		
I, 10	0.179 ¹ 0.181 ²	35 min. 0.210 50 min. 0.199	¹ After cutting left splanchnic. ² After cutting right splanchnic. Pleura accidentally punctured — clamped. Immediately after starting stim. stopped — artif. res. — recovery.
I, 13	0.086 0.140 ³	15 min. 0.144 34 min. 0.176	Slight reduction.	
I, 14	0.087 0.102	60 min. 0.104	Slight reduction.	Vagi cut. Oxygen administered.
I, 15	0.136	30 min. 0.148 60 min. 0.123	Reduction.	Urine at start reduced.
Average	0.130	0.158		

No hyperglycæmia is produced by stimulation of the cut nerve. In some of the experiments the urine acquired reducing properties, which, however, were feeble. These first experiments seemed, therefore, to confirm those of Eckhard, etc. By thus cutting both nerves, however, a marked fall in blood pressure resulted, and we could not be certain that this fall did not interfere with the glycogenolytic mechanism. The rise in blood pressure following stimulation of the nerve was only temporary. That a low blood pressure may have the above effect was first of all hinted at by Eckhard to explain the failure of morphine to produce glycosuria when the splanchnics are cut.²

Dolly and I³ have also suggested that the diminution in piqûre glycosuria which follows nicotine injections may also be due to fall of blood pressure. This question will be discussed in a future communication.

¹ The vagi were also cut in most of the experiments, the section being either in the neck or after the entry of the nerves into the abdomen. The object of cutting these nerves was to remove any possible effect of afferent vagal stimulation on the respiratory centre.

² ECKHARD, C.: *Beiträge zur Anatomie und Physiologie*, 1879, viii, p. 86.

³ DOLLY and MACLEOD: *Loc. cit.*

In two experiments (see Table VII) both splanchnics were cut, and the central end of the vagi then stimulated without the administration of oxygen or artificial respiration. Stimulation of the vagi under these conditions was not followed by hyperglycæmia. The interpretation of this result is, however, open to the same difficulty of fall in blood pressure.

TABLE VII.

STIMULATION OF CENTRAL END OF VAGI, WITH BOTH GREATER SPLANCHNICS CUT.

No. of exp.	Per cent sugar in blood.		Urine.	Remarks.
	Before stimulating.	After stimulating.		
II, 34	0.095	0.154 ¹	Sugar free Slowly excreted	{ Respiration markedly interfered with by stimulation. } ¹ Blood taken from heart after death.
II, 35	0.147	Sugar free	

To obviate this fall in blood pressure we have lately modified the experiment in that we merely exposed the greater splanchnic nerve on the left side and tied electrodes in position on it, then closing the wound in the abdomen. By this operation no fall in blood pressure is incurred. On stimulating the nerve periodically with the faradic current, a marked rise in blood pressure occurred each time the nerve was stimulated, and, as will be seen from Table VIII, a great increase in the amount of sugar in the blood very soon resulted. The administration of oxygen did not prevent this hyperglycæmia (No. II, 44). The mechanical irritation of the nerve involved in its dissection was not itself a sufficient stimulus (No. II, 44).

In one experiment (No. II, 45) no distinct increase in blood sugar was observed, even after stimulation of the splanchnic nerve for two hours. The dog in this experiment was well fed and in good condition, and there was no detail of the experiment, so far as I could notice, that was any different from those of the other experiments.

It will be noticed, in these experiments on the uncut splanchnic nerves, that glycosuria is quickly established as a result of the hyperglycæmia. Being unable at present to conduct more blood analyses, I have therefore performed a few experiments in which the reducing power of the urine alone was determined, after stimulation of the left greater splanchnic nerve. The results of the observations are given in Table IX.

TABLE VIII.

STIMULATION OF UN-CUT LEFT SPLANCHNIC NERVE, THE RIGHT ONE ALSO BEING INTACT.

No. of exp.	Per cent sugar in blood.		Per cent of sugar in urine.	Remarks.
	Before stimulating	After stimulating.		
II, 41	0.161	90 min. $\left\{ \begin{array}{l} 0.306 \\ 0.312 \end{array} \right.$	Secreted very rapidly. Sample collected 10 min. before last blood = 2.8 %	Effect on blood pressure at first small, afterwards very distinct. B. P. high. Resp. increased by stim. Diuresis.
II, 42	0.170	25 min. 0.240 100 min. 0.378	Secreted very rapidly at first, not so rapidly later. Sample collected 25 min. after commencing stim. = 1.6 %	Effect on B. P. and resp. marked. P. P. 130 + mm. Diuresis.
II, 43	90 min. = 0.302	Sample collected for 70 min. from beginning of stim. = 3.5 %	Effect on B. P. and resp. marked. B. P. 130 + mm.
II, 44	0.128 ¹	60 min. = 0.246	Sample collected 30 min. after start of stim. = 2.5 %	Large amount O ₂ given. ¹ 20 min. before taking this sample the left splanchnic was exposed and ligature placed in position.
II, 45	0.180	113 min. = 0.198	Urine very slowly excreted. Sugar free.	Blood-pressure averaged 130 mm. and reacted well to stimulation of splanchnics.
Average	0.159	0.280		

TABLE IX.

THE REDUCING POWER OF THE URINE AFTER STIMULATION OF THE LEFT GREATER SPLANCHNIC NERVE WITH BOTH OF THESE NERVES INTACT.

Time after commencement of stimulation at which urine showed distinct reducing power.	Per cent of sugar in urine collected immediately after reduction first noted.	Remarks.
30 min.	1.9	Diuresis.
30 "	6.2	Diuresis.
....	No sugar after 3 hours' stimulation of nerve	Almost complete anuria.
20 min.	2.0	Diuresis.
20 "	1.6	Diuresis.

Taking all the ten experiments recorded in Tables VIII and IX, we see that a positive result was obtained in eight and a negative result in two. In all the experiments giving positive results, diuresis accompanied the glycosuria, and in the two experiments giving negative results there was almost complete anuria.¹ The dog in one of the negative experiments had a very large goitre. The effect of the stimulation on the respiratory movements has always been, if anything, a quickening, but never tetanus. Oxygen was freely administered in most of the experiments. The percentage reducing power of the urine, as a result of splanchnic stimulation, is seen to be no higher than that frequently observed as a result of ether anæsthesia (see p. 396), but there is a very great difference in the length of time, after the beginning of the experiment, at which the sugar (?) attains this percentage in the two cases. Thus, after splanchnic stimulation, the urine acquires marked reducing powers in about thirty minutes; in ether anæsthesia, on the other hand, not till much later.

These ten experiments leave little doubt, I think, that in the greater splanchnic nerves fibres exist which control the production of sugar by the liver. In what way they do this, whether by merely affecting the hepatic circulation or by directly controlling the production of a glycogenolytic enzyme, is a question which will be considered in a future communication. In this connection we must of course bear in mind that, since no nerves are cut in these experiments, afferent stimulation of the medulla is possible; but, inasmuch as oxygen administration prevents hyperglycæmia following stimulation of the central end of the vagus and does not materially affect that following stimulation of the splanchnic, I think we may exclude such a mechanism.

Before concluding, I have to express my best thanks to Dr. C. E. Briggs, who assisted me in some of the experiments, and to Messrs. J. D. Knox and J. M. Harsh, who rendered valuable aid in the analyses of the blood for sugar.

CONCLUSIONS.

1. Stimulation of the central ends of the vagus nerves in the neck (both nerves being cut) causes hyperglycæmia when no precautions are taken against asphyxia.

¹ The diuresis is probably due to the excess of sugar in the blood.

2. When asphyxia is carefully guarded against (by the free administration of oxygen and artificial respiration), stimulation of the central end of the vagus nerve in the neck does not cause hyperglycæmia.

3. When asphyxia is carefully guarded against (as above described), stimulation of the spinal cord at any level does not cause hyperglycæmia.

4. Stimulation of the peripheral end of the greater splanchnic nerves, when these nerves on both sides are cut, does not cause hyperglycæmia.

5. Stimulation of the left splanchnic nerve, when no nerves are cut, usually produces marked hyperglycæmia and glycosuria.

6. In many of the above experiments the urine acquired distinct reducing properties without there being any hyperglycæmia.

7. It is concluded from the above research that, when every precaution is taken against asphyxia, glycogenolytic fibres are demonstrable with certainty only in the greater splanchnic nerves. In the spinal cord it is impossible to obtain evidence of their existence, possibly because of the shock which the experiments employed for their detection entail.

There is no evidence that stimulation of the central end of the vagus nerve can reflexly produce hyperglycæmia.

VASO-DILATOR FIBRES TO THE SUBMAXILLARY GLAND IN THE CERVICAL SYMPATHETIC OF THE CAT.

By A. J. CARLSON.

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

IT is commonly assumed that the cervical sympathetic nerve in the cat has the same vaso-motor action on the salivary glands as the cervical sympathetic of the dog, but, so far as I know, no one has actually measured the flow of blood through the cat's salivary glands on stimulation of the sympathetic. Heidenhain¹ speaks of only vaso-constrictor action of the cervical sympathetic to the salivary glands, including those of the cat. So does Langley,² who has not only worked specially on the salivary secretion and the salivary secretory nerves in the cat, but who is the recognized authority on the sympathetic nervous system in general. In his recent review of the subject of vaso-motor innervation, Bayliss speaks of the sympathetic as supplying only vaso-constrictor fibres to the salivary glands.³ Since the work of Dastre and Morat⁴ it is known that the cervical sympathetic contains vaso-dilator fibres to the bucco-facial region of the dog. Vaso-dilator fibres to the retinal blood vessels and the blood vessels of the brain have also been described in the cervical sympathetic of mammals, but, so far as I know, none to the salivary glands. The sympathetic vaso-motor innervation of the salivary gland has been studied in detail in the dog. One would hardly look for any difference in the vaso-motor nerves to the salivary glands of two mammals as closely related as the dog and cat. In the course of some experiments on relation of the blood flow to the composition of saliva,

¹ HEIDENHAIN: HERMANN'S Handbuch der Physiologie, 1883, v, p. 41.

² LANGLEY: Journal of physiology, 1879, i, p. 96; SCHÄFER'S Textbook of physiology, 1898, i, p. 494; 1900, ii, p. 617; Ergebnisse der Physiologie, 1903, ii, 2, p. 818.

³ BAYLISS: Ergebnisse der Physiologie, 1905, v, p. 327.

⁴ DASTRE and MORAT: Comptes rendus de l'académie des sciences, 1880, xcii, p. 393.

undertaken in conjunction with J. R. Greer and F. C. Becht, I was therefore greatly surprised to find that the cervical sympathetic in the cat contains not only vaso-constrictor but also vaso-dilator fibres to the submaxillary gland, and that on simultaneous stimulation of both systems of fibres with the weak tetanizing current the dilators usually overpower the constrictors, so that the stimulation results in an increased flow of blood through the gland that may nearly equal that following the stimulation of the chorda tympani.

1. The technique of the experiments consisted simply in measuring the blood flow through the gland by allowing the blood to drop from a cannula placed in the external jugular vein or its external maxillary branch, after tying off all the contributory veins, save the one from the submaxillary gland. The submaxillary vein in the cat is fairly constant in position, issuing from the hilus or mesial phase of the gland and joining the external maxillary vein near the bifurcation of the external jugular. The three-way cannula was connected with the sodium citrate or magnesium sulphate bottle for washing out occasional thrombi. The sympathetic fibres were stimulated both in the neck, and in that case always isolated from the vagus, and directly by isolating and stimulating the branch passing from the superior cervical ganglion to the submaxillary gland along the submaxillary artery. When it was desired to prolong an experiment, the loss of blood was compensated for by injection of defibrinated blood or Locke's solution into the femoral vein.

The rate of blood flow through the gland was recorded in drops on the kymograph by means of an electro-magnetic signal operated by an assistant.

No morphia was used, the animals being kept under light ether anæsthesia during the experiments.

2. The usual effect on the flow of the blood through the submaxillary gland on stimulation of the central end of the divided cervical sympathetic with the weak interrupted current is augmentation. This result was obtained in eight out of twelve animals worked. In the other four specimens the stimulation diminished the blood flow through the gland.

The augmentation of the blood flow following stimulation of the sympathetic varies from two to five times the normal, different animals exhibiting considerable variations in this regard. If the intensity of the interrupted current is carefully graduated up to the point where it just suffices to stimulate the vaso-dilator fibres, it is

possible in some specimens to obtain marked vaso-dilation without any secretion from the gland. In some animals, however, I failed to secure this separation, as the minimal stimulus for the dilators was sufficient to stimulate the secretory fibres. In specimens giving the usual augmented blood flow on stimulation of the sympathetic with a weak interrupted current, the same results were usually obtained

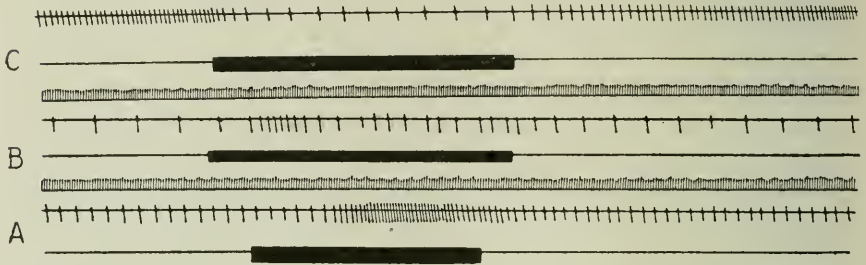


FIGURE 1. — Four sevenths the original size. Records of the blood flow (in drops) from the submaxillary vein on stimulation of the cervical sympathetic. *A* and *B*, cat. *C*, dog. Showing the opposite vaso-motor action on the gland in the two animals. Time, seconds.

with currents of medium and of very great intensity, currents too strong to be applied to the tongue, but occasionally the very strong interrupted produced vaso-constriction (Fig. 7 *B*).

The latent period of the augmented flow varies from five to ten seconds, depending on the strength of the stimulus. In general the stronger the stimulus the shorter the latent period of the augmented flow. But in some cases this relation is obscured apparently by the simultaneous stimulation of the vaso-constrictor fibres by the stronger current, the vaso-constrictors being apparently less excitable than the dilators. The augmented flow is usually of brief duration (Fig. 2). It may persist only for ten to twenty seconds, even though the stimulation is kept up. Occasionally it may persist for a minute or more, and may even outlast the stimulation by twenty to sixty seconds. The dilation may or may not run parallel with the rate of secretion from the gland. Records illustrating this typical augmented blood flow following stimulation of the cervical sympathetic are reproduced in Figs. 1 and 2.

While the above effects are the usual and typical ones following the stimulation of the sympathetic in the neck or on the submaxillary artery, deviations from this type are frequent. In no instance have I observed a diminution of the blood flow following the augmentation

either on cessation of the stimulation or on the continuing the stimulation in the cases where the augmentation was of brief duration. The flow returned to the normal either abruptly or gradually. But occasionally the stimulation produced no change in the output of blood from the gland during its progress, but a greatly augmented flow followed the cessation of the stimulation (Fig. 3 *B*). The stimulation may augment the flow, and a still greater augmentation follows immediately on cessation of the stimulation (Fig. 3 *C*). And again the stimulation may produce a slight diminution in the blood flow followed by a great augmentation as soon as the stimulation is discontinued, or even when the stimulation is continued (Fig. 3 *A*).

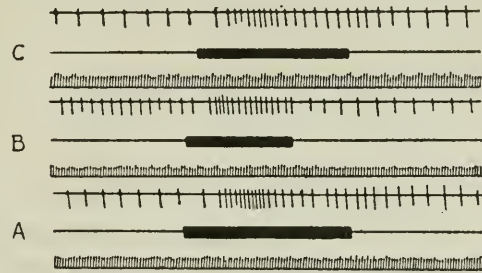


FIGURE 2. — Three fifths the original size. Records of the blood flow (in drops) from the submaxillary vein of the cat on stimulation of the sympathetic nerve. *A, B*, sympathetic stimulated in the neck. *C*, sympathetic stimulated on the submaxillary artery. Showing the typical vaso-dilation. Time, seconds.

These variable reactions are obtained more frequently in some animals than in others. They do not depend on the strength of the stimulus, because the same strength of interrupted current may give the typical pure augmentation at one time, and five minutes later augmentation only on discontinuing the stimulation. In general, however, these variable reactions are more frequently obtained with stronger than with weaker intensities of the stimulating current, — a fact suggesting that they are at least in part due to the varying effectiveness of the dilator and constrictor actions on the arterioles.

In some cases when the sympathetic was stimulated with strong currents either in the neck or on the submaxillary artery a more or less rhythmical alteration of periods of augmented and retarded flow

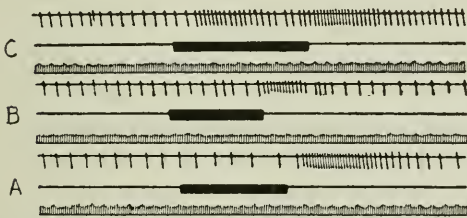


FIGURE 3. — One half the original size. Records of the blood flow (in drops) from the submaxillary vein of the cat on stimulation of the sympathetic in the neck. Showing vaso-dilation on cessation of the stimulation. Time, seconds.

was obtained. Two typical records of this kind are reproduced in Fig. 4. They are probably only extreme types of the variable reactions noted above, and due to the same causes, namely, the varying irritability, rate of fatigue, and perhaps actual number of the dilator and constrictor fibres, as well as the varying condition of the gland itself.

There can be no question, therefore, that the stimulation of the cervical sympathetic in the cat with the weak interrupted current usually

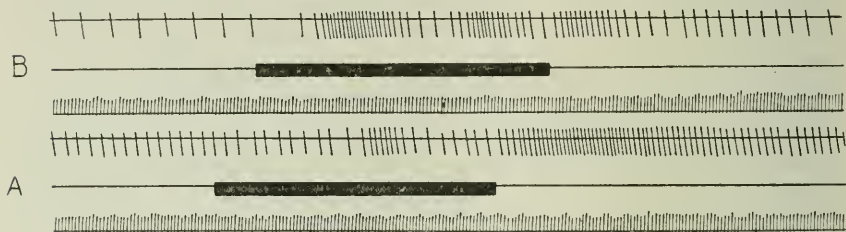


FIGURE 4. — Four fifths the original size. Records of the blood flow (in drops) from the submaxillary vein of the cat on stimulation of the neck sympathetic with the strong interrupted current. Showing periodic variation of the dilator action. Time, seconds.

augments the blood flow through the submaxillary salivary gland at the same time that it causes the gland to secrete. I have so far attributed the augmented blood flow to the stimulation of the vaso-dilator nerves. What is the evidence that such is the case? It is conceivable that such an augmented output of blood from the gland might be obtained on stimulation of the sympathetic in the absence of the vaso-dilators to the submaxillary gland, provided the vaso-constrictors to the gland were few in number and weak in action, as in that case the vaso-constriction in the head region might shunt enough blood through the submaxillary gland to more than compensate for the slight constriction of the arterioles. But the fact that the stimulation of the sympathetic branch that passes from the superior cervical ganglion to the gland gives the same results renders this explanation untenable.

It has already been noted that the augmented blood flow is usually of brief duration. May it not be due to the sudden constriction of the arterioles by action of the vaso-constrictors, thus forcing a few drops of blood out of the gland in more than the normal rapidity at the beginning of the stimulation? That this cannot be the mechanism of the augmentation is shown by the fact that it is not followed

by a diminished flow, which should necessarily be the case if it was due to vaso-constrictor action. Furthermore, this mechanism would not explain the not infrequent cases of prolonged augmentation, nor the augmentation sometimes following the cessation of the stimulation. For these very same reasons the augmentation cannot be due to the contraction of musculatures in the gland other than those in the walls of the blood vessels.

May not the augmented flow be due to the dilation of the capillaries in the absence of actual vaso-dilator nerves, according to the mechanism suggested by Henderson and Loewi¹ for the salivary glands? Henderson and Loewi have advanced the hypothesis that part of the augmented blood flow through the active sali-

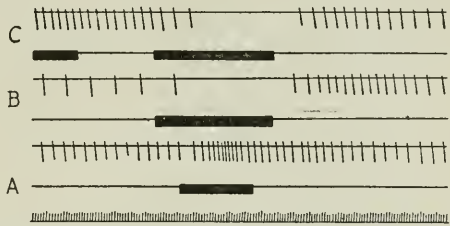


FIGURE 5. — Two thirds the original size. Records of the blood flow (in drops) from the submaxillary vein of the cat on stimulation of the sympathetic on the submaxillary artery. *A*, normal, or before administration of atropin; *B*, after administration of atropin (5 mg.); *C*, record taken ten minutes later than *B*. Showing paralysis of the vaso-dilators by atropin. Time, seconds.

vary glands is due to dilation of the capillaries, not as a result of the greater pressure owing to the dilated arterioles, but in consequence of the action of some substance developed by the active salivary cells and passed into the lymph. These investigators ascribe the diminished vaso-dilation produced by the chorda after atropin paralysis of the chorda secretory fibres as due to the absence of this active capillary dilating substance, rather than to depression of the dilator nervous mechanism. It is not the purpose to enter into any criticism of this interesting hypothesis at this time, but the following facts go to show that this mechanism fails to account for the augmented blood flow through the cat's submaxillary. It is possible to obtain the augmented blood flow by stimuli too weak to cause secretion. And the secretory action of the sympathetic may be paralyzed by atropin before the dilator mechanism is paralyzed, so that even the strongest stimulation of the sympathetic in the neck fails to cause secretion, although it produces an augmented blood flow. It is therefore evident that *the cervical sympathetic in the cat contains vaso-dilator fibres to the submaxillary gland.*

¹ HENDERSON and LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liii, p. 62.

3. The cervical sympathetic in the cat also contains vaso-constrictor fibres to the submaxillary gland. As already stated, in four out of twelve animals worked on the stimulation of the cervical sympathetic produced a diminution instead of an augmentation of

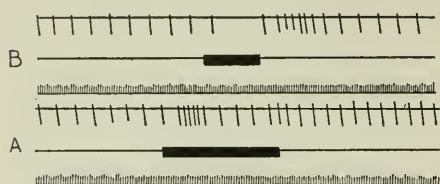


FIGURE 6. — Two thirds the original size. Records of the blood flow (in drops) from the submaxillary vein of the cat on stimulation of the sympathetic after administration of atropin. *A*, stimulation of sympathetic in the neck; *B*, stimulation of sympathetic on the submaxillary artery. Showing opposite effects on the blood flow of stimulation of the sympathetic fibres on either side of the superior cervical ganglion after atropin. Time, seconds.

the blood flow. The presence of vaso-constrictor fibres is also indicated by the peculiar variations in the dilator action of the sympathetic illustrated in Figures 3 and 4. The sympathetic causes pure vaso-constriction in the gland at a certain stage of atropin poisoning. One to two milligrams of atropin sulphate injected into the vein of a medium-sized cat paralyzes the secretory fibres of the chorda, diminishes the secre-

tory action of the sympathetic, and also diminishes the dilator action both of the chorda and of the sympathetic. Three to five milligrams paralyzes the secretory action of the sympathetic and greatly reduces its vaso-dilator action, but the latter always persists for some time after the abolition of the former. If just enough atropin is injected to paralyze the secretory fibres of the sympathetic when stimulated in the neck, and the sympathetic branch from the superior cervical ganglion to the gland isolated and stimulated directly, the result is just as copious a secretion of saliva from the gland as on stimulation of the sympathetic in the neck before the administration of the atropin. There is no evidence of paralysis of the gland cells or secretory fibres between the superior cervical ganglion and the gland. The primary paralysis is therefore in the superior cervical ganglion. This nicotin action of atropin was first noted by Langley.¹ A similar action of atropin was noted by myself in the case of the cardio-inhibitory fibres in *Limulus*.² Atropin paralyzes these fibres, and the point of the action is somewhere in the cardiac ganglion. A stronger dose of atropin abolishes the action of

¹ LANGLEY: *Journal of physiology*, 1878, i, p. 96.

² CARLSON: *This journal*, 1905, xiii, p. 237.

the sympathetic secretory fibres, even when stimulated between the superior cervical ganglion and the gland.

After a dose of atropin that has completely blocked the sympathetic secretory path in the superior cervical ganglion but left the vaso-motors functional, the stimulation of the sympathetic on the submaxillary artery usually produces the opposite vaso-motor effect in the gland to that following the stimulation of the sympathetic in

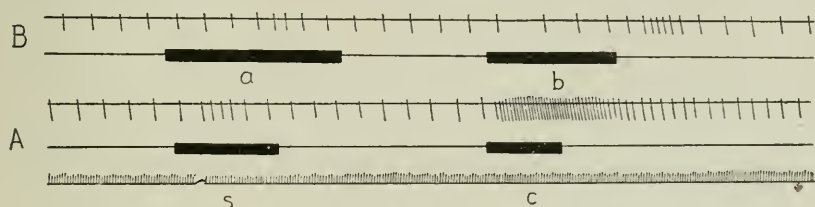


FIGURE 7. — Two thirds the original size. *A*. Records of the blood flow (in drops) from the submaxillary vein in the cat, on stimulation of the sympathetic and the chorda by the strong interrupted current after paralysis of both chorda and sympathetic secretory fibres by atropin. *c*, stimulation of chorda. *s*, stimulation of sympathetic in the neck. Showing relative vaso-dilator action of chorda and sympathetic at that stage of atropin action. Time, seconds. *B*, record of the blood flow from the submaxillary vein of the cat on stimulation of the nerve leading from the superior cervical ganglion to the gland. *a*, weak, interrupted current. *b*, strong interrupted current. Showing the opposite vaso-motor action of the strong and the weak stimulation.

the neck. Stimulation of the latter results in a slightly augmented flow; stimulation in the former gives a pure vaso-constrictor action. In no case have I failed to obtain pure vaso-constrictor effects on stimulation of the sympathetic branch to the gland at a certain stage of the atropin action. When this stage in the atropin action is reached, the secretory action of the sympathetic on the gland may or may not be abolished.

In four experiments I obtained a fairly copious saliva flow synchronously with the great vaso-constriction. But even in these cases further administration of atropin abolished the secretion, while the vaso-constrictor action persisted. At this stage of the atropin action stimulation of the chorda still produces vaso-dilation in the gland. The vaso-constrictor action of the sympathetic after atropin may be so great as to completely stop the passage of the blood through the gland. The explanation of these changes in the vaso-motor action of the sympathetic on the submaxillary gland is probably as follows. The sympathetic contains both vaso-constrictor and vaso-dilator

fibres to the gland. Under normal conditions when both are stimulated simultaneously with the weak interrupted current the dilator action prevails. Atropin has a greater depressor action on the dilator than on the constrictor mechanism, so that at a certain stage of atropin poisoning the constrictors prevail over the dilators. The pure vaso-constriction obtained after administration of atropin is therefore not necessarily an indication of complete paralysis of the dilator mechanism. It simply indicates a sufficient degree of depression of the dilator mechanism for the vaso-constrictor action to gain the upper hand. And as the relative power of the dilator and constrictor mechanisms varies somewhat from one animal to another, it is to be expected that the reversal of the sympathetic vaso-motor action by atropin would require different strength of dose in different animals, as is actually the case.

It seems more difficult to account for the opposite vaso-motor action of the neck sympathetic and of the branch from the superior cervical ganglion to the gland at this stage of the atropin poisoning. According to Langley both the secretory and the vaso-motor fibres to the salivary glands make "relays" in the superior cervical ganglion. The vaso-dilator to the submaxillary gland in the cat has, of course, not been investigated regarding this point. The dilator fibres may pass through the ganglion without connecting with the cells. They may make such connections in the ganglion, but the "synapse" of the dilators be less readily blocked by atropin than that of the constrictors. Or we may possibly have to ascribe some nerve centre function to the superior cervical ganglion in the way of transformation or shunting of the impulses.

The vaso-motor action of the cervical sympathetic on the parotid and sublingual gland in the cat has not been investigated. The fact that the cervical sympathetic carries both vaso-dilators and vaso-constrictors to the cat's submaxillary gland makes it highly desirable that the sympathetic vaso-motor innervation of the salivary glands be re-investigated in the mammals in general. It would be singular if the cat should be exceptional in this respect. Vaso-dilators to the salivary glands may be present in the cervical sympathetic in the dog and other mammals, but the constrictors are in preponderance, so that on simultaneous stimulation of the two sets of fibres only the constrictor action is apparent.

MAY REFLEX CARDIAC ACCELERATION OCCUR INDEPENDENTLY OF THE CARDIO-INHIBITORY CENTRE?

By DONALD R. HOOKER.

[From the *Physiological Laboratory, Johns Hopkins University.*]

THERE are two theoretically possible modes of reflex cardiac acceleration: (1) through a depression of the vagus action, and (2) through a stimulation of the accelerator centre. Of these the first is by far the more strongly supported in the literature, so that the recent text-books of physiology¹ are inclined to assume, provisionally at least, following the explanation advanced by Hunt,² that the accelerator centre acts merely as a tonic driving force to the heart, which is curbed by the action of the vagus centre. According to this view, then, reflex retardation or acceleration is mediated entirely through the vagus centre. It was the idea of throwing further light upon this point which led Dr. Howell to suggest the present research.

The careful and complete survey of the literature of the subject in the article by Hunt above referred to makes repetition unnecessary. I shall, therefore, have occasion to refer only to the more important articles or to those bearing upon the special points of this paper.

Roy and Adami³ conclude, from an extensive study of the mammalian heart, that cardiac acceleration is wholly dependent upon the activity of the vagus centre. This conclusion was based upon the observation that even direct stimulation of the accelerator nerves when the heart was beating rapidly or after vagus section caused only an augmented beat without acceleration.

MacWilliam⁴ similarly states that "reflex acceleration is not essentially dependent upon excitation of the augmentor nerves." He

¹ HOWELL: 1905.

² HUNT: This journal, 1899, ii, p. 395.

³ ROY and ADAMI: *Philosophical transactions of the Royal Society, London*, 1892.

⁴ MACWILLIAM: *Proceedings of the Royal Society, London*, 1893, liii, p. 464.

observed that if, after vagus section, the heart was slowed to "a moderate rate" by peripheral vagus stimulation, the application of stimuli to sensory nerves never caused acceleration; and Hunt¹ states that "the view that reflex acceleration is caused by inhibition of the cardio-inhibitory centre is confirmed; no evidence could be found that the accelerator nerves are ever thrown into action reflexly." This author also stimulated the vagi peripherally to slow the heart after vagotomy, but found no acceleration to result upon stimulation of sensory nerves. The slowing in the experiments referred to by Hunt equalled the rate existing before section of the vagi. He cites numerous experiments to show that reflex acceleration occurs before vagus section, but was never able to obtain it after these nerves were cut. And even when the activity of the accelerator centre was excluded by cutting the accelerator nerves, reflex acceleration occurred in much the same way. A tracing is published of such an experiment.

On the other hand, François-Franck² states that while the vagus action normally predominates over the accelerator action, yet in some cases the reverse is true, especially when artificial conditions modify the vagus action.

No tracings are given, nor are any experiments described in support of this view. Barbéra³ describes an experiment in which, after vagotomy, stimulation of the depressor nerve in a rabbit caused the heart rate to increase from 240 to about 270 in a minute. In this experiment, the tracing of which is not given, the blood pressure remained unchanged. Von Cyon⁴ has published a tracing of another part of the same experiment, showing the effect of stimulation of the central stump of the cervical sympathetic. The cardiac acceleration is marked, but apparently continued throughout the rest of the experiment. Bayliss⁵ has given a tracing of a similar experiment in which, after cutting both vagi, stimulation of the depressor caused an accelerated heart rate, approximately double the rate before stimulation, which returned to normal after removal of the stimulus.

In all of the experiments described by Bayliss the vagi were first cut. He states that "under these circumstances it is frequently seen that (following depressor excitation) there is instead (of a slowing)

¹ HUNT: *Loc. cit.*

² FRANÇOIS-FRANCK: *Travaux du laboratoire de Marey*, 1878-1879, p. 73.

³ BARBÉRA: *Archiv für die gesammte Physiologie*, 1897, lxxviii, p. 434.

⁴ VON CYON: *Archiv für die gesammte Physiologie*, 1898, lxx, p. 126.

⁵ BAYLISS: *Journal of physiology*, 1893, xiv, p. 303.

a cardiac acceleration sometimes very marked." Hunt has objected to this experiment and to that of von Cyon as evidence of reflex cardiac acceleration, on the ground that the acceleration represents practically a doubling of the heart rate, a condition which might result from a change in the auriculo-ventricular sequence, the rhythm changing from a 2 to 1 to a 1 to 1 rhythm. There is no doubt that such a change may occur during experimental procedure. Thus Roy and Adami¹ found that stimulation of the vagi frequently altered the ventricular response to the auricular rhythm. It may justly be asked, however, why such a condition does not occur more frequently in experimental work if it is to be used as an argument against the experiment of Bayliss? It was never observed in the experiments to be described below, and presumably Hunt himself did not observe it, since he makes no reference to any experiments.

Finally, Hering,² from a series of apparently carefully conducted experiments upon rabbits, reaches the conclusion that "the increased heart rate after muscular exercise is chiefly dependent upon the integrity of the accelerator nerves. This action is aided, in normal animals, by a coincident depression of the irritability of the depressor nerves." A comparison of the rates before and after vagotomy, in the experiments of this author, shows that the acceleration in the normal animal after exercise is approximately equal to the difference between the resting rate before and after vagotomy. Anthanasin and Carvallo³ from similar results, obtained with dogs, come to opposite conclusions.

Hering, in another series of experiments,⁴ observed in rabbits that the cardiac acceleration following muscular exercise was very much less after extirpation of the last cervical and first thoracic sympathetic ganglia than before the operation. This work represents the only positive evidence found in the literature in favor of reflex cardiac acceleration through the accelerator nerves, although Tigerstedt⁵ maintains that the reflex may occur either by way of the vagi or by way of the accelerators.

The results of these investigators, together with those of Roy and Adami,⁶ would indicate that there is a physiological maximum rate

¹ ROY and ADAMI: *Loc. cit.*

² HERING: *Archiv für die gesammte Physiologie*, 1895, lx, p. 429.

³ ANTHANASIN and CARVALLO: *Archives de physiologie*, 1898, x, p. 552.

⁴ HERING: *Centralblatt für Physiologie*, 1894, viii, p. 75.

⁵ TIGERSTEDT: *Physiologie des Kreislaufes*, 1893, p. 289.

⁶ ROY and ADAMI: *Loc. cit.*

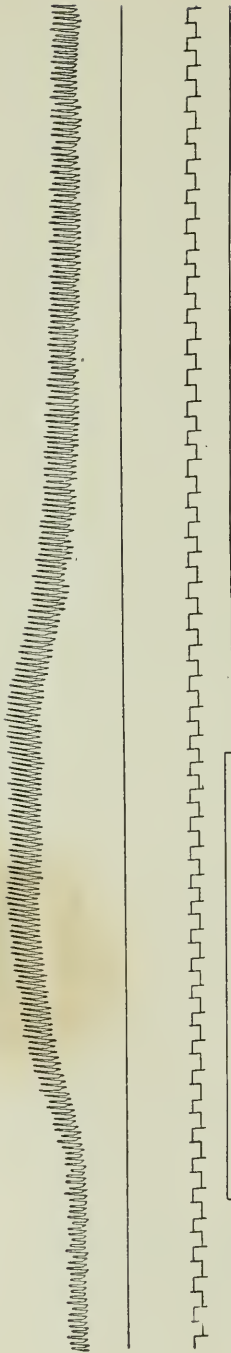


FIGURE 1. — Seven twelfths the original size. April 10, 1907. Dog. Hürthle manometer. Chest open. Both vagi cut. Stimulation of left vagus centrally. To show reflex acceleration without first slowing the heart rate.

of the heart which is usually reached after section of the vagi and beyond which it can only with difficulty be increased. If this is true, the limited amount of evidence in the literature favoring reflex cardiac acceleration through the accelerator centre is easy of explanation.

METHODS.

The assumption of the existence of a physiological maximum heart rate at once makes it evident that, in order to study the reflex activity of the accelerator centre, it is necessary, after excluding the action of the vagus centre by vagotomy, to maintain a heart rate which is slow enough to allow easily of acceleration. That, however, it is not always necessary to have recourse to artificial slowing is shown by the first tracing given in Fig. 1.

In this experiment, after vagus section, stimulation of the left vagus centrally caused an acceleration of 2 beats in ten seconds.¹

The experiments were performed upon dogs and rabbits, with the usual complete anæsthesia. Cats were found unsuited to the work, because of the ease with which their vagi fall out of tone. The heart rate was recorded either by tambours, the transmitting tambour being attached to the heart by a thread, or by the Hürthle manometer. When

¹ It may be mentioned in this connection that Dr. DAWSON has recently demonstrated in the class-room reflex cardiac acceleration after section of the vagi without preliminary slowing of the heart rate.

dogs were used, the pulse was counted while the animal was under the effects of morphia alone. The rate thus obtained served as the rate to be approximated in the control periods (40 or 50 in a minute; this rate is not far from the normal in resting dogs, so far

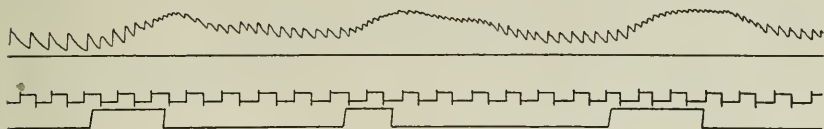


FIGURE 2.—Four sevenths the original size. April 5, 1907. Rabbit. Hürthle manometer. Chest not open. Both vagi cut. Heart rate slowed by peripheral vagus stimulation. To show reflex acceleration through the vagus.

as it has been counted in connection with this work). After the animal was completely under the influence of ether, tracheotomy was performed, and the thorax opened, after first ligating the internal mammary vessels, or not opened, as the experimental conditions required. Artificial respiration was, of course, employed in the

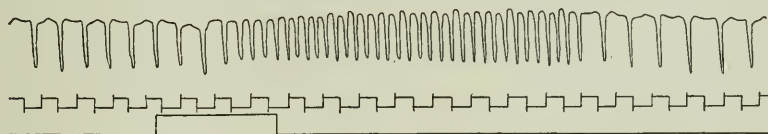


FIGURE 3.—Two thirds the original size. March 30, 1907. Dog. Tambours. Chest open. Both vagi cut. Heart rate slowed by peripheral vagus stimulation. To show reflex acceleration through the splanchnic.

experiments with the thorax opened. The operation was made as bloodless as possible, experience having taught that a successful experiment depended largely upon the condition of the animal. Both vagi were then cut, and one of them was stimulated peripherally with induction shocks to slow the heart. The best results were had when the rate thus obtained approximated the rate counted when the animal was under the influence of morphia alone. It seemed, however, to be a matter of no importance so long as the rate was distinctly retarded, it being safest to err on the side of slowness. Both Hunt¹ and MacWilliam² made use of this method of slowing the heart with negative results. The only apparent explanation for their failure seems to be that the slowing was not sufficient. Under such conditions it appears not enough to slow the heart to that rate which

¹ HUNT: *Loc. cit.*

² MACWILLIAM: *Loc. cit.*

existed before section of the vagi. This is especially true of dogs under the influence of morphia. The administration of ether causes an enormous difference in the rate, — an increase of from 50 to 100 or even 200 per minute. It is possible that the effect of the morphia

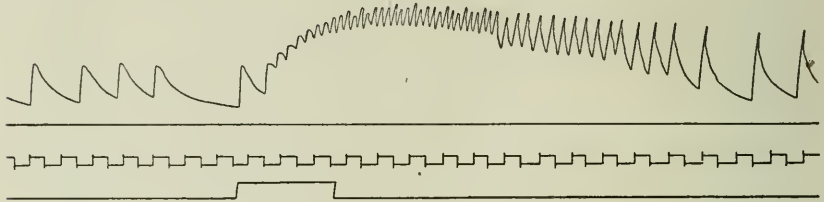


FIGURE 4. — Four sevenths the original size. April 4, 1907. Dog. Hürthle manometer. Chest not open. Both vagi cut. Heart rate slowed by peripheral vagus stimulation. To show reflex acceleration through the sciatic.

on the heart is such that the accelerator centre is thoroughly taxed to keep up the heart rate required, and therefore cannot respond further to a sensory demand. Be this as it may, it is unusual to get evidences of reflex acceleration without a primary slowing of the heart by peripheral vagus stimulation, and when it does occur, it is

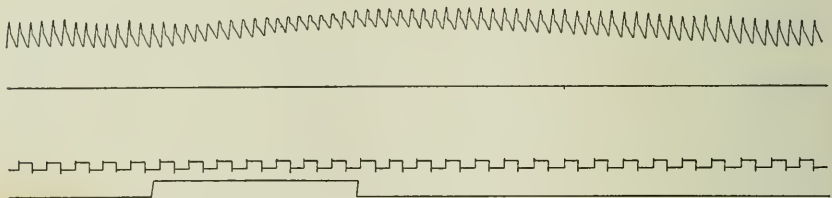


FIGURE 5. — One half the original size. April 9, 1907. Dog. Hürthle manometer. Chest open. Both vagi cut. Heart rate slowed by peripheral vagus stimulation. To show rise in blood pressure without cardiac acceleration upon central stimulation of vagus.

slight — 2-3 beats in ten seconds. This point is well brought out by the work of Roy and Adami¹ and of Hering² above referred to. Furthermore, on *a priori* reasoning it seems clear that the physiological mechanism of acceleration would become progressively more irritable as the rate of the heart made it more and more important that it come into play.

After the application of the peripheral vagus stimulus, the following nerves were stimulated: vagus centrally, saphenous, sciatic, and

¹ ROY and ADAMI: *Loc. cit.*

² HERING: *Loc. cit.*

³ TIGERSTEDT: *Physiologie des Kreislaufes*, 1893, p. 289.

splanchnic. This list might, to advantage, have been extended, but it serves the present purpose, namely, to show that reflex cardiac acceleration may occur independently of the cardio-inhibitory centre.

In brief, the success of the method depends upon (1) having the animal in good condition; extensive hemorrhage or any approach to shock invariably ruined the experiment; and upon (2) having the heart rate slow enough to make acceleration easily possible.

RESULTS.

This work represents the results of seven successful experiments, and comprises twenty-four determinations of reflex cardiac acceleration after the influence of the cardio-inhibitory centre had been excluded by section of both vagi. No record was regarded as evidence of reflex acceleration unless, without alteration of position or movement of the electrode on the peripheral stump of the vagus, and without change in the strength of the stimulus, acceleration occurred on applying a constant stimulus to a sensory nerve and, upon removal of this stimulus, passed off, leaving the heart rate approximately equal to that of the control period.

The accompanying table gives the results of these experiments in condensed form. The rate before, during, and after stimulation of the sensory nerves is counted for ten seconds; the three remaining columns indicate time in seconds. It will be seen that the latent period varies from less than one to thirty-eight seconds, averaging from one to six seconds in the more satisfactory determinations. The duration of stimulation of the sensory nerves and the strength of current used varied so much in the different determinations that the figures for the duration of acceleration serve only to indicate in a general way that the duration of stimulation and the duration of the resultant acceleration are roughly the same. The rate counted after the acceleration had disappeared corresponds well with that of the control period before stimulation of the sensory nerves.

The three tracings chosen to illustrate the results (Nos. 2, 3, and 4) are typical, and are sufficiently explained by the descriptions beneath them.

It will be noted that there is in both the records obtained with the Hürthle manometer distinct evidence of a coincident rise in blood pressure. That the rise in blood pressure is not the causal factor in the acceleration is evidenced by numerous records in which a rise of

pressure occurred independently of acceleration (see tracing No. 5) — and by the work of Schmiedeberg¹ and of Martin.² Schmiede-

TABLE I.

Date.	Nerve.	Stimulation of sensory nerve during peripheral vagus stimulation.			Latent period.	Duration of stimulation of sensory nerve.	Duration of acceleration.
		Before accel'n.	During accel'n	After accel'n.			
1907					sec.	sec.	sec.
April 5	Vagus *	10	30	15	1½	2	1½
" " *	15	35	15	0½	2¼	7½
" " *	15	35	16	1½	5¼	7
March 28	9	24	10	12	16	28
" "	7½	24	9	38	46	45
April 10	8	15	7	8	13	16
" 9	6	15	7	3½	7	3
" "	7	15	8	1	5½	5
" 4	7	13	4	6½	15	25
" 10	6	17	Inhibition	6	12½	28
March 27	6½	12	8	18	27	16
" "	8	13	7½	13	24	20
	Average	(9)	(20)	(10)	(9)	(16)	(17)
April 4	Sciatic	4	7	6½	3	19	14
" "	6	23	6	4	13	22½
" "	5	25	4	3	6	25
	Average	(5)	(12)	(5+)	(3)	(33)	(21)
March 30	Splanchnic	7	18	6	4	7	18
" "	8	14	9	3	6	4
April 4	4	18	5	1	3	9
March 30	6½	10	4	5	11	8
April 4	4	11	4	2	6	34
	Average	(6)	(12)	(6)	(3)	(7)	(13)
April 5	Saphenous *	8	45	10	1½	2	5
" " *	10	35	15	1½	3	4
" " *	9	18	10	2	8½	10
" 4	7	14	4	9	36	26
	Average	(9)	(28)	(10)	(4)	(12)	(11)

* Indicates the experiments with rabbits.

¹ SCHMIEDEBERG: Berichte der könig. Sächs., Gesellschaft der Wissenschaften zu Leipzig, Math.-Phys. Cl., 1870, xxii, p. 130.

² MARTIN: Physiological papers, 1895, p. 12.

berg has shown that the heart rate following direct stimulation of the accelerator nerves is entirely independent of blood-pressure changes, and Martin has shown upon the isolated heart that variations in arterial pressure, within wide limits, have no effect upon the pulse rate.

CONCLUSION.

The results of this investigation appear to furnish positive evidence that reflex cardiac acceleration may occur independently of the cardio-inhibitory centre.

THE EFFECT OF EXPRESSED TISSUE JUICES OF MUSCLE ON THE MAMMALIAN HEART BEAT.

By J. J. R. MACLEOD.

[From the *Physiological Laboratory, Western Reserve University.*]

IN the prosecution of a research¹ concerning the general question of the presence of poisonous compounds in the tissues as the result of infective processes, some facts which seem to me of interest from the physiological standpoint have been encountered. These facts refer to the action of expressed undiluted tissue juices on the beat of the mammalian heart.²

The tissue juices employed included those of cardiac and skeletal muscle. To prepare the extracts, the heart or muscle was finely minced and then rubbed up in a mortar with purified infusorial earth³ so as to form a mass of the consistency of a stiff paste, which, after being enclosed in stout sail-cloth, was placed in a large Buchner press. By raising the pressure to between three hundred and four hundred atmospheres, a suitable quantity of tissue juice (30 c.c.) could be thus obtained from about 150 gm. of muscle. The resulting juice was of a reddish color, at first clear but afterwards becoming slightly turbid. After standing, a buff-colored layer of fat sometimes separated from the extracts prepared from skeletal muscle but not from those of cardiac muscle. The extracts were acid in reaction towards litmus.

These extracts were injected, in 5 c.c. quantities, into the coronary circulation of a dog's heart perfused by Langendorff's method.⁴ In using this method, I have found that the pressure necessary for transfusion can be most conveniently obtained from a cylinder of

¹ In collaboration with Dr. G. W. CRILE.

² A preliminary communication of this work was given before The Society of Experimental Biology and Medicine, March 10, 1907.

³ The infusorial earth did not give any flame test for potassium.

⁴ LANGENDORFF: *Archiv für die gesammte Physiologie*, 1895, lxi, p. 291.

compressed oxygen, as recommended by Gottlieb and Magnus.¹ The following is a brief description of the method as used in the present research. The cylinder of oxygen is connected with a large Woulf bottle from which runs a tube, provided with a three-way tap, communicating with the top of the blood reservoirs. Each reservoir has a capacity of about 1200 c.c. The reservoirs are submerged in a large tank containing water at a temperature of 38° to 40° C., and in this tank is also placed an inverted bottomless two-litre bottle with its neck connected by tubing through the bottom of the tank with a vessel for the collection of the perfused blood. The bottom of each blood reservoir is connected by tubing to the side tube of the aortic cannula, the top of the cannula being closed by a thermometer. By having the blood reservoirs and the heart chamber submerged in the same water bath, constancy of temperature is assured. Manometers are connected both with the Woulf's bottle and with the aortic cannula. The pressure is kept constant by connecting the Woulf bottle with a valve consisting of a straight glass tube submerged to a given depth in mercury contained in a cylinder. The cylinder is firmly corked, so that the oxygen which escapes through this valve can be conducted, by means of a second tube through the cork, to the blood-collecting vessel under the tank. By this arrangement the escaped oxygen can be utilized for arterializing the blood.

The pressure can be very readily adjusted to any desired level by the above arrangement. Such a pressure was chosen in the experiments as to give a maximal beat, the rate of perfusion being, for a medium-sized heart, about 5 c.c. per minute. The pressure in the aortic cannula necessary to give this outflow is quite small at the outset of the experiment, but, as the coronary vessels become constricted, it has to be gradually raised to 100 mm. The perfusion fluid consists of the dog's defibrinated blood mixed with two volumes of Locke's solution.

The record of the heart beat is obtained by placing a cardiac sound in the left ventricle (through the auriculo-ventricular orifice) and connecting it with an air-tight Brodie's tambour. The sound consists of a finger of a rubber operating-glove tied on to a glass tube, a piece of wire bent in a loop being inserted in the tube so as to prevent the rubber membrane closing up its end. I have found this method of recording the heart beat extremely simple and useful, and

¹ GOTTLIEB und MAGNUS: *Archiv für experimentelle Pathologie und Pharmacologie*, 1902, li, p. 30.

only once or twice have I found that the manipulation of the heart, necessary for the introduction of the sound, produces fibrillary contraction.

To mix the extracts with the perfusion fluid, a large hypodermic needle is inserted through the rubber connections of the aortic cannula and the extract is injected by means of a syringe.

The first experiments were performed with extracts from the hearts of dogs that had received large doses of diphtheria toxins about

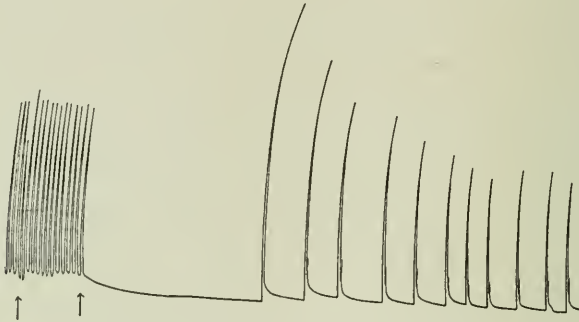


FIGURE 1.— For explanation see Fig. 2.

twenty-four hours previously. The animals were highly fevered and appeared quite sick, lying about the room and refusing to move or take food. They were shot, and the heart immediately excised, opened up, the blood removed, and the extract prepared as above described. The extracts were usually injected into the Langendorff heart within an hour or so after their preparation, being meanwhile kept on ice. In several instances they were neutralized (towards litmus) with sodium carbonate solution, and they were always warmed to body temperature before injection.

By referring to Fig. 1, it will be seen that the injection caused almost immediate inhibition of the heart beat in diastole. In some cases the inhibition was followed, in the course of from thirty seconds to a minute, by marked fibrillation of the ventricles (Fig. 2), the auricles however resuming their normal beat. In other cases the whole heart gradually began to beat normally again, the beats being as a rule feeble at first, but afterwards getting stronger until they became quite normal. Occasionally the injection had no effect on the heart beat, but I have found in these cases that a second injection of the same

extract immediately following the first one usually produced inhibition followed by fibrillation.

In this place it should be mentioned that similar injections of blood, of physiological saline, or of strong diphtheria toxin solution¹ had no immediate effect on the heart beat.

At first sight these results seem to indicate that some toxic compound had been produced in the heart as the result of the toxin inocu-

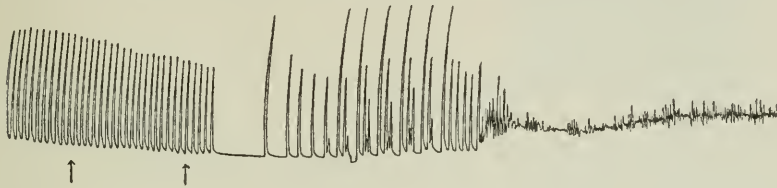


FIGURE 2.—Langendorff heart preparations. Between ↑ ↑ in each tracing was injected 5 c.c. of a Buchner's extract of the heart of a dog that had received a large dose of diphtheria toxin twenty hours previously. Read from left to right.

lation. It is known, from the recent experiments of Crile and Dolley² and others, that diphtheria toxin after its injection into the animal body soon fixes itself in the tissues, so that replacement of the infected dog's blood by normal blood does not curtail the course of the intoxication. The fact that cardiac failure is a common cause of death in diphtheria, as well as the fact that diphtheria toxins themselves do not affect the heart beat, led me to think that the injected toxins might possibly become combined with some chemical constituent of cardiac muscle to produce a compound having a paralyzing effect on the cardiac mechanism. The experiments described above seem at first sight to justify such a conclusion.

By performing the same experiments with extracts prepared from the hearts of normal dogs it was soon found, however, that such a conclusion is unjustified, for the normal extracts have just as great a paralyzing effect as have those from the hearts of inoculated dogs. The tracing in Fig. 3 shows the effect of such an extract.

Such results with the normal heart extract led to the question

¹ CLEGHORN states that when 25 per cent fresh diphtheria toxin is perfused through the apex of the dog's heart by PORTER'S method, slowing of the beat occurs. CLEGHORN: *This journal*, 1899, ii, p. 290. ROLLY found such injections to have no effect on the heart prepared by HERING'S method. ROLLY: *Archiv für experimentelle Pathologie und Pharmacologie*, 1899, xlii, p. 283.

² CRILE and DOLLEY: *Proceedings of the Society of Experimental Biology and Medicine*, 1907, iv, p. 65.

whether the paralysis is not due to some normal constituent of muscular tissue present in relatively excessive amount in the extract. I therefore injected a similar extract from normal skeletal muscle, with

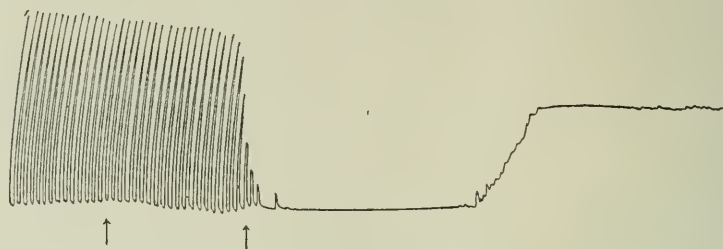


FIGURE 3.—Langendorff heart preparation. Between \uparrow \uparrow 5 c.c. of a Buchner's extract of the heart of a normal dog was injected. Read from left to right. It was attempted to remove the fibrillation by raising the perfusion pressure.

the result shown in Fig. 4. The result, it will be seen, is identical with those obtained by injection of the heart muscle extract.

The active principle in these extracts was found to be unaffected by heat, for the clear, watery, almost proteid-free solution obtained by boiling the extract and then filtering, gave the same result.



Figure 4.—Langendorff heart preparation. At signal 5 c.c. Buchner extract of normal skeletal muscle was injected into aortic cannula. When fibrillation appeared, the perfusion pressure was raised (\uparrow). Read from left to right. Time in seconds.

In the tissue juices, prepared as above by Buchner's press, it is certain that there must be a relatively large percentage of potassium, and the well-known inhibitory effect of this metal on the cardiac beat indicated the next step in the investigation, namely, to employ a watery solution of the ash of the extract. For this purpose a measured quantity of the extract was evaporated to dryness in a large platinum

crucible, the residue being then completely incinerated. After cooling, the ash was dissolved in water so as to make a quantity of

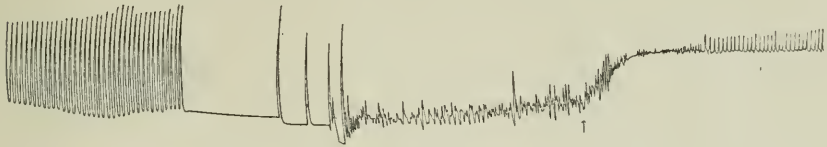


FIGURE 5.—Langendorff heart preparation. 5 c.c. of a watery solution of the ash of a Buchner extract of a normal dog's heart was injected into perfusion fluid (depression in signal line). At the arrow the perfusion pressure was increased. Time in seconds. To be read from left to right.

fluid equal to that of the extract originally employed. On injecting 5 c.c. quantities of this fluid into the heart preparation, as above described, the results recorded in Figs. 5 and 6 were obtained,—

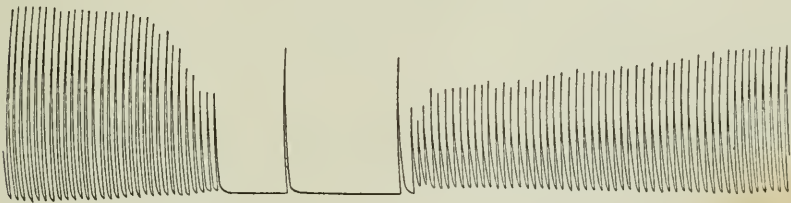


FIGURE 6.—Langendorff heart preparation. At signal 5 c.c. of a watery solution of ash of Buchner extract of normal muscle injected into aortic cannula. Read from left to right. Time in seconds.

results identical with those following the injection of the extracts. From these we must conclude that some inorganic constituent of the extracts is the cause of their remarkable toxicity towards the heart. Of the inorganic constituents possessing such an action, potassium is of course the best known.

This action of potassium on the mammalian heart has been thoroughly investigated by several workers, the most recent work being by Gross,¹ Braun,² and Howell and Duke.³ In the papers of these investigators will be found full references to the previous literature bearing on this subject. The effect described by these workers corresponds with that obtained above, with one important exception, namely, that in the present experiments fibrillation of the ventricles so frequently followed the initial inhibition. According to Gross, the inhibitory effect of potassium is removed by rapid transfusion with Ringer's solution. In the conclusions to his paper Braun also makes the same assertion, but in the context this writer states that "nach lethalen Kaliumdosen stirbt das Herz zumeist flimmernd ab" (p. 486).

The sudden inhibition of the beat, the frequent recovery of the heart, and the fact that smaller doses than 5 c.c. or slow injection of this dose had practically no effect on the heart beat, all agree with the conclusion that the potassium in such extracts is the toxic constituent. On the other hand, the fibrillation, so frequently observed, does not seem to be a common condition following potassium poisoning; indeed, perfusion with solutions containing an excess of potassium has been recommended as a method of removing fibrillation. It is claimed that the fibrillating heart is in this way quickly brought to standstill, and that, on again perfusing with a solution containing only the usual amount of potassium, it starts to beat normally.

Fibrillation is observed more frequently in a dog's heart than in that of the other laboratory animals, and once established in the dog's heart it is almost impossible to remove it. Its cause is ascribed by some to a sudden anæmia of the myocardium, and by others to irritation of the neuro-muscular mechanism concerned in the control of the heart beat.⁴

It does not seem to me that either explanation is satisfactory. Langendorff,⁵ in his first experiments on the isolated mammalian heart, pointed out that a sudden cessation of the coronary circulation in such a preparation could not be the cause of fibrillation, since it

¹ GROSS, E.: *Archiv für die gesammte Physiologie*, 1903, xcix, p. 99.

² BRAUN, L.: *Ibid.*, 1904, ciii, p. 476.

³ HOWELL and DUKE: *Journal of physiology*, 1906, xxxv, p. 131.

⁴ PORTER: Article "Circulation," *American text-book of physiology*, 2d ed., i, p. 182.

⁵ LANGENDORFF: *Loc. cit.*, p. 291.

does not follow sudden interruption of the perfusion. On the other hand, Porter's experiments,¹ in which the coronary circulation was suddenly interrupted without any mechanical injury to the heart itself, would seem to show that sudden anæmia does have something to do with the production of fibrillation. The most recent papers by Winterberg² and by Gewin³ on this difficult subject refer to the fibrillation following electrical stimulation of the heart and the influence of the cardiac nerves on its appearance, intensity, and duration, but there is nothing in these papers that helps us to explain its cause.

In the experiments recorded above, the fact that some of the extracts were opaque, from fat in them, led me to suspect that blocking of the coronary capillaries by fat globules might be responsible for the fibrillation. The absolutely clear filtered solution of the ash of such extracts had the same effect as the extracts themselves, so that such a supposition is unfounded.

Without attempting any further discussion on the cause of fibrillation it may be well to state concisely what were noted in the above experiments as interesting features of its appearance. These are:

1. It never appeared as a result of the injection unless this produced a preliminary complete inhibition. Sometimes, however, inhibition occurred without subsequent fibrillation.
2. When the inhibition affected only the strength and not the rhythm of the heart beat, fibrillation was not produced.
3. During and after the appearance of fibrillation the perfusion fluid continued to pass through the heart. I have not, however, made accurate measurements of the relative rate of outflow for a given perfusion pressure before and after fibrillation had set in.
4. The fibrillation was usually confined to the ventricles, the auricles resuming their normal beat after the inhibition.
5. Having once appeared, the fibrillation could not be made to entirely disappear again from the ventricles, although, as shown in Fig. 5, it could be made much less marked and a certain amount of normal beat might reappear.

The cause of the inhibition must, I think, be set down to the relatively high percentage of potassium in muscular tissue as compared with that in blood plasma. According to Abderhalden,⁴ the per-

¹ PORTER: *Journal of experimental medicine*, 1896, i, p. 46.

² WINTERBERG: *Archiv für die gesammte Physiologie*, 1907, cxvii, p. 223.

³ GEWIN: *Archiv für Physiologie, Supplement-Band*, 1906, p. 247.

⁴ ABDERHALDEN: *Lehrbuch der physiologischen Chemie*, 1906, p. 592.

centage of potassium in the blood plasma of the dog is 0.025. In muscle the percentage is, according to Katz,¹ 0.3125.²

We do not know what proportion of this amount of potassium in muscle will pass into the Buchner extract, but we can certainly expect a higher concentration in this than in saline extracts. This point would require to be determined by analysis of the extract, which as yet I have not found time to perform. It is to a similar influence of potassium that Langendorff³ ascribes the failure of the beat of the isolated mammalian heart on perfusion with the laked blood of animals whose erythrocytes contain large amounts of potassium, *e. g.*, rabbit; whereas, in employing the blood of animals whose erythrocytes do not contain a much greater amount of potassium than the plasma, *e. g.*, cat and dog, laking has little effect.

In connection with the above results, attention should be directed to the recent work of Howell⁴ and Dixon.⁵ Howell believes that "the inhibitory influence of the vagus and the augmentory influence of the sympathetic upon the heart may be exerted through some intermediate effect upon the potassium and calcium compounds in the heart tissue." The fact that by merely expressing the tissue juices of the heart, an extract is obtained containing sufficient potassium to produce inhibition would certainly lend support to Howell's view.

Dixon has recently claimed that from a cat's heart that had been inhibited for some time by vagus stimulation, he could prepare an extract which, on application to the frog's heart, produced inhibition of the beat. He states that potassium is not responsible for the result. In one or two experiments I prepared a Buchner extract from the heart of a dog after prolonged vagus stimulation, but did not find the resulting extract to have any greater inhibitory influence than a normal extract. It is, however, very difficult to make quantitative comparisons in the above experiments.

CONCLUSIONS.

The expressed tissue juice of fresh cardiac or skeletal muscle of the dog when injected into the fluid perfused through the heart of another

¹ KATZ, J.: *Archiv für die gesammte Physiologie*, 1896, lxiii, p. 46.

² The figures refer to the metal (K).

³ LANGENDORFF: *Ergebnisse der Physiologie*, IVer Jahrgang (1905), *Biophysik*, p. 7.

⁴ HOWELL: *This journal*, 1901, vi, pp. 181-206; *Ibid.*, 1906, xv, pp. 280-294.

⁵ DIXON: *Proceedings of the Physiological Section, British Medical Association*, Toronto, Canada, August, 1906.

dog, prepared by Langendorff's method, usually causes complete inhibition. The inhibition is sometimes recovered from, but, as frequently, it is followed by marked fibrillation of the ventricles. Since watery solutions of the ash of the extracts have the same effect as the extracts themselves, it is concluded that the large amount of potassium in them is the cause of their toxicity.

ŒSOPHAGEAL PERISTALSIS AFTER BILATERAL VAGOTOMY.

BY W. B. CANNON.

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

ALTHOUGH the wave of contraction passing along the œsophagus resembles in appearance the peristaltic wave of the stomach or small intestine, it has been assumed that peristalsis of the œsophagus, unlike that of the alimentary canal below the cardia, is wholly dependent on external nervous influences. In other words, the progress of a constriction throughout the length of the œsophagus is assumed to be due exclusively to impulses discharged from the central nervous system by way of the vagi. Thus Meltzer states,¹ "We know further that the section of the vagi wipes out peristalsis, while stimulation of their peripheral ends produces a simultaneous contraction of the entire œsophagus, but not a peristaltic wave. The movements therefore cannot be caused by the passing food, nor is their cause located within the gullet itself." And again,² "It is now generally assumed that the orderly progress of the peristalsis in the œsophagus is exclusively of central origin." More recently Starling has declared,³ "Section of the nerves going to the œsophagus abolishes its peristaltic contractions"; and last year,⁴ "The orderly progression of the peristaltic wave of inhibition plus contraction along the walls of the tube (œsophagus) is dependent on the integrity of the branches of the vagus nerve, by which the medullary centre is united to the gullet. Division of these nerves destroys the power of swallowing." Starck also has stated that bilateral vagotomy above the hilus of the lungs (in dogs) causes paralysis of the muscle of the

¹ MELTZER: New York medical journal, 1894, lix, p. 392.

² MELTZER: This journal, 1899, ii, p. 266.

³ STARLING: SCHÄFER'S Textbook of physiology, 1900, ii, p. 320.

⁴ STARLING: Recent advances in the physiology of digestion, Chicago, 1906, p. 132.

gullet, — peristaltic activity ceases, and solid pieces of food stop in the tube.¹

The evidence adduced to prove that peristaltic contraction of the œsophagus is managed through the vagi, is found, as the above quotations indicate, in the effects of cutting these nerves. The first of these effects is immediate cessation of peristalsis. For example, Meltzer² has lately noted that when fluid or air is introduced into the œsophagus, without laryngeal initiation a peristaltic wave starts close above the substance and pushes it into the stomach. On cutting the vagi, however, peristalsis at once fails, and the substance introduced is no longer moved downward. This stasis of the food in the œsophagus after vagus section was recorded by Reid in 1839.³ Reid's observations were quoted by Volkmann,⁴ and Volkmann's article is referred to by Kronecker as a basis for stating: "Since Volkmann's time we have known that in animals with vagus nerves sectioned in the cervical region deglutition becomes impossible."⁵ Kahn also in a recent paper refers to Volkmann as authority for declaring that "after bilateral vagus section the œsophagus is paralyzed."⁶ An examination of Reid's original paper, as well as Volkmann's reference to it, reveals no support for the denials of œsophageal activity above quoted. According to Reid's observations, the arrest of food in the œsophagus after vagotomy was prominent in rabbits, but in dogs the disturbance was temporary, and was followed by what seemed a free movement to the stomach.⁷ Reid's results receive some confirmation in a single observation by Sinnhuber. He records a case of bilateral vagotomy in which the œsophagoscope revealed nothing in the œsophagus after the dog had eaten solid food.⁸ This evidence of the possibility of the functional efficiency of the œsophagus after bilateral vagotomy does not accord with current views and with the

¹ STARCK: Münchener medizinische Wochenschrift, 1904, li, p. 1514.

² MELTZER: Zentralblatt für Physiologie, 1906, xix, p. 993.

³ REID: Edinburgh medical and surgical journal, 1839, li, p. 274.

⁴ VOLKMANN: WAGNER'S Handwörterbuch der Physiologie, 1844, ii, p. 586.

⁵ KRONECKER: Article "Deglutition," Dictionnaire de physiologie (Riche), 1900, iv, p. 753.

⁶ KAHN: Archiv für Physiologie, 1906, p. 355.

⁷ REID: *Loc. cit.*, p. 274. It was REID's observation of the improvement of the function of deglutition developing after a temporary defect of that function, that led to VOLKMANN'S statement in WAGNER'S Handwörterbuch (1844, ii, p. 588), that after vagus section the primary disturbance of digestion was followed by a more or less normal condition.

⁸ SINNHUBER: Zeitschrift für klinische Medicin, 1903, l, p. 114.

statements above quoted as to the paralysis of the gullet after that operation.

Two important considerations seem to have been slighted in modern investigations of the innervation of the œsophagus, — first, the difference between the immediate effects of vagus section and the later possible recovery of a normal state; and second, the muscular structure of the lower fourth or fifth of the tube, which in many animals is composed largely or entirely of non-striate fibres, well supplied with a myenteric plexus, and resembling in all essentials the muscular wall of the stomach and intestine.¹ In a recent paper I have shown that there may be for some time after vagus section a total absence or notable inefficiency of gastric peristalsis, with a subsequent remarkable restoration of function. The local mechanisms, at first inert after removal of vagus influence, later prove able to continue gastric peristalsis in an almost normal manner.² If it is possible for the stomach thus to recover from a primary paralysis, may not the œsophagus, at least that part of it similar in all essential respects to the stomach structure, be able to recover likewise from a primary paralysis?

An answer to the question just stated was found by cutting in cats the two vagus nerves³ — the right below the origin of the recurrent laryngeal, the left in the neck — and subsequently studying, by means of the Röntgen rays as already described,⁴ the movements of food in the œsophagus. The phenomena seen in the following case have been verified by observations on other cats, and may be regarded as typical.

On October 27 the right vagus nerve was cut below the origin of the recurrent laryngeal branch. Two days later the left vagus was cut in the neck. Before the second operation a small bit of meat wrapped about some bismuth subnitrate was given to the animal, and was seen, by means of the Röntgen rays, moving regularly along the œsophagus into the stomach.

On the day after the severance of the second vagus nerve the animal received meat ground fine and mixed with a small amount of subnitrate of bismuth and enough water to make a mush. The first mass swallowed

¹ OPPEL: *Lehrbuch der vergleichenden mikroskopischen Anatomie*, 1898, ii, pp. 134, 142, 146.

² CANNON: *This journal*, 1906, xvii, p. 429.

³ In all operations involving nerve section the animals were, of course, under complete general anæsthesia.

⁴ CANNON: *Loc. cit.*, p. 430.

appeared quickly below the glottis, and was pushed slowly and regularly to the top of the thorax. At that position the continuous movement stopped; only gradually and apparently by means of rhythmic changes of pressure due to respiration, was the mass worked down to a position about midway between the top of the thorax and the diaphragm. There it rested. Other masses arriving later came together in this region, and the accumulation was pushed onward until the œsophagus was filled to the diaphragm. The animal was watched continuously for forty-five minutes, but no movement of the œsophagus was seen, and no food entered the stomach. The entire œsophagus seemed to be paralyzed.

On the second day after cutting the left vagus nerve nothing was found in the œsophagus from the day previous. As it is common during the first few days after operation for these animals with bilateral vagotomy to regurgitate food they have swallowed (a fact Reid noted in dogs), it was possible that the œsophageal accumulation had been discharged. But in this case no evidence was found that such regurgitation had occurred. On this second day a single spoonful of a mush made of crumbled crackers, water, and bismuth subnitrate was given at 4.32 P. M. In four minutes it had been slowly worked down from the top of the thorax until it spread as a long slender mass even to the diaphragm. No movement of the œsophageal wall was seen; the spreading seemed to result from respiratory alterations of intra-thoracic pressure. The mass lay without further change for four minutes. Then a second spoonful of the mush was given. When this new material was pressed into the thoracic œsophagus, the lumen was enlarged to almost twice its former diameter. Immediately a constriction of the œsophageal wall occurred at the level of the lower half of the heart. This constriction moved toward the stomach and was followed by others that also moved downward. The first waves failed to drive food through the cardia; the food slipped back through the moving ring. Later waves, however, were more effective, and pushed food into the stomach. The remnant of the mush in the gullet was now extended again in a slender strand. During ten minutes more of continuous observation no further change was seen. The animal was placed in a clean dry cage for the night. The next morning there was no food in the cage and none in the œsophagus. The waste was in the large intestine.

On the morning of the third day at ten o'clock, after two spoonfuls of the mush had been given, the animal was placed again in the clean cage. At 3.45 P. M. there had been no regurgitation; the œsophagus was found empty. Now the animal took three spoonfuls of the food, which filled the thoracic œsophagus to stretching. Immediately, at the level of the lower half of the heart, a constriction appeared that passed downward, causing as it moved a marked bulging of the tube in front. Some of the food surely escaped backward through the advancing ring.

This wave was immediately followed by a second, starting from the heart level and pushing downward in a manner similar to the first. The second wave forced food into the stomach. The remnant became extended to the diaphragm; but only after four minutes did another ring start at the heart level and push the lower end of the column into the stomach. Again the remnant was extended to the diaphragm. Except occasional deep stationary constrictions, at the heart level, there was no change for eight minutes. Then a ring, formed just above the diaphragm, and pushed food into the stomach, and another ring, immediately above, cut off the lower end of the remaining mass and likewise forced this bit of food through the cardia. The rest of the œsophageal accumulation was now but a slight string in the upper thoracic region. For thirty-eight minutes of observation it remained unmoved in that situation.

On the seventh day the thoracic œsophagus was filled, from a rubber tube attached to a syringe, with thin starch paste (3 gm. to 100 c.c. water) mixed with bismuth subnitrate. At once after the injection one constriction after another formed in rapid succession, each cutting off the lowest part of the mass and moving it through the cardia. As judged by gently feeling the larynx, there was no swallowing in this process, — the action was a local response to the presence of material in the gullet. Thus, by repeated reductions from the lower end, the column of food was gradually carried away until only a slender remnant was left. This was slowly moved below the heart, but there it stayed for half an hour. At the end of that period a small bit of meat, with bismuth subnitrate adherent, was fed. The meat moved smoothly through the cervical region, but stopped at the top of the thorax. Now the slender mass below was gathered together and swept into the stomach. Sixteen minutes were required for the meat to come to the level of the lower half of the heart. Again nothing interpretable as a constriction was seen in the thoracic œsophagus above the heart. Below the heart, however, the meat, which had been separated into two pieces, was carried by peristalsis into the stomach.

Twenty-three days later the animal was again given starch paste as before, with the same results. While there was still a considerable amount of the paste above the heart level, swallowing movements were caused by tickling the larynx. Most careful scrutiny showed no sign of the passage of a wave over the food in the upper thoracic region.

Three days later — thirty-two days after severing the left vagus — the animal was etherized, and fitted with a tracheal cannula. Ligatures were tied with crushing tightness around the right vagus in the neck, and also around the left vagus below the point of severance. The introduction of starch paste into the œsophagus was followed temporarily by marked peristaltic waves in the lower thoracic region. When the waves stopped and

while food still filled the œsophagus, the nerves were stimulated with a tetanizing current. There was no effect on the thoracic œsophagus—degeneration of the nerves was evidently complete.

Now the left intercostal and the left internal mammary arteries were tied, and under artificial respiration the left chest wall removed. More paste was introduced, whereupon strong peristaltic constrictions were seen by direct inspection starting from the heart level and passing downward. When they had stopped, and while the paste was still distending the gullet, the right vagus was stimulated as before. There was no contraction of the wall of the thoracic œsophagus.

The right vagus was carefully examined after death. No connection was found between the cervical and the thoracic vagus trunk.

The facts that in this case the evidence of complete section of both vagi was clear at the time the second nerve was severed (faster heart beat, slower respiration), and also at autopsy; that subsequent stimulation of both vagi caused no constriction of the tube; and that peristalsis of the lower œsophagus persisted after crushing what remained of the right vagus, all combine to prove that peristalsis of the lower œsophagus can act without the support of the vagi.

In the foregoing record there are several points that stand out significantly:

(1) Immediately after operation and for twenty-four hours at least thereafter, it is easy to gather evidence of complete paralysis of the œsophagus. In one instance during this first period food was observed to stagnate in the gullet for five hours, and in another instance for seven hours after feeding. Evidently in the cat a distinction must be made between the primary paralysis of the whole œsophagus after bilateral vagotomy, and the secondary recovery of certainly the lower half of the thoracic portion.

(2) After a return of peristaltic activity in the lower œsophagus, an important factor for arousing that activity seems to be the stretching of the œsophageal wall. A slender mass spread along the œsophagus may lie for some time unmoved; the addition of a second mass, which causes a stretching of the wall, results in the instant appearance of constriction rings and peristaltic movements. And similarly after repeated reductions of the content of the œsophagus, the strand of food as it becomes more attenuated lies for longer periods unaffected by œsophageal contractions. This reaction of the œsophageal wall to the presence of a stretching mass is a local reaction, occurring without centrally initiated movements of deglutition.

It is thus similar to movements of the alimentary canal below the cardia. The lower œsophagus seems to become more responsive to the presence of contained material as time elapses, for the material is driven into the stomach with increasing rapidity, and even slender masses are sufficient cause for peristalsis.

(3) A difficulty in forcing food through the cardia explains to some extent the slower emptying of the gullet during the first days after operation. That the cardia of the cat offers an obstacle to easy passage to the stomach after bilateral vagotomy is proved by the fact that strong peristaltic waves, so strong as to produce a very marked bulging of the tube in front of them as they advance, have failed to force food into the stomach. Indeed, three days after cutting the second vagus nerve I have seen almost exactly the same repetition of deep constrictions and vigorous peristaltic movements in the lower œsophagus as occur in the small intestine in case of obstruction.¹ The opposition at the cardia was also noted when in these animals attempts were made to pass a tube into the stomach. These observations are in accord with the observations of Bernard² and Schiff,³ that cutting both vagi in the neck is followed after a few hours by strong contraction of the lowest part of the œsophagus. But they are not in accord with the observations of Krehl⁴ that after vagus section the cardia is patulous, nor are they in accord with Katschkowsky's⁵ assumption to the same effect. It may be that this conflict of evidence can be explained by the temporal factor. Thus Sinnhuber⁶ concludes, from a critical review of the literature and from his own experiments, that though cutting the vagi may cause the cardia to enter a cramp-like contraction, this is only a temporary state. Starck⁷ also does not believe that vagus section produces any lasting hindrance to the passage of food through the cardia. In my experience the increased tonus of the cat's cardia after bilateral vagotomy usually does not persist as a considerable obstacle, and the forcing of food into the stomach by œsophageal peristalsis becomes in time not difficult. But there have been a few instances under my observation

¹ CANNON and MURPHY: *Annals of surgery*, 1906, xliii, p. 522.

² BERNARD: *Comptes rendus de la Société de Biologie*, 1849, i, p. 14.

³ SCHIFF: *Leçons sur la physiologie de la digestion*, 1867, i, p. 350; ii, p. 377.

⁴ KREHL: *Archiv für Physiologie*, 1892, Supplement-Band, p. 286.

⁵ KATSKHKOWSKY: *Archiv für die gesammte Physiologie*, 1901, lxxxiv, pp. 29, 30.

⁶ SINNHUBER: *Loc. cit.*, p. 117.

⁷ STARCK: *Loc. cit.*, p. 1514.

in which there was a continued trouble in passing a tube into the stomach; the œsophagus in these cases suffered a marked dilatation and became filled with food which decomposed.

(4) Throughout these observations on animals with vagi severed a marked contrast was noted between the activity of the lower half of the thoracic œsophagus and the inactivity of the upper half. Very careful observation of fluid starch paste which filled the upper thoracic part of the tube revealed no sign of a wave sweeping over this easily mobile mass, even when the animal was repeatedly caused to swallow. This absence of peristalsis from the region above the heart was as true a month after the second vagus was severed as it was during the first twenty-four hours. Is there any difference of condition between these two parts of the thoracic œsophagus which might account for their difference of action after vagus section? Leaving one recurrent laryngeal nerve still provides innervation for the cervical œsophagus,¹ but cutting off all vagus supply, except one recurrent laryngeal, destroys the extrinsic innervation of the gullet between the base of the neck and the cardia. In this thoracic region the œsophagus is provided with two different kinds of muscular fibres. Dr. J. L. Bremer has kindly made a histological examination of the œsophagus of the animal on which were made the detailed observations reported above. He found that the musculature of the upper half of the thoracic region was composed predominantly of striped fibres, whereas the musculature of the lower half, over which peristalsis continued after vagus section, was composed almost wholly of unstriped fibres. Since the difference between the œsophagus in the neck, which acted normally, and the œsophagus in the upper thorax, which failed to act, was that the former had in all cases a recurrent laryngeal supply, while the latter had no outside nerve connection, the conclusion is justified that that part of the tube which is composed of striped muscle fibres is paralyzed when vagus impulses are removed from it. The general conclusion, however, that the entire œsophagus is put out of action by severance of the vagi must be modified. That part of the tube which is composed of unstriped muscle is, like other similar parts of the alimentary canal, capable of quite perfect peristaltic activity without the aid of extrinsic nerves.

The validity of these conclusions was confirmed by observations on the rabbit and the monkey (rhesus). In the rabbit no œsophageal peristalsis was seen at any time after severance of the second vagus

¹ KAHN: *Loc. cit.*, p. 361.

nerve, although one animal was kept alive and examined from time to time for two weeks after the operation. In the monkey, on the other hand, the results were similar to those in the cat. Three hours after the second vagus was sectioned, mashed banana mixed with subnitrate of bismuth was swallowed by the monkey and at once carried to the upper thoracic œsophagus, where it rested. More banana forced some of the mass in the gullet to the level of the heart. As soon as food reached beyond this level, it was promptly separated and carried slowly into the stomach. There was no evidence of obstruction at the cardia. For further assurance the animal was etherized, the right vagus also cut in the neck, the left thoracic wall widely opened, and the œsophagus watched directly as water was introduced through a tube into the cervical portion. Peristaltic waves appeared where the vessels of the left lung crossed the gullet, and they moved slowly downward until they went out of sight behind the diaphragm. The point where the waves were first seen was marked by making a deep cut, and the animal was then killed. Through the kindness of Dr. S. B. Wolbach, the œsophagus of the rabbit longest observed, and the œsophagus of the monkey, received careful histological examination. Striped muscle, almost exclusively, was seen throughout the length of the rabbit's œsophagus. The part of the monkey's œsophagus above the cut, the part which was paralyzed by bilateral vagotomy, was composed entirely of striped fibres; the part below the cut had only a few scattered striped fibres, the rest was all smooth muscle.

Meltzer's investigation¹ indicated two sorts of œsophageal peristalsis: one in which the progression of the wave follows a sequence of changes in the central nervous system — as if a succession of impulses were sent to the tube in an orderly manner from point to point along the entire vagus distribution; the other in which the wave is forwarded by reflexes through the vagi, reflexes started from and returning to successive points in the œsophagus. To these two varieties of peristalsis requiring vagus support must be added a third, which is seen when a portion of the œsophagus is composed of non-striated muscle fibre. The peristalsis of this portion, like peristalsis below the cardia, is capable of autonomy. In many cases which I have observed it has been sufficient without vagus support to clear the œsophagus of any ordinary food which had been carried into the thoracic segment.

¹ MELTZER: This journal, 1899, ii, p. 266.

THE ACTION OF QUININE SULPHATE ON HUMAN BLOOD.

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I. THE ACTION OF QUININE SULPHATE ON UNICELLULAR ORGANISMS.

THE obvious morphological resemblance between the polymorphous neutrophiles of human blood and certain rhizopoda would naturally suggest that these unicellular organisms are very similar in their pharmacological reactions to quinine sulphate. Although cinchona bark, the source of quinine, was introduced into Europe by the Duchess of Chinchon¹ in 1640, from Peru, where it had previously been employed by the Indians for the treatment of ague, it was not until 1867 that its destructive action on protozoans and bacteria was investigated. C. Binz² found that 0.2 per cent paralyzed three different species of unicellular animals in a few seconds, and that they soon became rounded, granular, and the protoplasm darkened. Similar changes in *amœba navicula* were seen with 0.1 per cent quinine hydrochloride. His studies on the white corpuscles of cats showed that with half the latter strength, when examined in a Schultze moist chamber at a temperature of 38° C., these likewise soon lost their motion, became granular, and sometimes divided unequally.

In the same year Helmholtz³ injected doses of 4 c.c. of a saturated solution of quinine sulphate into his nostrils as a cure for hay fever, from which he had suffered for twenty-one years, and found that certain vibrios which he claimed were previously abundant in his nasal secretions, could not be detected microscopically after the administration of the drug. Two years later, Binz showed that a

¹ BAILEY: *Encyclopedia Americana*, Hort. V., i, p. 316.

² BINZ: C. SCHULTZE, *Archiv für mikroskopische Anatomie*, 1867, iii, p. 383.

³ BINZ: *Archiv für pathologische Anatomie*, 1869, xlvi, p. 67.

strength of 0.8 per cent had strong bactericidal powers, in that it rendered contaminated water odorless for one month. In the same paper he refutes Schwalbe,¹ who maintained that no change in the percentage of the white corpuscles was produced in an animal poisoned by quinine. Binz took two brother cats, killed one with 0.1 per cent quinine injection, and used the other as a control. In the quinized animal there was almost an entire absence of white corpuscles from its blood, taken at the time of its death, while in the other the leucocytes remained unchanged. In the same paper he claims that he was able to recover unaltered from the urine 66 per cent of the quinine administered.

Some time later² the same investigator, while observing the action of this alkaloid on the white corpuscles seen in the capillaries of an exposed mesentery of a frog, noticed similar phenomena to that seen by him in the mammal's blood studied in the moist chamber, and the additional finding that all leucocytic migration was stopped. He thinks that this loss of motion is due to the quinine holding more firmly than normal the oxygen in the red corpuscles. This, he claims, is further corroborated by the work of Kühne³ on the ciliary movements of the gill of anodon. He found that when hæmoglobin is absent, hydrogen and carbondioxide cause the cessation of ciliary motion.

In his last paper⁴ on this subject, called forth by the incorrect results of Köhler,⁵ Binz supplements his work concerning the influence of quinine in preventing the migration of white corpuscles during inflammatory reactions of the exposed frog's mesentery, by making several additional experiments. He also cites confirmatory results from other experimenters. His own animals were kept curarized during the investigation, and quinine hydrochloride in doses of from 0.01 per cent to 0.005 per cent of their body weight was injected every two or three hours, as long as the animals survived. He found that in the quinized frogs, with the circulation intact, the migration of the leucocytes was prevented; while in the quinine-free curarized frogs a marked accumulation of pus was plainly

¹ SCHWALBE: *Deutsche Klinik*, 1868, xx, p. 325.

² BINZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1873, i, p. 29.

³ KÜHNE: *Archiv für mikroskopischen Anatomie*, 1866, ii, p. 374.

⁴ BINZ, C.: *Archiv für experimentelle Pathologie und Pharmakologie*, 1877, vii, p. 275.

⁵ KÖHLER, H.: *Zeitschrift für praktische Medicin*, 1877, p. 44.

visible, while microscopically the vessels were whitened with the leucocytes hugging the walls.

II. THE MODIFIED METHODS USED IN EXAMINING CERTAIN HUMAN BLOOD FACTORS.

a. A statement of the chief purposes of this research. — The known action of quinine sulphate on unicellular organisms and its leucopœnic effect when administered internally, led to this study of its influence on the phagocytic factors of human blood. It has been found that at least 90 per cent¹ of the quinine administered can be extracted unaltered from urine, and it was supposed that the drug would have an inhibitive effect on phagocytosis.

The general purpose of this research was to study *in vivo* the action of quinine sulphate, sodium salicylate, nucleinic acid, potassium iodide, and calomel on opsonic index and certain other factors; then, as a sort of control, to examine *in vitro* the phagocytic effect of these drugs. The work with quinine sulphate was done on volunteer students almost entirely during the summer of 1906, but other problems delayed the completion and publication of the results then obtained.

It was thought desirable to first ascertain the daily variation of a healthy individual in regard to seven hæmic factors: (1) the number of red and (2) white corpuscles; the percentage (3) of hæmoglobin and (4) of serum; (5) the differential count; (6) the coagulation time; finally, the average number of bacteria taken up by a single polymorphous neutrophile.

b. A comparison between defibrinated and non-defibrinated blood. — In ascertaining certain of these variables, it is preferable to employ non-defibrinated blood, and various schemes of preventing coagulation were considered. The cooling of the blood to about zero was discarded, because it was feared that clotting might occur before blood for the first five factors could be secured. The mixing of sodium citrate or other precipitant of calcium with the 1 c.c. of blood employed for each experiment was also very unsuitable for part of the work, and its use was likewise rejected.

The plan finally adopted was to employ defibrinated blood and determine, once for all, the variation, if any, in the five values obtained from the non-defibrinated blood and those from the defibri-

¹ CUSHNY: Text-book on pharmacology, 4th ed., 1906, p. 362.

nated. This determination was made as follows: When all apparatus required was ready and arranged conveniently for very rapid work, 1 c.c. of blood was aseptically withdrawn from the mediana cubiti of a healthy student, and at once transferred to a small tube resting on cracked ice. The difficult work of securing and properly manipulating the quantity of blood requisite for the determination of each separate factor before clotting occurred, was successfully ac-

TABLE I.

Factors.	Non-defibrinated.	Defibrinated.	Percentage difference of defibrinated contrasted with non-defibrinated.
Percentage of liquid	Plasma 54.7 per cent	Serum 56.2 per cent	+2.6
Hæmoglobin	94.0 " "	92.0 " "	-2.0
Erythrocytes	4272000.0	4032000.0	-5.6
Leucocytes	5500.0	4950.0	-10.0
Polymorphs	63.6 " "	67.5 " "	+6.1
Lymphocytes	27.1 " "	25.0 " "	-7.7
Large mono-nuclears	5.8 " "	3.5 " "	
Eosinophiles	2.5 " "	2.0 " "	
Basophiles	1.0 " "	2.0 " "	

complished. The blood remaining in the reception tube was next defibrinated, and the examination of the two kinds completed in the ordinary way. In spite of the fact that the defibrinated blood had just begun to clot spontaneously before defibrination commenced, and that a certain number of the formed elements were thus withdrawn from the determination, the results agree very fairly as regards percentage of liquid hæmoglobin erythrocytes, polymorphous leucocytes, and lymphocytes.

There is, then, no warrant for concluding that defibrinated blood gives values, for the five factors mentioned, materially different from those similarly obtained from the unclotted blood. In fact, none of the percentage differences go beyond the limit of error occurring in successive blood examinations as ordinarily made on the same individual.

c. **The technique employed in the blood examinations.** — The blood for all the experiments following was secured in the same manner as above mentioned, and transferred to a small crucible securely and conveniently held by a claw clamp, and defibrinated by means of a small glass rod. The times in seconds of the first appearance of the blood in the needle, and the first signs of the clot in the crucible were carefully noted. A small portion of the defibrinated blood was reserved for the hæmoglobin determination, the red, the white, and the differential estimations. Enough of the remainder was transferred to a long, narrow tube to occupy a length of 100 mm., and both portions were corked. The enumeration of the reds and the whites was made in the ordinary way, but special care was taken to reject all counts which failed to show homogeneous suspension of the corpuscles.

Since the Haldane method of hæmoglobin determination is the only one which contrasts the identical colored compound (carbon monoxide hæmoglobin), it was employed in this work. Instead, however, of shaking the reception tube, as Haldane recommends, the fluid in it was made uniform in color by using a medicine dropper of finely drawn out bore, and alternately drawing in and expelling the fluid from this pipette. In estimating the percentage of the different kinds of whites, 2 cubic millimetres of blood were spread evenly on each cover glass and stained according to my method.¹ The 2 cubic millimetres of blood contain about ten million reds, and on an ordinary slip of 500 square mm. would average 20,000 erythrocytes per square mm., so that 50 of them could lie on a small square of an ordinary hæmocyto-meter without being crowded. This number would be considerably reduced by some blood clinging to the tissue paper, and some accumulating more or less on the edge of the slip last touched in the act of making the smear. Care was always taken to count a portion of the smear containing at least 250 leucocytes, in strips running parallel with the direction of the spread. This latter precaution, of necessity, better insured a correct result.

The defibrinated blood in the long tube was centrifuged at a uniform speed for forty-five minutes, and the percentage of serum noted. The results are given in Table II.

d. **Manner of securing the leucocytes for work on opsonins.** — For the purpose of uniformly sensitizing the bacteria which were em-

¹ This paper will be published in *Journal of experimental medicine*, 1907, ix, no. 6.

ployed, it was incumbent to preserve a portion of the serum. The whites also required to be completely freed from serum, and, as far as feasible, segregated from the erythrocytes. A few leucocytes, sufficient for opsonic determination, are usually partially separated from the reds by collecting them from the surface of a specimen sedimented in a straight or U tube. As, however, it was considered of some value to ascertain the best means of making a somewhat

TABLE II.

mm. of sediment taken	Leucocytes in specimen 1 (Poppen). ¹	Leucocytes in specimen 2 (McGrath). ¹
60 to 50	21500	18250 (64 to 50 m.m.)
50 to 40	7075	11650
40 to 30	4700	6350
30 to 20	2250	4600
20 to 10	2100	1050
10 to 0	250	150

¹ In Poppen's blood there were 4600 white corpuscles per cubic millimetre, while in McGrath's there were 6200.

complete separation of the formed elements of the blood, two experiments were made thereon before proceeding further with the main problem. About 1 c.c. of blood was defibrinated and centrifuged for an hour in a tube of such diameter that the quantity had a length of 100 mm. The serum was carefully syphoned off, and a white count made of the blood in each succeeding 10 mm. of the 60 per cent sediment which remained. The results are tabulated below.

It thus appears that the upper third of a sediment is four or five times richer in leucocytes than is the lower two-thirds of it. It was also found later, that a greater percentage of whites appears in the upper third when the centrifugalization is continued for some time, even after the sediment has well formed. The upper third of the sediment, equal in volume to 0.2 c.c., was washed with 20 c.c. of salt solution for ten minutes, thereby diluting the small quantity of serum it contained more than a hundred times. After removing the supernatant fluid, it was again washed as before, thus reducing

the serum present in it to less than one ten-thousandth part of the original. The sediment was finally washed in the long, narrow tube, and a still greater concentration of whites was obtained by withdrawing the upper portion of it. The latter was used for phagocytosis.

e. The solution employed for washing the corpuscles. — Instead of using the ordinary 0.9 per cent sodium chloride for washing the corpuscles, a solution was employed containing the principal ingredients of plasma, which could, without transformation or precipitation, be autoclaved. It was, in fact, somewhat similar to that already claimed by the writer. One litre of it had the following composition :

Glucose	1.5 gm.
Urea	0.5 “
NaCl	5.85 “
Na ₂ CO ₃	1.75 “
NaHCO ₃	0.25 “
Na ₂ HPO ₄	0.5 “
KCL	0.03 “

To this was added the calcium citrate and sodium chloride derived from previously dissolving 0.2 gm. of calcium chloride, and 0.3 gm. of sodium citrate. The resulting alkaline solution was found injurious to the white corpuscles, as were also all solutions whose alkalinity exceeded 0.02 per cent. For this reason the above solution was almost neutralized by adding 55 c.c. of N/4 hydrochloric acid. After filtering, it was placed in small flasks and sterilized. The clear brown solution having $a\Delta$ of 525, and $a\lambda^5$ * of 82.4, prevented to a considerable extent the formation of vacuoles, and better preserved the granules of the phagocytes. In other words, it apparently nourished the corpuscles during their work of engulfing the bacteria, with which they were afterward brought in contact.

f. The making and examination of blood suspensions. — The streptococci employed for each experiment were kept somewhat uniform in concentration by inoculating with approximately equal quantities and into the same volume of bouillon containing a little lactose, and by always growing them for twenty-four hours at 37° C.

A modified medicine dropper (shown in Fig. 1) containing, when filled to the mark *M*, about 25 cubic millimetres, was employed in making blood suspensions.

* λ^5 = conductivity expressed in reciprocal ohms at 5° C. $\times 10^8$.

For each opsonic index determination there was mixed in a fine tube 40 per cent of bacteria, the same percentage of washed corpuscles, and 20 per cent of serum, secured by filling the pipette once with serum, and twice with each of the other two ingredients which had been previously shaken. A count of one of these suspensions gave 636,000 reds, and 7300 whites per cubic millimetre, or a ratio of 87 reds to 1 white corpuscle, whilst the blood of this student contained 6,264,000 reds and 6260 whites per cubic millimetre respectively, or a ratio of 1001 to 1. So that, by the method adopted

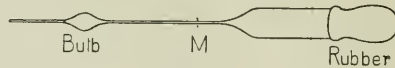


FIGURE 1.

for separating these corpuscles, there was an increase of the whites over the reds of eleven and one-half times. The homogeneous suspension was next placed in a thermostat at 37° C. for sixty minutes, care being taken during this period to shake the mixture every fifteen minutes.

After incubation, smears were made on cover slips by taking about 2 cubic millimetres for each slip. The specimens were air-dried, thrice passed through the flame, stained according to my method, and mounted in Canada balsam.

A much greater percentage of eosinophiles, as compared to the polymorphs was present in the smears than that found in normal blood. In one case the eosinophiles were as high as 8 per cent, whereas in normal blood they were only 1 per cent. This result indicates that the eosinophiles have a lower specific gravity, and perhaps are less viscous than the polymorphous neutrophiles, and hence more readily could gain the upper layers of the sediment.

The bacteria in 50 polymorphs were counted in each experiment, and the average for one corpuscle obtained therefrom.

III. THE ACTION OF QUININE ON HUMAN BLOOD IN VIVO.

a. The control experiment. — In the control experiment to which reference was previously made, a specimen of blood was taken from a healthy medical student, about one hour after breakfast, on each of five successive mornings. The results are given in the first part of the subjoined table:

TABLE III.

Initials of student.	Coagulation time.	Percentage of serum	Percentage of hæmoglobin.	No. of whites per mm. ³	Thousands of reds per mm. ³	Differential count.					Phagocytosis per polymorph.	Virulence of the streptococcus.
						Polymorphous neutrophiles.	Lymphocytes.	Large mononuclears.	Eosinophiles.	Basophiles.		
W. V.	5.7	45	105	4900	5464	53.2	39.6	3.2	4.0	0.0	7.3	8.2
W. V.	5.0	46	108	4200	6058	42.5	47.5	3.0	1.5	0.5	6.8	8.2
W. V.	4.7	46	110	4350	5400	46.5	47.5	1.5	4.0	0.5	8.1	8.2
W. V.	4.5	46	110	4600	6168	53.0	38.5	2.5	2.5	0.5	8.1	8.2
W. V.	4.7	47	109	5650	5560	56.5	37.0	3.0	3.0	0.5	6.9	8.2
D. D.	0.0	45	7900	5736	63.8	29.2	4.0	2.5	0.5	29.9	2.0
D. D.	46	7400	5568	66.5	29.0	2.0	1.5	1.0	38.2	2.0
Dr. S. A. M.	3.5	46	6600	5296	53.3	39.3	4.6	0.6	2.0	21.3	2.0
Dr. S. A. M.	3.9	46	6500	5480	60.0	33.0	4.0	2.0	0.6	24.1	2.0
J. L.	5.0	51	6050	6148	62.5	24.3	11.3	1.3	0.6	31.8	2.0
J. L.	3.0	53	6500	6304	49.0	43.0	52.0	2.0	1.0	30.2	2.0
C. S. M.	3.2	44	100	6500	5796	56.4	36.4	2.4	4.4	0.4	19.7	2.0
C. S. M.	3.5	47	95	6050	5552	51.6	36.8	7.2	4.0	0.4	18.1	2.0
A. P.	3.0	40	100	5500	6336	46.8	36.4	7.6	2.0	2.8	12.1	4.9
A. P.	4.2	49	105	6200	6848	50.0	42.0	4.8	0.8	0.4	12.6	4.9
C. G. P.	5.0	45	95	5050	6150	65.0	28.0	4.0	1.0	2.0	8.6	4.9
C. G. P.	3.7	45	85	5350	6000	67.0	38.0	2.5	1.5	1.0	8.9	4.9
E. B.	3.5	43	6900	5824	75.0	21.0	2.5	1.0	0.5	7.4	4.9
E. B.	2.5	45	6800	6048	54.0	41.0	4.0	1.0	0.0	11.0	4.9
E. McG.	3.5	33	100	6200	6264	61.0	33.0	5.0	1.0	0.0	10.8	4.9
E. McG.	4.6	36	94	6400	6116	68.0	25.3	3.6	2.6	0.5	13.5	4.9
C. T. B.	3.6	44	101	5350	5128	73.0	21.5	2.5	1.5	1.5	8.0	4.9
C. T. B.	4.0	38	107	5750	5648	65.5	25.5	4.0	1.5	0.5	10.7	4.9
T. M. W.	4.5	50	92	6500	4392	60.0	30.8	3.2	5.6	0.4	8.3	8.2
T. M. W.	4.5	48	98	6000	4976	48.4	42.0	1.2	7.2	1.2	12.1	8.2

b. **The action of quinine on ten students.** — In the examination of the action of quinine sulphate on ten students, it was necessary to first make a complete determination of the different factors already dealt with in the control experiment. Then 15 grains of quinine in divided three-grain doses were administered during the course of the next twenty-four hours. At the expiration of this period the same determinations were again made from the blood extracted at the same time after the morning meal as on that of the previous day. The results are given in the second part of Table III.

The results in the first line coupled with each separate name were obtained from the blood before giving the quinine, while in the line following are those got twenty-four hours afterward. In the first case recorded, 9 grains of the drug produced such marked aural symptoms that the remaining 6 grains were not taken, yet the results obtained show a marked increase in the opsonic index.

In seven other cases there was also a slight increase, while in the remaining two a slight decrease was found.

c. **Standardizing the virulence of organisms.** — It will be noted that there is considerable range in the number of streptococci englobed on the average by each polymorph. The range was from 6.8 to 38.2 in sixty minutes. This was due, in large part, to the employment of three different strains of streptococci. In order to properly compare the results, it became necessary to standardize the virulence of these bacteria. In general, it may be stated that where the number and size of the organisms per unit volume remain constant, their virulence varies inversely with the percentage of the serum present, and also with the number of bacteria taken up in a unit of time. An organism is here considered to have a virulence equal to the unit, if 60 of them are taken up during sixty minutes' incubation at 37° C. by a single polymorphous neutrophile, in the presence of 20 per cent serum, the organisms present in the medium being from 0.5 to 5 millions per cubic millimetre. For example, an organism would have a virulence of 0.5, if 30 were taken up in fifteen minutes, since $60 \div (30 \times 4) = 0.5$. In the control experiment the first organism used in testing V.'s blood had a virulence of 8.3, since in one hour 7.3 bacteria per leucocyte were engulfed.

d. **The phagocytosis in polymorphous neutrophiles.** — The power of the polymorphous neutrophiles of taking up and digesting bacteria can be readily verified. The microscopic scrutiny of any of the various specimens of the blood treated as above described will reveal

many streptococci in different stages of digestion, within the cytoplasm of the phagocytes. Sometimes the digestion will have so far advanced, in the course of sixty minutes, that one is left in doubt whether the shadows seen are artifacts or really bacteria, almost wholly stripped of their blue staining material by the action of the enzymes within the white corpuscles. A halo is very often seen

TABLE IV.

PHAGOCYTOSIS OF EOSINOPHILES.					
No. of intact corpuscles counted.	No. of bacteria per corpuscle.	No. of ruptured corpuscles counted.	No. of bacteria per ruptured corpuscles.	Bacteria per polymorph.	Virulence of bacteria.
11	7.0	10	8.6	18.2	2.0
5	7.6	4	2.0	12.1	2.0
8	6.3	1	5.0	8.1	8.2
6	2.5	8.1	8.2
15	1.8	1	14.0	7.8	8.2
20	1.2	1	0.0	8.3	8.2
6	4.0	2	9.5	8.0	8.2
12	1.2	3	6.3	8.4	8.2

around each coccus, resulting either from a digested portion of the organism or a slight accumulation of what is probably digestive secretion surrounding the streptococcus.

e. The phagocytosis of eosinophiles. — There is considerable doubt raised as to whether the eosinophiles are actually phagocytes. A solution of this question was attempted by counting the bacteria in the intact eosinophiles, also those somewhat ruptured, in one series where the virulence was 2 units, and in other examples where it was 8.2. It was found that in all instances fewer bacteria were present in the eosinophiles where the virulence of the organisms was the greater.

These results in Table IV. show that the presence of the bacteria in the eosinophiles cannot be due to the mechanical gathering of the cocci in the crevices between the large acid-staining granules, since their presence under these conditions would be wholly independent

of the virulence of the organism. Moreover, it was found that when the fluid was rendered sufficiently alkaline to inhibit phagocytosis in the polymorphous neutrophiles, bacteria were no longer seen in these eosinophiles.

Basophiles were similarly found to be phagocytic, while lymphocytes were devoid of this power, although in long-standing specimens bacteria in some cases had invaded these cells.

IV. THE ACTION OF QUININE SULPHATE ON OPSONIC INDEX AS STUDIED IN VITRO.¹

In investigating the effects of this drug outside of the body, it becomes absolutely essential to have quinine sulphate the only variable present in each sample of a series. On the other hand, the number of bacteria and leucocytes and the percentage of serum must be kept constant throughout the experiment.

a. A graduated series of quinine sulphate solutions. — In a mortar with a little 0.9 per cent sodium chloride was ground half a gram of quinine sulphate. To the thoroughly mixed product was added enough 0.9 per cent salt solution to make 100 c.c., and the part undissolved evenly suspended by thorough shaking. From this volume 50 c.c. were transferred to a small bottle, and the remainder made up to 100 c.c. by the addition of more salt solution. After being uniformly mixed, half of it was transferred to a second bottle. The remainder was, on the addition of another 50 c.c. of the solvent, brought into complete solution. As in the case of the second solution, half of this third solution was transferred to a third bottle. A fourth solution was prepared similarly to the third, and so on with the rest. There thus resulted a series of eleven graduated dilutions of the alkaloid which may be conveniently represented thus: $q, 2^{-1}q, 2^{-2}q, 2^{-3}q, \dots, 2^{-10}q,$ ² where q is 0.5 per cent, or a dilution of 1 in 200.

The bacteria were got from a twenty-four-hour bouillon culture of streptococcus pyogenes found by my method³ to contain approximately one and one-half million bacteria per cubic millimetre.

The 2.5 c.c. of blood required for each experiment were drawn

¹ A note on this part of the paper was forwarded to "Science" on June 14, 1907.

² $2^{-1}q = q/2 = 0.25$ per cent; $2^{-3}q = q/2^3 = q/8 = 0.0625$ per cent.

³ WILSON: "On the recovery of bacteria from human blood," forwarded on June 7, 1907, to "Canadian journal of medicine and surgery."

aseptically from the mediana cubiti of students and afterwards defibrinated.

b. The making of blood suspensions. — A second series was next made by taking definite proportions of the defibrinated human blood, the suspension of bacteria, and the graduated series of quinine solutions. Into each of eleven small, clean test tubes of about 1 c.c. capacity, each provided with a sterile cotton plug, was carefully measured 0.15 c.c. of blood, and the same volume of streptococcic suspension. To the first tube was also added 0.2 c.c. of q specimen; to the second test tube the same quantity from $2^{-1} q$ dilution. Likewise from $2^{-2} q$ the same volume was placed in the third tube, and so on for the rest of the series.

Each member of this series has thus a total volume of 0.5 c.c. The blood in it amounted to 30 per cent, which would be equivalent to 20 per cent of serum and 10 per cent of corpuscles, since the serum forms about two thirds the volume of the blood.¹ The suspension (or solution) of quinine sulphate taken from the first series would amount to 30 per cent of the volume of the resultant blood mixture, and the bacterial suspension 40 per cent. The actual amount of the quinine present in these mixtures of blood might be indicated thus: Q , $2^{-1} Q$, $2^{-2} Q$, $2^{-3} Q$, . . . $2^{-10} Q$, and since the amount of quinine is 40 per cent of the blood suspension, the percentage of the drug present in Q specimen would be two fifths of that in q , and therefore in $2^{-3} Q$ the percentage of the sulphate would be $2/5 \left(\frac{0.5}{2^3} \right)$, or 0.025. A twelfth tube was a control, and contained the same quantities as each of the eleven tubes, with the exception that physiological salt solution replaced the quinine solution.

It should be noted that only specimen Q was supersaturated with quinine, and that $Q 2^{-10}$ contained less than could be tasted. The latter solution had only one part quinine in nearly 1,000,000 parts of the blood mixture. In other words, Q had more quinine than could possibly be present in human blood, whereas $Q 2^{-10}$ would have only the same percentage of quinine as would result from 0.05 grain of that drug circulating in the blood of a man weighing 70 kgm.

The series Q , $Q 2^{-1}$, $Q 2^{-2}$, etc., were mixed and placed in the incubator in some experiments for thirty minutes, in others for

¹ This journal, 1905, xiii, p. 130.

sixty minutes, at 37° C. Starting with the control specimen and ending with the 2^{-10} *Q* suspension, smears were quickly made on cover slips from each member of the series, by taking 2 cm. from the surface of the corpuscles sedimented by centrifugalization for five minutes. This minute quantity from each sample measured by a finely drawn out pipette was quickly spread on the previously prepared cover glasses, air-dried, thrice passed through the flame, and then stained. In each stained specimen the bacteria in 40 polymorphous neutrophils were counted, and the average for one corpuscle thereby obtained.

c. The effect of the quinine on phagocytosis. — The phagocytic action in the control ran to about 8 bacteria per white corpuscle during the half-hour incubation and about double this number for the sixty-minute period. There was marked inhibition with the strong dilutions until $Q 2^{-5}$, or $1/16000$, was reached. From this to the $1/500000$ dilution increased phagocytosis was observed. The maximum increase as compared with the control was about 25 per cent at $Q 2^{-7}$ dilution.

The percentage of quinine in $Q 2^{-7}$ specimen would correspond to about half a grain circulating in the blood of a man weighing 70 kgm., since approximately one twentieth of his body weight would consist of that fluid. This quantity would probably be taken into the circulation from a dose of from 2 to 3 grains of the sulphate. The results for two experiments are given in Table V.

In all suspensions which contain more than $1/20000$ of the alkaloid, a marked absence of the granules from the polymorphs was observed. The contour of the cell was frequently ragged. Vacuoles were in a large percentage of cases present, the nuclei swollen, and the cells generally showed markedly diminished staining powers. These features contrasted strongly with the quinine free samples, illustrating the destructive action of the quinine sulphate on undifferentiated protoplasm.

It would seem from these tentative experiments *in vitro*, that quinine sulphate in doses of from 2 to 3 grains increases the phagocytic action of the polymorphous neutrophils. It is impossible, however, to say whether or not this increase may be due, in whole or in part, to the quinine rendering the bacteria more susceptible to the action of the leucocytes. When more than 4 grains are circulating in the blood, the results are positively injurious to phagocytosis.

TABLE V.

Percentage of quinine sulphate in the suspensions, where Q = 0.2%.	Number of bacteria per polymorph in thirty minutes' incubation.	Number of bacteria taken up by one phagocyte in sixty minutes.
Q	0.35	0.2
2 ⁻¹ Q	0.45	1.1
2 ⁻² Q	2.8	7.4
2 ⁻³ Q	3.9	11.9
2 ⁻⁴ Q	6.4	16.4
2 ⁻⁵ Q	7.6	19.6
2 ⁻⁶ Q	7.9	21.7
2 ⁻⁷ Q	11.5	23.8
2 ⁻⁸ Q	7.5	18.1
2 ⁻⁹ Q	8.9	18.4
2 ⁻¹⁰ Q	8.9	17.3
Control	8.7	16.9

V. SUMMARY.

1. The number of red and white corpuscles, the percentages of hæmoglobin and of serum, and the differential count can be got accurately from the defibrinated in place of non-defibrinated blood.

2. Not more than 2 cm. of blood should be used for smears on 20 × 25 mm. cover slips, and differential percentages should be made by counting parallel with the direction followed in the spreading of the smear.

3. The concentration of the whites in the blood can be increased eleven and one-half times by the method of sedimentation above described.

4. The virulence of any organism may be conveniently standardized by dividing into the constant 60 the number of bacteria found in a single polymorphous neutrophile during one hour's incubation.

5. The eosinophiles have a lower specific gravity than the polymorphous neutrophiles.

6. Evidence is here advanced that eosinophiles and basophiles are phagocytic.

7. The effect of any drug on opsonic index may be studied in a few hours by the system of graduated dilutions above described.

8. The administration of 15 grains of quinine to healthy students seemed to cause a slight increase in their opsonic index.

9. Quinine sulphate *in vitro* has an inhibitory effect on phagocytosis in strong solutions, but apparently a stimulating influence in dilutions from 1/15000 to 1/1000000.

I desire to express my thanks to Dr. G. N. Stewart for aiding in the final revision of this paper, and to Messrs. Ball, Bell, Doseff, Loth, Matthews, McGrath, Menzies, Poole, Pollen, and Verity, for their kindness in subjecting themselves to experimentation for the work of part of this research.

METABOLISM IN PHOSPHORUS POISONING.¹

BY GRAHAM LUSK.

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PRESENT-DAY medical literature is frequently influenced by the idea of a reduced general oxidation within the body. It has long since been established that there is no reduced oxidation in anemia or in diabetes. It is, however, generally believed that there is a reduced metabolism in phosphorus poisoning. When the evidence for this belief is examined, it is found mainly to rest upon a single respiration experiment which lasted for two hours, and which was performed by Bauer in 1871. Bauer² found that the carbon dioxide output of a dog poisoned with phosphorus was 47 per cent less than it had been the day before, when the dog had been normal.

The only other respiration experiments on dogs are the recent ones of Welsch,³ who used a modification of Geppert's apparatus. Welsch finds that phosphorus reduces the carbon dioxide output from 24 to 29 per cent.

Welsch also finds a reduced metabolism in rabbits, and in this he agrees with A. Schneider.⁴ Schneider's paper has not been available for me, but his methods are criticised by Loewi in Von Noorden's *Handbuch der Pathologie des Stoffwechsels*, as being very crude.

Only Lo Monaco⁵ in experiments on mice finds that phosphorus has no influence whatever on the carbon dioxide output.

In the writer's laboratory⁶ a fasting dog poisoned with phosphorus has been observed, whose proteid metabolism had risen twofold and

¹ A paper presented at the Seventh International Physiological Congress, Heidelberg, August, 1907.

² BAUER: *Zeitschrift für Biologie*, 1871, vii, p. 63.

³ WELSCH: *Archives internationales de pharmacodynamie et de therapie*, 1905, xiv, p. 211.

⁴ SCHNEIDER: *Inaugural Dissertation*, 1905, Würzburg.

⁵ MONACO, LO: *Bullettino della Accademia di Roma*, 1893, xix, p. 39.

⁶ MANDEL and LUSK: *This journal*, 1906, xvi, p. 136.

ammonia output fourfold the simple fasting amounts, and whose urine contained considerable lactic acid,—all this without any apparently great depression of the vital activities. A further indication that there is no reduced oxidation in phosphorus poisoning, is offered by the literature cited by Arohnson,¹ which shows that fever is an accompaniment of this disease.

The plan of the present experiment was as follows. Two dogs, of the setter race and nearly the same weight, were each starved for periods of eight days. Their respective metabolisms were determined from the third to the eighth day of starvation by the means of six-hour respiration experiments accomplished in a replica of Voit's apparatus which stands in this laboratory. After these fasting periods each dog was brought back to nearly his former weight by giving a generous diet for three weeks. Then each dog was again fasted, and on the third day was put in the respiration apparatus for six hours, following which he received a dose of a one per cent phosphorus oil subcutaneously.

On the first day of the experiment Dog I was very violent during his confinement in the cage. After two hours of scratching and pawing the animal was removed from the cage and was beaten. The irregularity of the decline in the metabolism during the experiments on both dogs finds its explanation in the excitable disposition of the animals, and also because the experiments were not continued for twenty-four hours.

On the days when Dog I was seen to be shivering he was covered with cloths which prevented the continuance of the phenomenon. The dogs were both long-haired animals, and the variations in room temperature do not seem to have appreciably affected their metabolisms. The temperature of the respiration chamber was probably a degree or two higher than that of the room which is here given. Wherever the body temperatures are noted the figures obtained are for the times of entrance, and six hours later of leaving the cage.

The ventilation of the respiration chamber was at the rate of about a litre per second. The apparatus was frequently tested to prove that its passageways were air-tight.

Unfortunately the phosphorus oil first used on Dog I had no physiological value, and it was not until immediately after the end of the respiration experiment, on the seventh day of starvation, that an active oil was introduced. This caused a rise in temperature and a rise

¹ AROHNSON: *Zeitschrift für klinische Medizin*, 1907, xi, p. 180.

Date, 1907.	Day of fast.	Weight in kg.	Urine.				C. in resp.	Calories produced.				Temp. of dog.	Temp. of room.	Remarks.	
			In c.c.	N	NH ₃	Creatinin.		From protoid.	From fat.	Total.	Per kg.				
May 1	3	16.16	30	1.296	20.68	32.47	213.82	246.29	15.24	{ 20° 22° 20° 21.5° 20°	Dog violent.	
2	4	15.63	48	1.195	14.57	29.85	141.81	171.66	11.00	{ 20° 20°		
3	5	15.36	22	1.142	14.90	28.53	147.60	176.13	11.46	{ 20°		
4	6	15.24	36	1.011	14.63	25.25	149.32	174.57	11.46	{ 19.5°		
5	7	15.02													
6	8	14.90	36	0.951	13.63	23.76	138.00	161.76	10.86	{ 18°		
27	3	15.56	18	0.968	0.039	0.098	14.78	24.18	151.54	175.72	11.29	38.3°	{ 17°	P. oil (old).	
28	4	15.20	19	0.762	0.031	0.096	16.75	19.03	182.31	201.34	13.24	38.75°	{ 16° 17°	P. oil (old).	
29	5	15.10	21	1.018	0.039	0.089	14.65	25.43	148.34	173.77	11.51	39.0°	{ 16° 17°	P. oil (old).	
30	6	15.00	19	0.927	0.047	0.088	13.82	23.16	140.95	164.11	10.94	39.3°	{ 17° 18°		
31	7	15.06	34	0.955	0.077	14.83	24.96	152.64	177.60	11.79	38.9°	{ 18°	2 c.c. P. oil (new).	
June 1	8	14.81	20	0.861	0.057	0.107	16.48	21.51	175.79	197.30	13.32	{ 39.1° 39.3°	{ 18° 19°	Shivering at first. 6 c.c. P. oil.	
2	9	14.92	223	1.407	0.090	15.32	35.15	144.40	179.45	12.03	{ 40.9° 39.3°	{ 16°	S't shiv'g at first. 6 c.c. P. oil.	
3	10	14.38	89	2.693	0.157	0.089	15.85	67.27	110.79	178.06	12.45	{ 39.4° 39.7°	{ 16° 17°	C: N = 0.71 : 1. 10 c.c. P. oil. No shivering.	
4	11	13.88	123	2.684	0.076	0.093	15.20	67.06	102.79	169.85	12.23	{ 38.9° 38.6°	{ 18°	Death.	
5	12	13.16	15*	12.44	{ 37.6° 36.6°	{ 18°		

* Albumin.

in the total metabolism eighteen hours later, as is demonstrated in Table I. The proteid metabolism shows its first rise two days after the injection of phosphorus. On the third day of phosphorus the proteid metabolism had risen threefold, the ammonia output to the same extent; there was a high fever, and a high total metabolism. On the fourth day the same picture is maintained. On the fifth day the dog died two minutes after removal from the cage of the respiration apparatus. The urine of this last period was scanty and filled with albumin, and no urine was secreted during the last three hours. Estimation of the total metabolism was therefore impossible. The lowered output of carbon in the respiration is the proper accompaniment of the falling body temperature and the final *exitus mortalis*.

This case is a typical one of phosphorus poisoning. If one recalls the fact that the metabolism of a dog may fall 20 per cent during a ten-day fast, the significance of the high metabolism figures during the four days of phosphorus poisoning is very evident.

Determinations of the creatinin output in this dog showed a gradual fall in the amount eliminated, and one independent of the total nitrogen output and also independent of the tone and strength of the muscle,—a relation ascribed to it by Shaffer.¹

The results obtained in Dog II are not quite so satisfactory, for dosage with phosphorus at once damaged the kidney and produced albuminuria. The urinary nitrogen was obtained after precipitation of the albumin. Eighteen hours after administration of phosphorus oil there was an increased proteid metabolism, an increased total metabolism, and a febrile temperature, thus confirming the results with Dog I. On the second day the proteid metabolism rose, the total metabolism fell, but this was coincident with complete muscular rest, with a fall in body temperature, and was followed by the dog's death during the night.

In both dogs microscopical examination of the livers, *post mortem*, showed a large fat content.

The results of these experiments disprove the statement that phosphorus reduces the oxidative power of the organism. The reverse is rather the case. The metabolism is raised, and this partly on account of the fever and perhaps partly on account of the specific dynamic action of the increased proteid metabolism, in the sense of Rubner.

¹ SHAFFER: Proceedings of the American Society of Biological Chemists, Journal of biological chemistry, 1907, III, p. xiii.

TABLE II.
Dog II. Six-hour periods.

Date 1907.	Day of fast.	Weight. in kg.	Urine.			C. in Resp.	Calories produced.				Temp. of room.	Temp. of dog.	Remarks.
			In c.c.	N	NH ₃		Proteid.	Fat.	Total.	Per kg.			
May 13	3	16.50	22	0.884	15.62	22.08	164.46	186.54	11.30	19°	
14	4	16.40	16	0.736	16.65	18.39	181.82	200.21	12.21	{ 19° 22.5°	
15	5	16.24	12	0.834	14.49	20.83	152.15	172.98	10.65	20°	
16	6	16.01	.. ¹	0.751	13.26	18.76	139.60	158.36	9.90	20°	
17	7	15.90	11	0.668	13.78	16.69	148.70	165.39	10.41	19°	
18	8	15.76	..	0.685	14.55	17.11	157.57	174.68	11.08	19.5°	
June 7	3	17.84	23	0.916	0.026	14.97	22.88	155.48	178.35	10.00	{ 38.3° 38.1°	{ 38.9° 39.1°	3 c.c. P. oil.
8	4	17.18	28	1.109	0.028	16.94	25.70	173.69	199.39	11.61	{ 39.0° 38.3°	{ 39.0° 38.3°	3 c.c. P. oil. Death.
9	5	16.50	155	1.446	0.016	13.02	36.02	114.85	150.87	9.14	20°	

¹ Some lost. N averaged.

The calculations have been made in the ordinary way. The night urine of Dog I on June 3-4, which was free from albumin, was found to have the C : N = 0.71 : 1, which is not far from Rubner's normal for fasting urine of 0.728 : 1. It may, nevertheless, be objected that the metabolism in phosphorus poisoning yields abnormal products, which might invalidate the results. Investigations by Pfaundler,¹ however, show that phosphorus poisoning in dogs causes only a small increase of urinary amino-acid nitrogen. Furthermore, if amino-acids were retained in the organism, the heat production essential to their formation would not be recorded in the calculations, but this would scarcely aggregate a large quantity. Again, if lactic acid were eliminated through the urine, the error involved in the calculations would be on the side of a larger elimination of carbon from fat in the respiration, and hence an increased heat production.

Therefore, after taking into account all possible sources of error, it may be concluded that the figures obtained are properly representative of the metabolism as it actually occurs.

I wish here to reiterate a standpoint taken before in my laboratory.² Non-combustion of lactic acid causes a rise in proteid metabolism just the same as does non-combustion of sugar. If phlorhizin diabetes be induced in a starving dog, there is a large increase in proteid metabolism, as much as fivefold the starvation quantity: if now phosphorus oil be injected, *there is no further rise in proteid metabolism*, although the symptoms of phosphorus poisoning followed by death ensue. Phosphorus, therefore, does not act to permit limitless autolysis within the tissues, but acts, in my opinion, upon the enzyme which burns lactic acid, and the accumulating acid acts secondarily upon certain denitrogenizing enzymes, which leads to the accumulation of amino-acids. In substantiation of this Schryver³ has found that addition of lactic acid favors the accumulation of amino-acids in autolysis of the liver.

In diabetes the cause of the fatty infiltration of the liver and other cells lies in the non-combustion of sugar, whereas in phosphorus poisoning the non-combustion of lactic acid, which is the first derivative in the metabolism of dextrose, is the cause of a similar phenomenon. The sugar-hungry cells attract fat in greater quantity than they can burn it.

¹ PFAUNDLER: *Zeitschrift für physiologische Chemie*, 1902, xxx, p. 75.

² MANDEL and LUSK: *Loc. cit.*, p. 131.

³ SCHRYVER: *Biochemical journal*, 1906, i, p. 153.

I beg to acknowledge with gratitude the assistance of Professor J. R. Murlin, who made all the urinary determinations mentioned in this research.

SUMMARY.

There is no reduction in the amount of total metabolism in phosphorus poisoning, but there may be rather an increase due to fever and perhaps to the specific dynamic action of the increased proteid metabolism, in the sense of Rubner. The creatinin output is scarcely affected in a fasting dog poisoned with phosphorus.

HYDROLYSIS OF GLYCININ FROM THE SOY BEAN.¹

By THOMAS B. OSBORNE AND S. H. CLAPP.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

NEARLY all of the protein matter of the soy bean, *Glycine soja*, consists of a globulin which has been subjected to extensive fractionation in this laboratory.²

As this fractionation gave no evidence that the greater part of the globulin of this seed consisted of more than one substance, it was accordingly named glycinin.

With this glycinin is associated a small amount of legumelin and proteose, which can be easily separated, owing to the ready solubility of these proteins in water. There was also evidence of the presence of a small amount of globulin soluble in extremely dilute saline solutions which had a composition similar to that of phaseolin, but the amount in the fractions yielding this substance was too small to justify any definite conclusions.

In the preparation of the glycinin for hydrolysis we received valuable assistance from Mr. I. F. Harris, for which we wish to make acknowledgment.

The soy beans were coarsely ground in the laboratory, freed from the greater part of the outer seed coats, and then extracted with petroleum benzine until most of the oil was removed. After freeing from adhering benzine, the meal was finely ground and extracted with about three parts of ten per cent sodium chloride solution. Owing to the very viscid character of the extract, it was necessary to mix with the meal and solvent enough dry, finely divided filter paper to make it possible to squeeze out the extract in a powerful screw press. If the proper amount of paper was first incorporated in the mass,

¹ The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D.C.

² OSBORNE and CAMPBELL: Journal of the American Chemical Society, 1898, xx, p. 419.

about 80 per cent of the solvent applied to the meal could be pressed out in a condition so far free from solid matter that complete filtration was subsequently possible.

The perfectly clear extract thus obtained was then dialyzed for four days in running water. The globulin that separated was filtered out, dissolved again in sodium chloride solution, and precipitated a second time by dialysis. After repeating this process a third time the precipitated glycinin was washed thoroughly with water, absolute alcohol and ether, and then dried over sulphuric acid. By this repeated dialysis the glycinin was separated from the more soluble proteins, and had the properties and composition of the globulin formerly described under this name.

Five hundred grams of this glycinin, containing 0.76 per cent ash and 10.55 per cent moisture, were suspended in a mixture of 500 c.c. of water and 500 c.c. of hydrochloric acid of specific gravity 1.19. After solution had been partially effected by warming for some time on the water bath, the hydrolysis was made complete by boiling for twenty hours in a bath of oil.

As a preliminary removal of glutaminic acid materially simplifies the further examination of the protein, the hydrolysis solution was accordingly concentrated to about two thirds of the original volume, and after saturating with hydrochloric acid gas allowed to stand at 0°. The yield of glutaminic acid hydrochloride was 54.80 gm., equivalent to 44.4 gm. of the free acid, or about 10 per cent of the glycinin.

The substance decomposed at about 198°.

Carbon and hydrogen, 0.1962 gm. subst., gave 0.2926 gm. CO₂ and 0.1069 gm. H₂O.

Calculated for C₅H₉O₄N = C 40.81; H 6.12 per cent.

Found = C 40.67; H 6.05 " "

The filtrate from the glutaminic acid hydrochloride was concentrated to a very thick syrup under strongly reduced pressure, and the residue esterified with alcohol and hydrochloric acid gas in the usual way. After liberating the free esters with sodium hydroxide and potassium carbonate and shaking out with ether, the aqueous layer was saturated with hydrochloric acid gas, freed from inorganic salts, in the manner often described, and the esterification again repeated. After distilling off the ether on the water bath, the esters were fractioned as follows:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	85°	20.00 mm.	8.00 gm.
II	80°	0.48 "	38.68 "
III	100°	0.28 "	61.14 "
IV	130°	0.36 "	63.53 "
V	200°	0.36 "	41.77 "
Total			213.12 gm.

The undistilled residue weighed 53 gm.

Fraction I.—Temperature of bath up to 85°. Pressure, 20 mm. Weight, 8 gm.

This fraction yielded 2.64 gm. of glyocol as the hydrochloride of the ethyl ester. The melting-point was 144°.

Chlorine, 0.4689 gm. subst., gave 0.4813 gm. AgCl.

Calculated for $C_4H_{10}O_2NCl = Cl$ 25.45 per cent.

Found = Cl 25.38 " "

The remainder of this fraction seemed to contain alanine, but its isolation was not effected.

Fraction II.—Temperature of bath up to 80°. Pressure, 0.48 mm. Weight, 38.68 gm.

This fraction was saponified in the usual manner, and the dried amino acids extracted with boiling alcohol to remove the proline. The undissolved portion was then re-esterified with alcohol and hydrochloric acid gas, and yielded 1.67 gm. of glyocol as the hydrochloride of the ethyl ester. The melting-point was 144°.

In the filtrate from the glyocol the free amino acids were regenerated and submitted to fractional crystallization.

There were obtained 11.98 gm. of leucine and 3.03 gm. of aminovalerianic acid. The leucine decomposed at about 298°.

Carbon and hydrogen, 0.1792 gm. subst., gave 0.3612 gm. CO_2 and 0.1639 gm. H_2O .

Calculated for $C_6H_{13}O_2N = C$ 54.96; H 9.92 per cent.

Found = C 54.97; H 10.17 " "

The aminovalerianic acid gave the following analysis:

Carbon and hydrogen, 0.1895 gm. subst., gave 0.3562 gm. CO_2 and 0.1593 gm. H_2O .

Calculated for $C_5H_{11}O_2N = C$ 51.28; H 9.40 per cent.

Found = C 51.26; H 9.34 " "

Alanine seemed to be present in the filtrate from aminovalerianic acid, but we were unfortunately unable to isolate this substance in a state of purity.

Fraction III. — Temperature of bath up to 100°. Pressure, 0.28 mm. Weight, 61.14 gm.

This fraction consisted essentially of the esters of leucine and proline. It was saponified in the usual way, evaporated to dryness under reduced pressure and the residue extracted with boiling absolute alcohol. The insoluble part yielded 25.45 gm. of leucine.

Carbon and hydrogen, 0.2979 gm. subst., gave 0.6003 gm. CO₂ and 0.2539 gm. H₂O.

Calculated for C₆H₁₂O₂N = C 54.96 ; H 9.92 per cent.

Found = C 54.95 ; H 9.47 “ “

The proline solution of fraction III was united with that of fraction II. The yield of racemic proline copper salt was 2.86 gm.

Water, 0.1455 gm. subst., lost 0.0159 gm. H₂O at 110°.

Calculated for C₁₀H₁₆O₄N₂Cu · 2 H₂O = H₂O 10.99 per cent.

Found = H₂O 10.93 “ “

Copper, 0.1268 gm. subst., dried at 110°, gave 0.0351 gm. CuO.

Calculated for C₁₀H₁₆O₄N₂ = Cu 21.81 per cent.

Found = Cu 22.11 “ “

The weight of the copper salt of lævo-proline, dried at 110°, was 18.70 gm., equivalent to 14.75 gm. of proline. For identification the phenyl-hydantoin was employed. The substance melted at 142°–143°.

Carbon and hydrogen, 0.1476 gm. subst., gave 0.3603 gm. CO₂ and 0.0759 gm. H₂O.

Calculated for C₁₂H₁₂O₂N₂ = C 66.67 ; H 5.57 per cent.

Found = C 66.57 ; H 5.71 “ “

Fraction IV. — Temperature of bath up to 130°. Pressure, 0.36 mm. Weight, 63.53 gm.

From this fraction the ester of phenylalanine was separated by shaking out with ether in the usual manner. The yield of phenylalanine hydrochloride was 8.82 gm., equivalent to 7.22 gm. of free phenylalanine. The substance was identified as the copper salt.

Carbon and hydrogen, 0.1930 gm. subst., gave 0.3897 gm. CO₂ and 0.0913 gm. H₂O.

Copper, 0.1816 gm. subst., gave 0.0367 gm. CuO.

Calculated for C₁₈H₂₀O₄N₂ Cu = C 55.16; H 5.11; Cu 16.24 per cent.

Found = C 55.07; H 5.25; Cu 16.15 " "

The aqueous layer was saponified by heating with an excess of baryta. It separated on standing 5.83 gm. of aspartic acid as the barium salt.

Carbon and hydrogen, 0.2114 gm. subst., gave 0.2810 gm. CO₂ and 0.1047 gm. H₂O.

Calculated for C₄H₇O₄N = C 36.09; H 5.26 per cent.

Found = C 36.25; H 5.50 " "

The aspartic acid reddened, but did not decompose, at 300°.

The filtrate from barium aspartate was freed from barium, concentrated under reduced pressure to small volume, and saturated with hydrochloric acid gas. After prolonged standing at 0°, there had separated 4 gm. of nearly pure phenylalanine hydrochloride, while no glutamic acid could be obtained. The fraction yielded 16.08 gm. of air-dry copper aspartate.

Copper, 0.1076 gm. subst., gave 0.0311 gm. CuO.

Nitrogen, 0.4268 gm. subst., required 2.1 c.c. 5/7 N-HCl.

Calculated for C₄H₅O₄N Cu · 4½ H₂O = Cu 23.07; N 5.08 per cent.

Found = Cu 23.09; N 4.92 " "

In the filtrate from the copper aspartate no definite substance could be isolated.

Fraction V. — Temperature of bath up to 200°. Pressure, 0.36 mm. Weight, 41.77 gm.

From this fraction there were isolated 6.59 gm. of phenylalanine as the hydrochloride, 1.25 gm. of aspartic acid as the barium salt, 10.53 gm. of glutamic acid as the hydrochloride and 4.92 gm. of copper aspartate.

The glutamic acid decomposed at about 202° and gave the following analysis:

Carbon and hydrogen, 0.1714 gm. subst., gave 0.2556 gm. CO₂ and 0.0961 gm. H₂O.

Calculated for C₅H₉O₄N = C 40.81; H 6.12 per cent.

Found = C 40.67; H 6.22 " "

RESIDUE FROM DISTILLATION.

The residue remaining after distilling off the esters weighed 53 gm. It was taken up with water and saponified by boiling with an excess of baryta, and the glutaminic acid brought to separation as the hydrochloride. The yield of glutaminic acid hydrochloride was 3.52 gm., which makes the total yield of glutaminic acid obtained in this hydrolysis 57.74 gm., or 13.04 per cent of the protein, which falls considerably below the 19.46 per cent obtained by Osborne and Gilbert.¹

TYROSINE.

Fifty grams of glycinin equal to 44.34 gm., moisture and ash free, were digested on a water bath with a mixture of 150 gm. of sulphuric acid and 300 c.c. of water until the protein was nearly all dissolved and then boiled in an oil bath for twelve hours. The solution was then freed from sulphuric acid with baryta, and the filtrate and washings from the barium sulphate were concentrated to small volume. The crude tyrosine which separated when re-crystallized weighed 0.825 gm., equal to 1.86 per cent.

Nitrogen, 0.1923 gm. subst., required 1.5 c.c. 5/7 N—HCl.

Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.

Found = N 7.80 " "

The filtrate and washings from the tyrosine were worked up for bases according to the method of Kossel and Patten with the following results :

HISTIDINE.

The solution of the histidine was made up to 500 c.c.

Nitrogen, 100 c.c. required 3.34 c.c. of 5/7 N—HCl = 0.0334 gm. N = 0.1670 gm. N in 500 c.c. = 0.6154 gm. histidine, or 1.39 per cent.

The histidine was converted into the dichloride and re-crystallized, but unfortunately not enough of the pure substance was obtained for analysis.

It crystallized in the characteristic prisms which melted at about 232°, and gave on heating a pronounced biuret reaction.

¹ OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

ARGININE.

The solution containing the arginine was made up to 1000 c.c. and nitrogen determined in it.

Nitrogen, 50 c.c. required 3.54 c.c. 5/7 N—HCl = 0.0354 gm. N = 0.708 gm. in 1000 c.c. = 2.1976 gm. arginine. Adding 0.072 gm. for the solubility of the silver arginine gives 2.2696 gm. arginine, or 5.12 per cent.

The arginine was converted into the copper nitrate double salt.

Water, 0.2096 gm. subst., air-dry, lost 0.0218 gm. H₂O at 110°.

Calculated for C₁₂H₂₈O₄N₃Cu (NO₃)₂ · 3 H₂O = H₂O 9.16 per cent.

Found = H₂O 10.40 “ “

Copper, 0.1514 gm. subst., dried at 110°, gave 0.0226 gm. CuO.

Calculated for C₁₂H₂₈O₄N₃Cu (NO₃)₂ = Cu 11.87 per cent.

Found = Cu 11.93 “ “

LYSINE.

The lysine was isolated as the picrate, of which 3.09 gm. was obtained, equal to 2.71 per cent. The lysine picrate gave the following analysis:

Nitrogen, 0.1113 gm. subst., gave 10.2 c.c. moist N₂ at 30° and 756.7 mm.

Calculated for C₆H₁₄O₂N₂ · C₆H₃O₇N₃ = N 18.67 per cent.

Found = N 18.69 “ “

The results of this hydrolysis calculated to a moisture and ash free basis were the following:

	Per cent.		Per cent.
Glycocoll	0.97	Serine	not isolated
Alanine	not isolated	Tyrosine	1.86
Valine	0.68	Arginine	5.12
Leucine	8.45	Histidine	1.39
Proline	3.78	Lysine	2.71
Phenylalanine	3.86	Ammonia	2.56
Aspartic acid	3.89	Tryptophane	present
Glutaminic acid	19.46	Total	54.73

HYDROLYSIS OF THE CRYSTALLINE GLOBULIN OF THE SQUASH SEED (*CUCURBITA MAXIMA*).¹

BY THOMAS B. OSBORNE AND S. H. CLAPP.

[*From the Laboratory of the Connecticut Agricultural Experiment Station.*]

THE material for this hydrolysis was prepared from fresh seeds which were ground in the laboratory and then extracted with petroleum benzine until nearly all the oil was removed. The fine meal thus obtained was extracted with ten per cent sodium chloride solution, the extract filtered clear and dialyzed until nearly free from chlorine. The globulin thus deposited, which consisted of a mixture of crystals and spheroidal forms, was washed with water, dilute and absolute alcohol, and dried over sulphuric acid.

Weighed portions of this crude globulin were then dissolved in one-tenth saturated ammonium sulphate solution, the insoluble part filtered out, and the clear solution diluted with water heated to 50° until the solution contained about two per cent of protein and two per cent of ammonium sulphate. After slowly cooling to 20° about forty per cent of the total globulin separated in octahedral crystals, which were washed thoroughly with water, dilute and absolute alcohol, and dried over sulphuric acid. In this way a large quantity of perfectly crystallized globulin was obtained which contained 7.98 per cent of moisture and 0.15 per cent of ash.

We wish to acknowledge here the valuable assistance which Mr. I. F. Harris rendered in the preparation of this material.

After the material for this hydrolysis had been prepared, Abderhalden and Berghausen² published the results of their hydrolysis of this protein. As their results were confined to the monoamino acids, and as their hydrolysis was made with a comparatively small

¹ The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D.C.

² ABDERHALDEN and BERGHAUSEN: *Zeitschrift für physiologische Chemie*, 1906, xlix, p. 15.

amount of the globulin, we decided to continue our work as first planned.

Seven hundred grams of the crystallized globulin were suspended in a mixture of 700 c.c. of water and 700 c.c. of hydrochloric acid of specific gravity 1.19. After warming for some time on the water bath, the hydrolysis solution was boiled in the oil bath for twenty-two hours.

As the amount of glutamic acid present in this globulin is relatively small, no preliminary removal of this substance was made; but the hydrochlorides of the amino acids were at once esterified with alcohol and dry hydrochloric acid gas in the usual way. As the yield of ester from one esterification is far from quantitative, the whole operation was again repeated, and the aqueous layer from the first esterification freed from inorganic salts and esterified as before.

The united esters were separated into the following fractions by distillation under reduced pressure:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	80°	11.0 mm.	12.30 gm.
II	96°	11.0 "	38.81 "
III	100°	0.52 "	97.46 "
IV	150°	0.58 "	120.38 "
V	200°	0.43 "	90.91 "
Total			359.86 gm.

The undistilled residue weighed 88 gm.

Fraction I. — Temperature of bath up to 80°. Pressure, 11 mm. Weight, 12.3 gm. This fraction yielded 1.36 gm. of glycooll as the hydrochloride of the ethyl ester. The melting-point was 144°.

Chlorine, 0.3446 gm. subst., gave 0.3529 gm. AgCl

Calculated for $C_4H_{10}O_2NCl = Cl$ 25.45 per cent.

Found = Cl 25.32 " "

The remainder of the fraction consisted largely of alanine, of which there were isolated 2.26 gm.

Fraction II. — Temperature of bath up to 96°. Pressure, 11 mm. Weight, 38.81 gm.

This fraction was saponified by boiling with water, evaporated to dryness under reduced pressure, and the residue extracted with boiling alcohol to remove the proline. The undissolved portion yielded on

esterification 2.35 gm. of glycocoll as the ethyl ester hydrochloride. In the filtrate from the glycocoll, there were obtained by systematic fractional crystallization of the free amino acids 3.64 gm. of leucine, 10.09 gm. of alanine, and 1.69 gm. of aminovalerianic acid. The latter substance gave the following analysis:

Carbon and hydrogen, 0.2218 gm. subst., gave 0.4190 gm. CO₂ and 0.1873 gm. H₂O.

Calculated for C₅H₁₁O₂N = C 51.28; H 9.40 per cent.

Found = C 51.51; H 9.38 " "

The alanine decomposed at about 292°.

Carbon and hydrogen, 0.1624 gm. subst., gave 0.2419 gm. CO₂ and 0.1200 gm. H₂O.

Calculated for C₃H₇O₂N = C 40.45; H 7.86 per cent.

Found = C 40.62; H 8.21 " "

Fraction III. — Temperature of bath up to 100°. Pressure, 0.52 mm. Weight, 97.46 gm.

This fraction was boiled with water until the alkaline reaction had ceased. The solution was then evaporated to dryness under strongly reduced pressure, and the proline extracted with boiling absolute alcohol. The insoluble portion yielded 43.40 gm. of leucine. The substance decomposed at about 298°.

Carbon and hydrogen, 0.1160 gm. subst., gave 0.2333 gm. CO₂ and 0.1053 gm. H₂O.

Calculated for C₆H₁₃O₂N = C 54.96; H 9.92 per cent.

Found = C 54.85; H 10.09 " "

In the filtrate from the leucine, there were further isolated 2.87 gm. of copper-aspartate.

The proline solutions of fractions II and III were united. The yield of racemic proline copper salt was 1.39 gm., while of the amorphous lævo-proline copper salt, dried at 110°, there were obtained 21.78 gm. equivalent to 17.18 gm. of proline. For identification the phenyl-hydantoin was employed. The melting-point was 142°–143°.

Carbon and hydrogen, 0.2081 gm. subst., gave 0.5066 gm. CO₂ and 0.1005 gm. H₂O.

Calculated for C₁₂H₁₂O₂N₂ = C 66.67; H 5.57 per cent.

Found = C 66.39; H 5.37 " "

Fraction IV. — Temperature of bath up to 150°. Pressure, 0.58 mm. Weight, 120.38 gm.

This fraction was treated with five volumes of water and the ester of phenylalanine shaken out with ether. The yield of phenylalanine hydrochloride was 12.14 gm., equivalent to 9.94 gm. of free phenylalanine.

The substance decomposed at about 277° (uncorr.) on rapid heating.

Carbon and hydrogen, 0.1711 gm. subst., gave 0.4093 gm. CO₂ and 0.1019 gm. H₂O.

Calculated for C₉H₁₁O₂N = C 65.45; H 6.66 per cent.

Found = C 65.24; H 6.62 “ “

The aqueous layer was saponified with baryta, and the racemized aspartic acid separated as the barium salt. The yield of aspartic acid was 10.40 gm.

Carbon and hydrogen, 0.1474 gm. subst., gave 0.1944 gm. CO₂ and 0.0707 gm. H₂O.

Calculated for C₄H₇O₄N = C 36.09; H 5.26 per cent.

Found = C 35.97; H 5.33 “ “

The filtrate from barium aspartate was freed from barium, concentrated to small volume under reduced pressure, and saturated with hydrochloric acid gas. On prolonged standing at 0° the solution separated 4.60 gm. of nearly pure phenylalanine hydrochloride. There were further isolated from this fraction 19.52 gm. of air-dry copper aspartate.

Copper, 0.1042 gm. subst., gave 0.0303 gm. CuO.

Nitrogen, 0.3229 gm. subst., required 1.6 c.c. 5/7 N-HCl.

Calculated for C₄H₅O₄N Cu 4½ H₂O = N 5.08; Cu 23.07 per cent.

Found = N 4.95; Cu 23.23 “ “

Fraction V. — Temperature of bath up to 200°. Pressure, 0.43 mm. Weight, 90.91 gm.

From this fraction there were isolated 7.61 gm. of phenylalanine as the hydrochloride, and 8.51 gm. of glutaminic acid as the hydrochloride. The free glutaminic acid decomposed at about 202°–203°.

Carbon and hydrogen, 0.2145 gm. subst., gave 0.3205 gm. CO₂ and 0.1198 gm. H₂O.

Calculated for C₅H₉O₄N = C 40.81; H 6.12 per cent.

Found = C 40.75; H 6.21 “ “

TYROSINE.

One hundred grams of the globulin equal to 89.92 gm. dry and ash-free were digested on the water bath for three hours with a mixture of 300 gm. of sulphuric acid and 600 c.c. of water and then boiled in an oil bath for twelve hours. The solution was then freed from sulphuric acid with an equivalent quantity of barium hydroxide and the filtrate and washings concentrated to about 300 c.c. and allowed to stand for some time, when the tyrosine that separated was filtered out and washed. The filtrate and washings were decolorized with bone black, concentrated to a syrup at low pressure, and the tyrosine that separated was filtered out and washed. No more tyrosine could be obtained from the solution filtered from this second separation. The crude tyrosine thus obtained was united with the first crop and recrystallized, giving 2.76 gm. of tyrosine, or 3.07 per cent.

Nitrogen, 0.1831 gm. subst., required 1.44 c.c. 5/7 N-HCl.

Calculated for $C_9H_{11}O_3N = N$ 7.75 per cent.

Found = N 7.86 " "

CYSTINE.

The filtrate from the tyrosine was made strongly acid with sulphuric acid and treated with mercuric sulphate solution as long as a precipitate formed. The mercury precipitate was washed with 5 per cent sulphuric acid, decomposed with hydrogen sulphide and the solution concentrated *in vacuo* to small volume, acidified with acetic acid and about two volumes of alcohol added. On standing, a small precipitate gradually separated, which, after forty-eight hours, was filtered out and found to weigh 0.23 gm. When dissolved in hot dilute ammonia and recrystallized by adding an excess of acetic acid, the cystine separated in its characteristic hexagonal plates.

HISTIDINE.

Fifty grams of the globulin, equal to 44.96 gm. moisture and ash-free, were hydrolyzed by heating with 150 gm. sulphuric acid and 300 c.c. of water for three hours on the water bath, and then boiling for twelve hours in the oil bath. After freeing the solution quantitatively from sulphuric acid with barium hydroxide, the solution was

concentrated to about 500 c.c. and the bases separated according to the method of Kossel and Patten. The solution containing the histidine was made up to 500 c.c. and nitrogen determined in it.

Nitrogen, 100 c.c. solution required 5.9 c.c. 5/7 N-HCl. = 0.0590 gm. N = 0.2950 gm. N in 500 c.c. = 1.0871 gm. histidine, or 2.42 per cent.

The rest of the histidine solution was freed from sulphuric acid with an excess of barium hydroxide and from most of the excess of barium with carbonic acid. The histidine was then precipitated with mercuric chloride, the mercury precipitate decomposed with hydrogen sulphide, and the histidine obtained as the dichloride by evaporation. It separated in the characteristic prisms, which decomposed at about 232°-233°.

Chlorine, 0.0495 gm. subst., gave 0.0613 gm. AgCl.

Calculated for $C_6H_{11}O_3N_3Cl_2 = Cl$ 31.14 per cent.

Found = Cl 30.63 " "

ARGININE.

The solution of the arginine was made up to 1000 c.c. and nitrogen determined in it.

Nitrogen, 50 c.c. solution, required 10.34 c.c. 5/7 N-HCl = 0.1034 gm. N = 2.0680 gm. N in 1000 c.c. = 6.4191 gm. arginine.

Adding 0.072 gm. for the solubility of the silver arginine gives 6.4911 gm. arginine, or 14.44 per cent. The arginine was converted into the copper nitrate double salt.

Water, 0.2311 gm. subst., air dry, lost 0.0239 gm. H_2O at 110°.

Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2 \cdot 3 H_2O = H_2O$ 9.16 per cent.

Found = H_2O 10.34 " "

Copper, 0.1472 gm. subst., dried at 110° gave 0.0221 gm. CuO .

Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2 = Cu$ 11.87 per cent.

Found = Cu 11.99 " "

LYSINE.

The lysine was separated as the picrate, of which 2.29 gm. were obtained, equal to 0.8915 gm. of lysine, or 1.99 per cent.

Nitrogen, 0.0990 gm. subst., gave 17.25 c.c. of moist N₂ at 29.5° and 756.7 mm.

Calculated for C₆H₁₄O₂N₂ · C₆H₃O₇N₃ = N 18.67 per cent.

Found = N 18.93 " "

The results of this hydrolysis calculated to a moisture and ash-free basis were as follows:

Glycocoll	0.57	Serine	not isolated
Alanine	1.92	Tyrosine	3.07
Valine	0.26	Cystine	0.23
Leucine	7.32	Histidine	2.63
Proline	2.82	Arginine	14.44
Phenylalanine	3.32	Lysine	1.99
Aspartic acid	3.30	Ammonia	1.55
Glutaminic acid	12.35 ¹	Tryptophane	<u>present</u>
			55.77

¹ OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

HETEROTRANSPLANTATIONS OF BLOOD VESSELS.

By C. C. GUTHRIE.

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Saint Louis, Missouri.]

INTRODUCTION.

IT has been shown that segments of blood vessels removed from animals may be caused to regain and indefinitely retain their function of conveying blood.¹ This was accomplished by replacing them in the animals from which they had been removed, and suturing their ends to the ends of other blood vessels that had been divided. Good function has been observed in such cases for months after the operation.²

While Carrel and I were working together at the Hull Physiological Laboratory, we planned a series of experimental operations to study the results of heterotransplantations. Our plans included preliminary immunization of animals, if indicated, by injections of serum or tissue extracts. Lack of time, however, prevented us from engaging in the work together. Recently Carrel has reported the results of some heterotransplantations carried out by him at the Rockefeller Institute for Medical Research.³ He removed segments of arteries from dogs, and after keeping them in cold storage for considerable periods transplanted them into cats. Of five such operations two were successful, one transplanted segment of dog's carotid artery between the divided ends of a cat's abdominal aorta being free from clot or observable defect at the end of six days, while in another cat

¹ CARREL and GUTHRIE: *Comptes rendus hebdomadaires des séances de la Société de Biologie*, 1905. *American medicine*, 1905. *Surgery, gynecology, and obstetrics*, 1906, ii, pp. 14-15, etc.

² CARREL and GUTHRIE: *The American journal of the medical sciences*, 1906. *Report for the Physiological section of the British Medical Association*, Toronto, August, 1906.

³ CARREL, ALEXIS: *The journal of experimental medicine*, 1907, ix, pp. 226-228.

similarly operated upon the circulation appeared to be active after seventy-seven days.

The purpose of this note is to record the results of the transplantation of segments of the abdominal aortas of a cat and of a rabbit between the ends of divided common carotid arteries of dogs.

TECHNIQUE.

A segment of abdominal aorta was removed from the rabbit or cat under ether anæsthesia, washed free of blood with 0.9 per cent NaCl solution, after which it was smeared with vaseline both inside and out and wrapped in gauze moistened with the salt solution. It was then laid aside at room temperature for one to two hours, when it was transplanted. The actual technique of making the anastomoses was similar to that improved by Carrel and myself,¹ only serrefines (or bulldog forceps) were used more freely than we at one time thought advisable. The risk of injury to vessels the size of the common carotid arteries or external jugular veins in medium-sized dogs by the direct use of such forceps appears to be slight, provided care is taken to exclude all forceps that have very stiff springs.

In preparing the cut end of the vessel for anastomosis it is best to turn the external sheath back rather than remove it, as it is valuable for covering over the line of union of the vessels after the inner walls have been sutured. With a little care it can be included with them in the suture, and if this is done the anastomosis is much stronger and less liable to leak when the circulation is first re-established. If it is thought best to remove a portion of it, however, it can be most safely and quickly done by gently grasping and stretching with the fingers and snipping off with scissors or a very sharp knife. The use of forceps as recommended by Watts² would be less safe, at least in the hands of an inexperienced operator. As a rule, it is safer to use the fingers than instruments, when possible, in the manipulation of blood vessels. Single strands from Chinese twist silk, threaded into No. 15 sharps needles, then doubled and sterilized in vaseline or oil, were used. The wounds were not closed until all capillary oozing had ceased and the tissues had been thoroughly dried with gauze sponges.

¹ CARREL and GUTHRIE: *Surgery, gynecology, and obstetrics*, 1906, ii, pp. 3-4.

² WATTS, STEPHEN A.: *Bulletin of the Johns Hopkins Hospital*, May, 1907, p. 169.

Two planes of catgut sutures (Bartlett's twenty-day) were employed in closing the deep and subcutaneous tissues. The skin was closed with an ordinary continuous catgut suture, after which a large cotton dressing was applied directly over the wound and surrounding area and fastened in place by a suitable bandage. In almost all operations on dogs and cats not involving fractures, etc., a short roller bandage over the cotton, fastened with pins, and then the application of a suitable form of tailed bandage made from unbleached cotton or similar material, has given excellent results. Such a dressing is not removed by the animal, and will wear and stay in place for weeks. After the operation the animal is placed in a smooth-walled, open-topped cage, provided with a screen bottom raised several inches from the floor, where it is kept for from twelve hours to a week, depending on the magnitude of the operation. The screen bottom is very essential, as it prevents wetting of the dressings. It is a good plan to put the animal in a sack immediately after the dressing is completed and suspend it in the air until the animal becomes rational. This avoids the possibility of it injuring itself while coming out from the influence of the anæsthetic. For anæsthesia ether alone is used. After the animal is placed on the operating-table ether is administered from a Woulf's bottle by means of a rubber hood over the head or a glass tube passed through the larynx into the trachea and a rubber tube connected with the bottle through a respiration valve,¹ which is provided with an adjustable side opening, enabling one to quickly regulate the mixture of air and ether.

PROTOCOLS.

Dog No. 1. April 24, 1907. Large, adult, mangy, white bulldog. Removed 0.5 cm. segment of the right common carotid artery, and in its place put a segment 1.0 cm. long of adult tomcat's aorta removed posteriorly to the renal arteries. Circulation re-established through segment one and three quarters hours after removal from cat. Diameter of segment one third less than carotid. Closed wound in dog's neck and dressed; applied ointment for mange. Preserved specimens from aorta and carotid for comparisons.

April 26. Animal doing well. Wound dry.

April 29. Pulse appears same in both carotids.

May 1. Pulse same in both carotids. Wound healed. Mange gone.

¹ GUTHRIE, C. C.: The journal of the American Medical Association, 1907, xlviii, p. 1183.

May 29. Pulse same in both carotids.

June 13. Pulse same in both carotids. Made exploratory incision. Circulation through aortic segment excellent. Diameter of segment somewhat greater than of carotid. Tissue appears normal. Closed wound.

June 21. Wound healed. Pulse same in both carotids. Dog in fine condition.

Dog No. 2. May 15, 1907. Young adult, yellow cur bitch. Weight about 25 lbs. Short-haired, fair condition, except for large double goitre. Removed 0.5 cm. segment of the left common carotid artery, and in its place put a 2.5 cm. segment of adult buck rabbit's abdominal aorta. Circulation re-established through segment one and one-half hours after removal from rabbit. Arteries in dog's neck much enlarged (probably in connection with the goitre, as this is commonly observed in dogs). Diameter of aortic segment about one-half that of carotid. Dr. W. N. Sharpe assisted at this operation. Wound healed promptly.

May 29. Good pulse felt on both sides in the positions of the carotids, but owing to the large size of the thyroid vessels, no definite conclusion can be drawn from external examination.

June 15. Made exploratory incision. Circulation in aortic segment excellent. Segment about 3.0 to 3.5 cm. long and of the same diameter as the carotid. Both in appearance and to the touch it is very similar to the artery. Dr. Willard Bartlett directly examined the segment at this time.

The circulation was reversed in the right inferior thyroid vein by ligating and dividing both it and the right common carotid artery and anastomosing the central end of the artery to the peripheral end of the vein. The immediate results were similar to those previously described with Carrel,¹ namely, an increase in the size of the gland with an active circulation of arterial blood accompanied by a strong pulsating in the peripheral portion of the vein operated upon.

June 21. Good pulse in position of left common carotid artery. Strong pulse with thrill in right inferior thyroid vein. Right thyroid lobe greatly enlarged and very hard.

SUMMARY OF RESULTS.

A segment of cat's aorta transplanted between the ends of the divided common carotid artery of a dog was observed by direct examination at the end of fifty days to be adequately performing its

¹ Surgery, gynecology, and obstetrics, *loc. cit.*, p. 10. CARREL and GUTHRIE: Comptes rendus hebdomadaires des séances de la Société de Biologie, 1906, lx, pp. 582-583.

new circulatory function, and apparently to be in good condition; while a segment of rabbit's aorta similarly placed in another dog showed similar results at the end of thirty-one days.

In this connection it may be mentioned that a segment of aorta from a cat preserved in formaldehyde solution for about a month, then washed in very dilute ammonia water, partially dehydrated in alcohol and impregnated with vaseline, when similarly transplanted into a dog, gave excellent temporary results. On killing the animal with ether and examining the segment, it was found to resemble the artery of the dog in a much greater degree than before being transplanted, being more pliant and having a flesh color, the latter due, no doubt, largely to the presence of blood that got into or between the coats from the outside. The union of the intimas was excellent, and they both had the characteristic glistening appearance. My thanks are due Dr. Bartlett for assistance with this operation. A series of operations are being made with the view of determining the permanent results of similarly prepared and transplanted blood vessels.

DISCUSSION.

The apparent differences in diameter of the aortic segments before and after transplantation present some interesting features. It has been shown in horse, ox, etc.,¹ and man² that on removal from the body the walls of peripheral arteries (brachial, carotid, basilar, middle cerebral, renal, etc.) may undergo enormous tonic contraction, amounting to as much as 100 per cent in the size of the lumen and 30 per cent in the thickness of the media. Similar observations have been made on the thoracic and abdominal aorta of cats.³ This tonic contraction may be elicited by mechanical stimulation in arteries from amputated limbs after twenty-four to forty-eight hours, and after even a longer interval by chemical stimulation.⁴

In view of this, it is not permissible, with our present data, to attribute the observed increase in size of the transplanted aortic segments to a structural change in the walls. Neither is it profitable to speculate as to the probability of re-establishment of vaso-motor con-

¹ MACWILLIAM, J. A.: Proceedings of the Royal Society, 1902, lxx.

² MACWILLIAM, J. A., and MACKIE, A. H.: Journal of physiology, 1906, xxxiv, pp. xxxiv-xxxv.

³ GUTHRIE and PIKE: Science, 1906, N. S. xxiv, p. 53; American journal of physiology, 1907, xviii, p. 29.

⁴ MACWILLIAM and MACKIE: *Loc. cit.*

nections or functions in the segments, as these points will be put to the experimental test. Subjecting such arterial segments to cold, heat, ammonia vapor, or the employment of other relaxing measures, may lead to an improvement of our present technique, as a contracted blood vessel is less easily sutured than a relaxed one.

CONCLUSIONS.

From these results it would seem that arteries from rabbits or cats may be safely transplanted into dogs.

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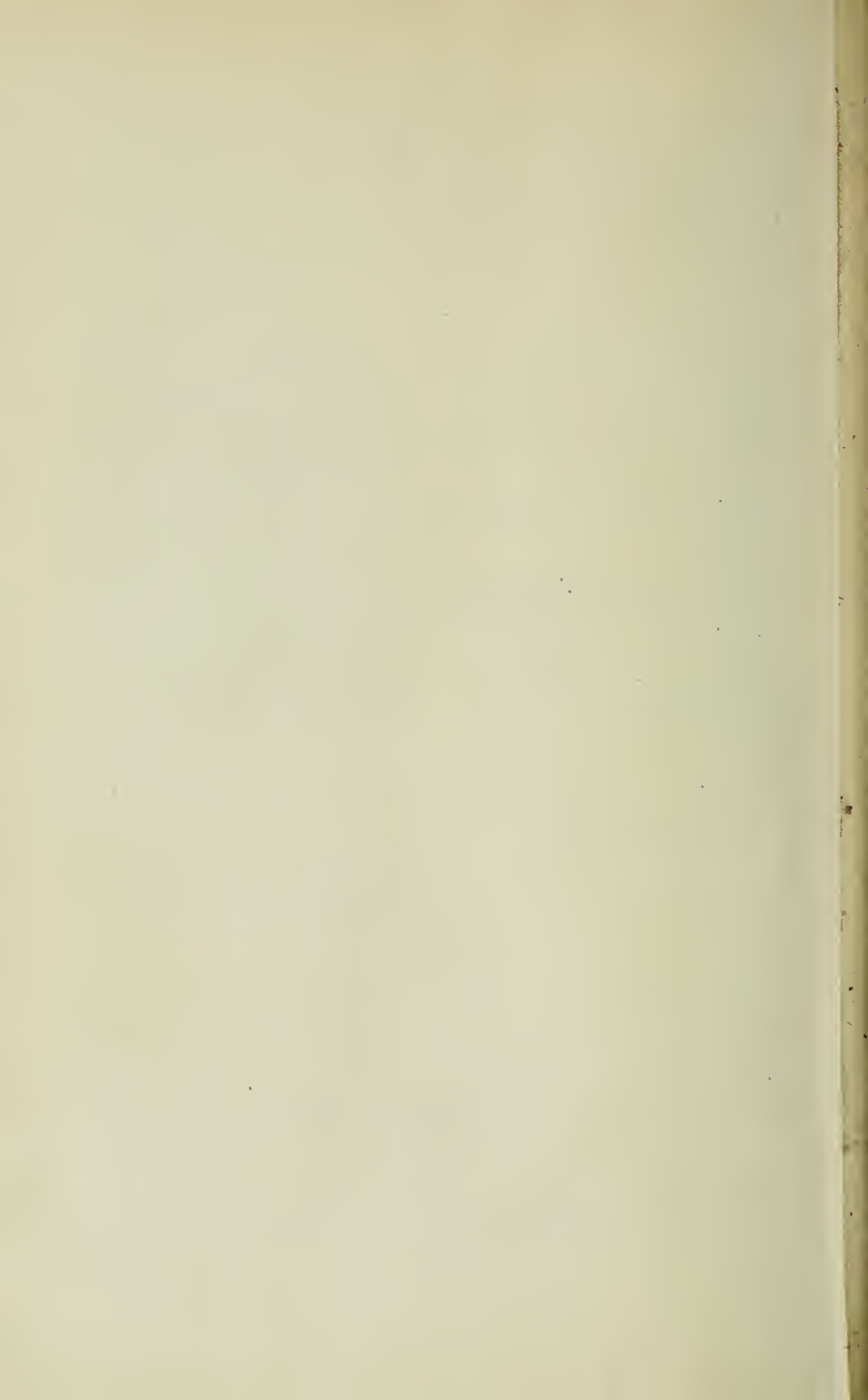
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PROCEEDINGS OF THE AMERICAN PHYSIO-
LOGICAL SOCIETY.

SEVENTH SPECIAL MEETING.

IN CONNECTION WITH THE

CONGRESS OF AMERICAN PHYSICIANS AND
SURGEONS.

WASHINGTON, D. C., MAY 7, 8, 9, 1907.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL
SOCIETY.

ON THE CHEMICAL RELATION OF COLLAGEN TO GELATIN.

BY A. D. EMMETT AND WILLIAM J. GIES.

THE authors have been unable, by Hofmeister's method of continuous drying at 130° C., to convert either pure gelatin or commercial gelatin to collagen. The resultant desiccated products were somewhat less soluble than the original gelatin, both in water and in dilute sodium carbonate solutions at 40° C., but were digested with apparently the same readiness in neutral trypsin solution at 40° C., in which even very minute fragments of collagen fibres remained unaffected for hours.

It appears that Hofmeister attached too much significance to the difference in solubility between the unmodified gelatin and the desiccation product. The observed difference may have been due to *decomposition* instead of simple *dehydration*. Besides, he did not apply the tryptic digestion test to his products to ascertain whether they resembled collagen in resistance to tryptolysis.

When fresh tendon, ossein shavings, and pure collagen from bone and tendon were boiled in water about two hours for the production of gelatin, considerable ammonia was liberated from each. When gelatin was subjected to the same treatment, ammonia was not eliminated.

The authors believe that the so-called collagen obtained by Hofmeister from gelatin by desiccation at 130° C. was not collagen, and that his conclusion, and the prevalent view, that gelatin is a simple hydrate of collagen, are not well founded. That gelatin results from an intramolecular rearrangement of collagen on treating the latter with boiling water, and that the resultant gelatin is not a simple hydrate of collagen, are shown by the fact that ammonia is liberated from the collagen when the latter is converted into gelatin.

VAGUS INHIBITION FROM RISE OF PRESSURE IN THE AORTA.

By J. A. E. EYSTER AND DONALD R. HOOKER.

TEMPORARY ligation of the descending aorta in dogs and rabbits causes quite constantly a decrease in pulse rate when the vagi are intact. Ligation of the ascending aorta, on the other hand, causes usually no change or an increase. After section of both vagi, the rate is practically unaffected by ligation of either the ascending or the descending aorta. Slowing of the pulse was produced experimentally by artificial increase of pressure in an isolated loop of the aorta in animals with intact vagi, and also by inflow of Ringer's solution, at a pressure greater than blood pressure, into the cerebral arteries. It would seem, therefore, that the slowing of the heart that results from a rise of arterial pressure is both reflex and direct. The efferent path of the reflex is the vagus, the afferent through nerves distributed to the aorta, probably also fibres of the vagus. The rise of blood pressure seems also to affect the cardio-inhibitory centre in the medulla directly.

MAY REFLEX CARDIAC ACCELERATION OCCUR INDEPENDENTLY OF THE CARDIO-INHIBITORY CENTRE?

By DONALD R. HOOKER.

THE action of the cardio-inhibitory centre was excluded by section of both vagi. The resultant rapid heart rate was reduced to about 50 per minute by peripheral vagus stimulation. While the heart rate was thus reduced, provided that the animal was in good condition, stimulation of sensory nerves never failed to show cardiac acceleration. Dogs and rabbits were used. The nerves investigated were: the vagus centrally, saphenous, sciatic, and splanchnic. The heart rate was recorded either with the Hürthle manometer or by means of tambours. With the former the records all showed a coincident rise of blood pressure. The possibility that this rise of pressure was the causal factor in the acceleration was excluded by records in which, after section of the accelerator nerves, stimulation of sensory nerves caused a similar rise in blood pressure without an accelerated heart rate.

THE INHIBITION OF TETANY PARATHYREOPRIVA BY
EXTRACTS OF THE PARATHYROID GLAND.

BY S. P. BEEBE.

THE acute symptoms following the extirpation of the parathyroid gland in dogs have been completely inhibited by the hypodermic administration of the nucleoproteid of beef parathyroid. The symptoms may be alleviated for three days by a single injection, but it has not thus far been possible to save the animal from ultimate death by the continued injection of this proteid. If the alkaline solution of the proteid is first boiled, it fails to act. The active principle is not destroyed by peptic or tryptic digestion for forty-eight hours.

PROTEIN METABOLISM IN CYSTINURIA.

BY C. G. L. WOLF AND PHILIP A. SHAFFER.

METABOLISM experiments upon a case of cystinuria confirm the findings of Alsberg and Folin, that the sulphur of hair, or protein-cystin, fed to a cystinuric individual, is normally oxidized to sulphuric acid, and contradict the conclusion of Loewy and Neuberg, that cystinuric individuals are unable to oxidize ingested cystin. Both cystein and cystin prepared from the patient's urine were likewise oxidized when given by mouth. The cystin excreted in the urine is evidently not absorbed as such from the intestine, but must be absorbed in the form of a larger molecule; because cystin absorbed as such is oxidized. An increase of food protein leads to an increase of cystin excreted; but when food protein is hydrolyzed outside the body and the isolated cystin is given to a cystinuric patient, the sulphur of this cystin is oxidized to sulphuric acid.

Cystin injected subcutaneously was excreted in the form of neutral sulphur (probably as cystin). Cystein similarly injected led to an increase of the total sulphur of the urine, the increase being equally divided between inorganic sulphates and neutral sulphur.

The authors believe that the cystin excreted by subjects of cystinuria has a double source. A part, and perhaps a greater part, of the usual diet is derived directly from the food protein, and is therefore strictly exogenous. But a second part of the cystin appears to be

independent of the food protein, and is therefore not exogenous. At least one phase of the anomaly in cystinuria appears to consist of the inability to oxidize that part of the sulphur-containing protein which has *not* been split so far as the cystin stage in the intestine. That part which *is* absorbed as cystin, the cystinuric as well as the normal individual does oxidize.

PROTEIN METABOLISM IN THE DOG.

BY C. G. L. WOLF.

THE nitrogen and sulphur metabolism in dogs in an early stage of starvation was examined. The animals were fed on a non-nitrogenous diet of fat and carbohydrate containing 80 calories per kilogram. At the end of eight days a large quantity of protein in the form of casein was administered, and the metabolism followed during four days of subsequent starvation. In a second series 160 calories per kilogram were fed at the end of the 80 calorie period. This was done in an attempt to change the distribution of nitrogen and sulphur as a consequence of the high caloric value of the diet. The total nitrogen excretion was reduced to a level not heretofore observed in dogs of this weight. The ammonia nitrogen was relatively increased as a result of the starch and fat diet. The oxidized sulphur was lowered markedly, both absolutely and relatively. The relative excretion rose at once on the administration of the protein. The absolute creatinin excretion was uninfluenced by any change in diet. The undetermined nitrogen and neutral sulphur were increased with the administration of protein, but decreased relatively to the nitrogen and sulphur outputs respectively. There was no constant relation between the elimination of ethereal sulphur and indican.

PRODUCTION OF SHOCK BY LOSS OF CARBON DIOXIDE, AND RELIEF BY PARTIAL ASPHYXIATION.

BY YANDELL HENDERSON.

THE writer is led by a large number of experiments to advance the following hypothesis to explain the causation of shock by extreme pain: Pain induces violent and prolonged hyperpnœa. The carbon

dioxide content, not only of the arterial blood but of the tissues of the body as well, is thus greatly reduced. This condition of acapnia lowers the tonus of the peripheral blood vessels, and induces tachycardia, shallow respiration, failure of reflexes, and the mental condition characteristic of surgical shock.

The liability to shock which attaches to the exposure of the abdominal viscera is to be explained by the exhalation of carbon dioxide from the organs exposed, and the consequent loss of tonus in their blood vessels. Thus it was found that when a loop of intestine of a cat was exposed to a current of air warmed to 35° to 38° C. and saturated with moisture, an extreme congestion rapidly developed. When the loop was placed in saline saturated with carbon dioxide, the congestion rapidly disappeared. When the abdominal viscera of a dog under moderate anæsthesia were thus exposed for one hour, and moderate hyperpnœa was thereafter induced for ten minutes by stimulation of the sciatic nerve, shock developed. Arterial pressure fell to 60 mm. Respiration was very shallow. The animal was comatose, and wholly insensitive to stimulation of afferent nerves.

Rapid recovery was effected by assisting in the restoration of the normal carbon dioxide content of the body. The dead space of the respiratory tract was increased by attaching to the trachea a piece of hose a metre in length and 2 cm. in diameter. A moderate volume of saline saturated with carbon dioxide was perfused into the femoral vein. Within fifteen minutes arterial pressure had risen above 160 mm. Respiration was full and deep. The animal came out of the coma, and the reactions to sensory stimuli were restored to normal character and intensity.

All of the above quoted experiments have been performed repeatedly.

THE ACUTE EFFECTS OF GASTRIC AND PERITONEAL CAUTERIZATION ON BLOOD PRESSURE AND RESPIRATION.

BY TORALD SOLLMANN.

CORROSION, or violent or mild irritation of the gastric mucosa, submucosa and serosa, or of the parietal peritoneum, does not change the general circulation or the respiration, in anæsthetized animals, the observations extending over an hour.

THE OSMOTIC PRESSURE OF COLLOIDS AND THE ACTION
OF ELECTROLYTES ON THE OSMOTIC PRESSURE OF
PROTEID SOLUTIONS.

By R. S. LILLIE.

THE osmotic pressure of solutions of colloids (egg-albumin and gelatin) is dependent on the nature and concentration of the electrolytes in the solution. Salts depress the osmotic pressure; the degree of this action depends on the character of the ions of the salt; solutions of alkali metal-salts depress to a less degree than equimolecular solutions of alkaline earth salts, and these to a less degree than salts of the heavy metals. The nature of the anions of the added salt is important; in general the order of decreasing depressant action for several anions has been found to be as follows: $\text{SO}_4 < \text{Cl} < \text{NO}_3 < \text{Br} < \text{I} > \text{CNS}$. Salts with plurivalent anions in general depress osmotic pressure to a greater degree than those with monovalent anions. The presence of non-electrolytes, on the other hand, has little or no influence on the osmotic pressure of the above colloids. The osmotic pressure of gelatin, but not of albumin, is greatly increased by the addition of small quantities of either acid or alkali.

RESULTS OF REMOVAL AND TRANSPLANTATION OF OVARIES
IN CHICKENS.

By C. C. GUTHRIE.

DURING August, 1906, three pure-bred Black S. C. Leghorn and three White S. C. Leghorn pullets were operated upon as indicated below, in the Hull Physiological Laboratory, University of Chicago.

B ₁	}	controls, not operated.
W ₁		
B ₂	}	ovaries removed and exchanged.
W ₂		
B ₃	}	ovaries removed and exchanged.
W ₃		

They began laying the first week in February. They were first mated Feb. 14, 1907. All eggs laid before mating were incubated and found to be sterile. The matings so far are as follows:

B₁ mated to pure-bred Black S. C. Leghorn cock.
 W₁ mated to pure-bred White S. C. Leghorn cock.
 B₂ } mated to pure-bred White S. C. Leghorn cock.
 W₂ }
 B₃ } mated to pure-bred Black S. C. Leghorn cock.
 W₃ }

The results are charted below :

No. of hen.	No. of eggs.	Fœtus and chick markings.
B ₁	42	Thirteen black with ordinary light breasts and throats.
W ₁	40	Seventeen ordinary solid white.
B ₂	52	Nine ordinary white; eleven white, with black spots on backs of heads and wings or on backs.
W ₂	11	One black and three white and one spotted.
B ₃	20	Four ordinary black and two black with white legs.
W ₃	38	Twelve white with black spots on backs of heads and wings or on backs.

CONCLUSIONS.

1. The transplanted ovaries appear to function in a normal manner.
2. The color characters of the fœtuses and chicks may be influenced by the foster mother.

EMBRYO-CHEMICAL STUDIES — THE PURINE METABOLISM OF THE EMBRYO.¹

By LAFAYETTE B. MENDEL.

IN connection with a more extended series of chemical investigations in embryonic growth conducted by the writer, Dr. P. H. Mitchell has studied the occurrence and development of the enzymes associated with the metabolism of the purines. The more important conclusions already reached may be summarized as follows :

1. The liver of the embryo pig contains *adenase*, but no *guanase*. In this respect it resembles the adult liver of the same species.

¹ The expenses of this investigation were shared by the Carnegie Institution of Washington, and the Sheffield Laboratory of Physiological Chemistry, Yale University. The data will later be published in detail.

2. An extract of embryo viscera exclusive of the liver, readily indicates the presence of guanase.

3. The view that guanase and adenase have an unlike distribution and are therefore specific enzymes receives new corroboration from such results.

4. Extracts of the organs of embryo pigs have failed to demonstrate any capacity for forming uric acid from free purines, even after prolonged digestions of five days' duration in the presence of oxygen. The added purine bases, although deamidized, could be quantitatively recovered. *Xantho-oxidase* was therefore not recognized in such extracts.

5. On the other hand, extracts of the liver of adult pigs, or of very young pigs, are capable of forming uric acid.

6. The *uricolytic enzyme* has not been identified in any extract of embryo organs under conditions in which its presence is easily demonstrated in adult tissues.

7. Both the xantho-oxidase and the uricolytic enzyme apparently begin to functionate either in the last stages of embryonic life or soon after birth.

The tardy appearance of some of these enzymes is of interest in view of the peculiar character of the physiological processes characteristic of young or growing organisms.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS — SOLUTIONS OF ELECTROLYTES.

BY TORALD SOLLMANN.

A SERIES of electrolytes were investigated. Solutions of the same freezing-point as 1 per cent NaCl are not always isotonic toward kidney cells, and some of the changes observed on perfusing such solutions are osmotic. In most cases, however, osmosis does not suffice to explain the phenomena, there being also chemical and vital factors. With few exceptions, the results throw but little light on the comparative diuretic effects of salts under the conditions pertaining in living animals.

The following communications were also presented :

THE CALCIUM AND POTASSIUM METABOLISM OF THE HEART DURING INHIBITION AND ACCELERATION OR AUGMENTATION. By WILLIAM H. HOWELL.

THE TEACHING OF PHYSIOLOGY IN THE LABORATORY. By VELVIEN E. HENDERSON.

RESULTS OF REMOVAL AND TRANSPLANTATION OF OVARIES IN CHICKENS. By C. C. GUTHRIE.

NOTES ON THE THYROID. (Read by title.) By REID HUNT.

ON THE OCCURRENCE OF FERMENTS IN EMBRYOS. By WALTER JONES and C. R. AUSTRIAN.

THE COMPOSITION AND CHARACTER OF THE HOURLY EXCRETIONS OF URINE. By A. B. MACALLUM and C. C. BENSON.

PROTEID SUSCEPTIBILITY and IMMUNITY. By V. C. VAUGHAN.

THE DISTRIBUTION OF SULPHUR AND PHOSPHORUS IN THE HUMAN BRAIN. (Read by title.) By WALDEMAR KOCH.

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