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FOURTEENTH ANNUAL MEETING.

UNIVERSITY OF CHICAGO, DECEMBER 30 AND 31, 1901.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

SOME NEW OBSERVATIONS ON BLOOD PLATES.

By GEORGE T. KEMP and O. O. STANLEY.

The experiments of Dutjen (Virchow's Archiv, 1901, clxiv, p. 239) were repeated and his observation of amœboid movements of the blood plates on agar-agar containing sodium hexa-metaphosphate $Na_6P_6O_{18}$ were corroborated. In the blood of animals into whose circulation methylene blue had been injected, the plates showed blue granules scattered through the colorless part which was making amœboid movements. These blue-stained granules resembled in staining properties the nuclei of leucocytes. Macallum's method for the detection of phosphorus in the cell showed the plates to be rich in phosphorus, the green color of the reaction being seen principally, if not entirely, in granules. From these experiments, together with the observation of Lilienfeld (corroborated by us), that the plates leave an insoluble residue after digestion with hydrochloric acid and pepsin, the authors conclude that the plates contain nucleo-proteid in the form of granules, and that they also contain a part (probably protoplasmic) which is capable of making amœboid movements.

The experiments of Arnold with blood in 10 per cent solution of KI were repeated and while the *Maulbeer* and *Stechapfel* forms were constantly present, the complete budding off of the structures which he took to be plates was not always observed. Such separated portions as were seen were thought by the authors to be artefacts, and to be not identical with the blood plates.

RELATION OF BLOOD PLATES TO THE INCREASE IN THE NUMBER OF RED CORPUSCLES AT HIGH ALTITUDES.

By GEORGE T. KEMP.

The red corpuscles and blood plates were counted first in Paris. The method employed is described in this Journal, 1901, v, p. iv. The mean of five counts on five consecutive days was as follows: red corpuscles

4,800,000 and blood plates 457,000. The ratio of plates to red corpuscles was 1 : 10.5. A journey was then made to the Gorner Grat, Switzerland, 10,290 feet above the sea-level. The journey was accomplished in forty-eight hours, and as the ascent of the mountain was made by the electric railway, the experiment was not complicated by physical exertion or fatigue. On the Gorner Grat, seventy-two hours after the last blood count in Paris there were 7,000,000 red corpuscles (mean of two counts), and 1,206,900 plates. The ratio of plates to red corpuscles was 1 : 5.8.

The number of plates had, therefore, increased more than one hundred per cent in seventy-two hours. The change in the character of the plates was most striking. The field was strewn with them and they were at least twice the average size of those seen at Paris. The number of small red corpuscles was very great. The large plates and the small red corpuscles overlapped in size. A careful examination failed to reveal any color in the plates. The small red corpuscles were always stouter than the plates and could not have been mistaken for them. Nothing that could be taken for a connecting form between them was observed. In spite of this, there was a marked resemblance to the *crise hématoblastic* described by Hayem.

NOTES ON THE PHYSIOLOGY OF THE CIRCULATORY SYSTEM OF THE CALIFORNIA HAGFISH, POLISTOTREMA STOUTI.

By C. W. GREENE.

Three distinct propelling organs are situated on the system of blood vessels in the hagfish. These are the hearts, which may be distinguished as the systemic heart, the caudal heart in the tail, and the portal heart on the portal venous system. The systemic heart is of peculiar interest in that it is free from all nervous regulation. Stimulation of the vagus nerves, and of various portions of the central nervous system, has no influence on the rate and force of the heart, though other muscles are profoundly influenced. The portal heart was first observed to contract in Myxine by A. Retzius and J. Müller. This organ contracts at a rate of from 40–50 per minute and maintains its rhythm for many hours after isolation from the body. The portal heart is also without a special nervous regulative mechanism.

Studies on the relation of the activity of the systemic heart and of the portal heart to the composition of the blood have revealed that the osmotic pressure of the blood is much higher than in bony fishes. Tested by the freezing-point method the hagfish serum depresses the freezing-point 1.934° C.–1.992° C., the equivalent of about 3 per cent sodium-chloride solution, *i. e.*, practically the same as the sea-water, 1.945° C., of Monterey Bay.

ON THE QUESTION WHETHER DEXTROSE ARISES FROM CELLULOSE IN DIGESTION.

By GRAHAM LUSK.

It was shown that neither twenty grams of cauliflower fed to a dog diabetic with phlorhizin, nor ten grams of paper fed to a phlorhizinized goat increased the dextrose in the urine. Since dextrose fed in phlorhizin diabetes is quantitatively eliminated in the urine, it follows that dextrose is not produced from cellulose in digestion.

THE ACTION OF ALCOHOL ON MUSCLE.

By FREDERIC S. LEE and WILLIAM SALANT.

Ethyl alcohol in various percentages was injected into frogs in quantities proportional to the weight of the animals. In each experiment one gastrocnemius muscle was protected from the action of the drug, and compared with the alcoholized gastrocnemius of the opposite side. It was found that in small quantities ($\frac{1}{2}$ minim of 10 per cent alcohol per gram of frog), the drug has no effect. Larger quantities (1 to 4 minims of 10 per cent alcohol per gram of frog) allow the muscle when stimulated to contract more quickly, relax more quickly, perform a greater number of contractions in a given time, and do more work than a muscle without alcohol, while the onset of fatigue is at the same time delayed. In some cases the increase in the amount of work performed is more than 10 per cent. Experiments in which curare and alcohol were used together make it for the present uncertain whether this seemingly beneficial result is to be attributed to the action of alcohol on the muscle protoplasm, or on the intramuscular nerve substance. Whether the alcohol exerts

its action by serving as a food, or in some other manner, is not decided.

In larger quantities than the above the alcohol is detrimental to muscular action, diminishing the whole number of contractions, inducing early fatigue, and diminishing the amount of work that the muscle is capable of performing, even to the extent of doing away entirely with contractile power. In such quantities the drug is distinctly poisonous. The after-effects of the administration of alcohol have not yet been studied.

EXPERIMENTS ON THE RELATION BETWEEN THE SPLEEN AND THE PANCREAS.

By LEO F. RETTGER (presented by LAFAYETTE B. MENDEL).

In investigating the problem of the possible functional relation between the spleen and the pancreas, a considerable number of observations on dogs have been made in repetition of the experiments of Herzen[1] and of Gachet and Pachon.[2] This preliminary report brings a confirmation of the observed influence of spleen extracts in vitro (Herzen), and in vivo (Gachet), in increasing the proteolytic power of pancreatic extracts. The specific effect of intravenous injections of spleen extracts in augmenting the trypsin content of the pancreas of splenectomized dogs has been controlled by comparable injections of other fluids, *e. g.*, physiological saline solution, boiled extracts of spleen, extracts of liver, pancreas, etc. In none of the latter cases was any specific "trypsinogenic" effect manifested. While the positive results obtained were perhaps not in every instance as pronounced as those reported by earlier investigators, the effects of the spleen reactions are characteristic, as indicated by the protocols and demonstrations presented.

EXPERIMENTS ON ALLANTOIN EXCRETION.

By LAFAYETTE B. MENDEL.

1. Thymus glands were finely divided, mixed with water, and introduced *per rectum* into dogs. The absorption of the "purin" constituents was demonstrated by the characteristic excretion of allantoin

[1] Herzen: Revue générale des sciences pures et appliquées, June 15, 1895.

[2] Gachet and Pachon: Archives de physiologie, 1898, xxx, p. 363.

in the urine eliminated during the succeeding day. The output of uric acid was also noticeably increased. The animals had previously been fed for a day or two on a diet of casein and lard, to exclude the influence of dietary nuclein compounds. The quantity of allantoin excreted was noticeable; and the masses of crystals separated directly from the concentrated urine were exhibited. The experiments were carried out by Mr. F. P. Underhill.

2. In an investigation of the physiological action of the nucleates and nucleic acid separated from wheat germs by the methods first described by T. B. Osborne, products containing about three per cent of phosphorus were fed to a cat in doses of five grams, with milk and cracker meal. The urine readily yielded crystals of allantoin, which were exhibited. These vegetable nucleates thus resemble the so-called nucleoproteids from animal tissues. The nucleic acid from wheat apparently shows a similar behavior in metabolism.

A study of the action of the vegetable products when introduced directly into the circulation has been begun. The results already obtained are in part comparable with those recently recorded by Bang for the guanylic acid and the nucleoproteid of the pancreas. These experiments have been undertaken by Mr. G. B. White.

THE RÔLE OF THE CELL NUCLEUS IN OXIDATION AND SYNTHESIS.

By R. S. LILLIE (presented by W. T. PORTER).

Sections of fresh and alcoholic tissues of the frog were treated with an alkaline solution containing para-diamido-benzene and alpha-naphthol. This solution, at first colorless, turns violet on oxidation from the formation of indophenol by condensation. Different tissues produce a more or less rapid appearance of the color according to their greater or less oxidative activity.

The chief results and conclusions were as follows. (1) In general the coloration is intensest and appears soonest in those organs and in those regions of organs which contain the most numerous and most densely aggregated nuclei, *e.g.* kidney, spleen, thymus, ventral gill-vestiges. Oxidations and their correlated syntheses appear therefore to depend chiefly upon the nuclear matter of the cell.

2. The formation of the color is prevented by acid, and by strongly

reducing substances; and is retarded by the presence of poisons which interfere with oxidation, such as hydrocyanic acid and its salts.

3. The formation of the color appears on microscopical examination to take place chiefly at the *surface* of the nucleus. The oxidative and synthetic activity of such organs as the kidney and liver thus probably depends largely upon their great extent of nuclear surface.

4. The mucosa of the intestinal tract has a marked oxidative activity; the color appears chiefly at the inner ends of the epithelial cells near the nucleus. The layer of closely aggregated nuclei resulting from the columnar arrangement of the cells must be regarded as of great importance in effecting oxidations and syntheses during absorption.

5. Leucocytes and lymphoid structures in general have a marked oxidative activity. This is probably of importance in the activity of leucocytes in phagocytosis.

6. In the kidney-tubule oxidation is effected chiefly by the glandular region; the Malpighian corpuscles are comparatively inactive.

STUDIES IN DIURESIS.

By J. T. HALSEY.

Nussbaum's investigations on the circulation in the frog's kidney with their far-reaching importance in explaining the physiology of diuresis, failed to receive anatomical confirmation when Adami repeated these experiments. Recently Marcuse, while studying phlorhizin diabetes, confirmed Nussbaum unreservedly. In the hope of possibly finding an explanation for the contradictory results the matter was again taken up by myself, and the results of these new investigations form the basis of this communication.

Every care was taken to carry out the investigation according to Nussbaum's directions. The only deviation made was that in addition to ligaturing the renal and genito-urinary arteries, all branches of the abdominal aorta were ligatured, in order to prevent, if possible, blood reaching the kidneys through anastomotic arterial circulation.

Canadian bullfrogs, male and female, females of several varieties of the small indigenous frogs, and female Rana Esculenta from Strassburg in Alsace, sixty-three frogs in all, were used in the course

of the investigation. After completion of the operation and a short time before death, a solution of ammonium carmine, or a suspension of granules of vermilion or prussian blue, was injected.

In every case, without exception, microscopic examination of the kidneys disclosed the coloring matter or the pigment inside the capillaries of a greater or less number of the glomeruli. A careful study of the sections, however, led to the belief that, as so few of the glomeruli were receiving blood, and as in these the blood stream appeared to be so sluggish, it was a justifiable conclusion that the glomeruli in the Nussbaum frog are a physiologically negligible factor.

The study of the behavior of various substances injected into the veins of such frogs gave results in entire accordance with those of Nussbaum. Urea and indigo carmine are excreted by the kidney under these conditions, whereas dextrose, egg albumin, peptones (Witte's), and ammonium carmine are not. Under the influence of phlorizin, dextrose was secreted as Marcuse had found. When theobromine (diuretin) was injected simultaneously with dextrose, the urine reduced Fehling's fluid so strongly that the presence of sugar seemed established. After injecting NaCl, Na_2HPO_4, and Na_2SO_4, these salts were regularly present in the urine.

It seems impossible at present to explain the results of these experiments in any other way than by attributing to the tubular epithelium an active power of excreting urea and the three salts mentioned. Under the influence of phlorizin or theobromine, the tubular epithelium seems to acquire the power of excreting dextrose, a power which it does not possess under normal conditions.

ON SALINE DIURESIS.

By ARTHUR R. CUSHNY.

Magnus has recently shown that the sulphate of sodium injected intravenously causes a more profuse diuresis than that produced by the chloride and that this difference is due to some local effect in the kidney and not to the changes in the circulation. He appears to consider that the sulphate stimulates the secretory elements of the kidney, a view also adopted by Sollmann. A simpler explanation may be given on Ludwig's theory, namely that the sulphate is not so readily absorbed by the renal tubules as the chloride, and thus

retains a larger amount of water. On the injection of chloride and sulphate together intravenously, the chloride and sulphate increase in the urine along with the fluid. As the diuresis decreases, the chloride falls much more rapidly than the sulphate, which increases in percentage. This may be explained by the chloride and water being absorbed, while the sulphate permeates the tubules less readily. If this view is correct the chloride should decrease still more rapidly on accelerating the absorption while the sulphate should be less affected. This may be done by compressing the ureter during the injection, and in a number of experiments the ureter was narrowed until the pressure within it rose to 20–30 mm. mercury. The urine from this ureter was compared with that from the normal one of the other side, when it was found that the water from the former was less by 66 per cent, the chloride by 82 per cent, and the sulphate by 30 per cent. This can be explained only by absorption in the tubules, and the sulphate therefore penetrates the epithelial cells much less readily than the chloride.

ON THE GLANDS OF THE OVIDUCT IN THE FOWL.

By ARTHUR R. CUSHNY.

The oviduct of the fowl is about 37–50 cm. in length and in the upper part possesses a thick wall with large villous projections. In the middle the wall is thinner and has fewer villi, while in the lowest 7.5–10 cm. (uterus) the wall is again thick and villous. Histologically the oviduct consists of a layer of unstriated muscle externally (thin except in the uterus), a thin connective tissue submucosa, a mucosa consisting largely of glands with little connective tissue, and a lining epithelium. The glandular layer is entirely absent in the first part of the duct towards the fimbriated tube. The lining epithelium is of the pseudotransitional variety and is covered with cilia whose function appears to be the propulsion of the ovum downwards in a spiral. From above downwards the glands may be divided into albumen-secreting, mucous, membrane-secreting and lime-secreting.

The albumen glands are very complicated, branching and coiling through the mucous coat. When they are not actively secreting, the cells are filled with fine granules which grow in size, coalesce, and form secretion vacuoles which are voided into the tubules and thence poured into the oviduct before the ovum passes. The lumen of the

gland is entirely closed before the secretion is formed, but is dilated with masses of albumen as the secretion vacuoles burst. The mucous part of the duct lies between the albumen and the membrane secreting glands and consists of typical mucous beaker cells with compressed ciliated cells between them. This part of the duct is 1–3 cm. long and secretes very actively, but the purpose of the secretion is unknown.

The membrane-secreting part of the oviduct possesses glands of which the cells are filled with secretion-vacuoles or granules which coalesce and are excreted into the lumen.

In the uterus the lime secreting glands are very complex, with few apertures. The gland cells are uniformly clear except towards the lumen, where they present granules staining strongly with eosin and with aniline blue.

EXPERIMENTS WITH ZYGADENUS VENENOSUS (POISON CAMASS).[1]

By REID HUNT.

By following the methods ordinarily used for the isolation of plant alkaloids a substance having the following properties was obtained from the alcoholic extract of Zygadenus ven.: as left from the evaporation of its solution in chloroform it was hard, glassy, transparent, slightly yellow in color, alkaline to litmus, easily soluble in alcohol and acidulated water, insoluble in distilled water. When dissolved in alcohol and the alcohol slowly evaporated, a semi-crystalline mass was left.

When this substance was treated with sulphuric acid a yellow color appeared; on warming, the color became violet, then orange and cherry red. The solution showed a green-yellow fluorescence. Warmed with hydrochloric acid a pink-red color was produced; the solution became fluorescent on the addition of acetic acid. When a little of the substance was warmed with oxalic acid a blood-red color resulted.

The substance had an intensely acrid burning taste which was very persistent. Dissolved in alcohol or chloroform and applied to the

[1] These experiments were performed last July in the chemical laboratory of the Montana State College of Agriculture. Most of the work was done in connection with Mr. V. K. Chesnut of the U. S. Department of Agriculture.

skin it caused a burning sensation which soon became painful; the pain continued long after the solution was washed off. Later the spot became almost anæsthetic. The dust of the dried plant as well as the alkaloid caused intense irritation and sneezing when applied to the nose. The substance is very poisonous, five or six milligrams pro kilo body-weight being fatal to rabbits. The immediate cause of death in animals poisoned by this substance was paralysis of the respiration; there was also a marked lowering of the blood pressure and a slowing of the heart. The latter occurred after the administration of atropine and was probably due to a direct action upon the cardiac muscle. Convulsions (probably due to asphyxia) occurred in all cases; the convulsions were followed by periods of great muscular weakness. The relaxation of the frog's muscle was greatly prolonged in animals poisoned by this substance.

Thus both the chemical and physiological properties of the active principle of Zygadenus venenosus agree very closely with those of the mixture of alkaloids known as "veratrine";[1] experiments to determine the exact character of the alkaloid (or alkaloids) present are now being made.

The alkaloid is excreted very rapidly in the urine, while it is absorbed rather slowly from the stomach. By the use of diuretics (diuretin or caffeine) the drug may be removed from the circulation so rapidly that few poisonous symptoms may result; rabbits and sheep invariably recovered from fatal doses of zygadenus when treated in this manner. Strychnine and atropine seemed to intensify rather than diminish the effects of the poison.

THE EXCRETION OF LITHIUM.

By C. A. GOOD.

Hüfner states that on giving 25 mgm. of lithium carbonate by the stomach, none of the metal could be found in the urine, although the spectroscopic method he employed permitted the recognition of 0.00003 mgm. When lithium was given in 50 mgm. doses the spec-

[1] After the completion of the work here reported Vejux-Tyrode published a note (Journal of medical research, Nov., 1901) in which he states that Pfaff has isolated from Zygadenus ven. a "white, crystalline, neutral body" which causes a change in the form of the contraction of the frog muscle similar to that produced by veratrine. Vejux-Tyrode obtained a similar body from Zygadenus frumentii.

trum was obtained from the urine. No quantitative estimations have hitherto been made. In a number of experiments on cats in which lithium chloride was injected hypodermically, the lithium was estimated by taking advantage of the insolubility of the phosphate in ammonia solution. The dose of 0.5 gram was found fatal, the animal dying after about a week. When large quantities (1–2 grams) were injected hypodermically, very considerable amounts were obtained from the stomach (by lavage), and from the bowel, and the saliva also contained appreciable quantities. In fatal poisoning more was found in the stomach and bowel contents than in the urine. In experiments in which small doses were administered repeatedly, more lithium was excreted in the urine than by the alimentary tract.

AN ATTEMPT TO OBTAIN REGENERATION OF THE SPINAL CORD.

By PERCY M. DAWSON and EDWIN N. RIGGINS.

A few years ago experiments were performed in this laboratory which led to the conclusion that regeneration of the dorsal-root fibres into the cord will take place under the proper conditions. The authors then stated the opinion that with proper technic a severed spinal cord might be made to regenerate its broken tracts both ascending and descending.[1]

Acting on this hope the following experiment was performed. In a young bitch anæsthetized with morphia and ether, the spinal cord was exposed and divided with a very sharp knife at the level of the lowest dorsal vertebra. The animal was then nursed with the greatest care for a period of one hundred and twelve days. During this time it was under constant observation. At autopsy the site of operation presented a very satisfactory appearance. The dura was adherent above to a mass of scar tissue but otherwise appeared quite normal. The cord observed by transmitted light, showed only a fine white line at the plane of section.

Although the animal had remained in excellent condition and the operation had been so successful from a surgical point of view, *there was never any conclusive evidence of conscious sensation or of voluntary motion in the "hind dog."*

[1] Regeneration of the dorsal root fibres of the second cervical nerve within the spinal cord: Baer, Dawson and Marshall. Journal of experimental medicine, 1899, IV, p. 29.

A STUDY OF METABOLISM IN A CASE OF LYMPHATIC LEUKÆMIA.

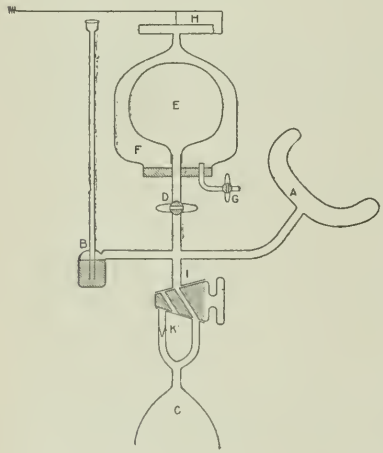
By YANDELL HENDERSON and G. H. EDWARDS.

By analyses of the urine of a case exhibiting all the characteristics of lymphatic leukæmia, the uric acid and phosphates were found rather below than above the normal. From this the conclusion was drawn in conformity with the views of Milroy and Malcomb that the nuclein metabolism of leukæmia, of this form at least, differs from the leucocytosis produced by injecting nuclein, in being due, not to an increased formation of leucocytes, but to a diminished katabolism. This may be due to an arrested development in the small leucocytes. The investigation is still in progress.

A NEW INSTRUMENT FOR DETERMINING SYSTOLIC AND DIASTOLIC BLOOD-PRESSURE IN MAN.

By JOSEPH ERLANGER.

Since the work of Howell and Brush has shown decisively that instruments which make use of the principle of Marey — viz., the pressure under which an artery will give its maximum pulsations is the mean pressure in that artery — give, not mean pressures, but minimum or diastolic pressures, and since the same authors have shown that the systolic and diastolic pressure may actually be affected in opposite directions, the importance of a sphygmomanometer that will give parallel records of the variations in systolic and diastolic pressures becomes evident.



Therefore the following instrument has been devised. An arm-piece like that employed in the Hill-Barnard sphygmometer is fastened about the arm above the elbow. Its rubber bag (A) communicates with a mercury manometer (B) and with a Pulitzer bag (C) for varying the pressure. The maximum pressure is obtained by noting the

pressure required to obliterate the pulse distal to the point of application of the pressure. With the rest of the apparatus the diastolic pressure is obtained. When the stopcock (D) is open the pressure in the apparatus is also transmitted to the rubber bag (E) enclosed in the glass bulb (F). The air space between these communicates with the exterior through the stopcock (G) and with the tambour (H) the lever of which writes upon a slowly revolving drum. The tambour is perforated by a minute opening. To determine the minimum pressure, the stopcock (G) being open, the pressure is quickly raised above the expected minimum. The three-way stopcock (I) is then closed and the valve (G) is closed. The lever will now record the pulsations. After, say, twenty pulsations have been recorded, the stopcock (I) is turned so as to communicate with the capillary (K). The pressure falls slowly and is stopped after a fall of 5 mm. Hg. The lever will quickly return to its original level on account of the equalization of pressure permitted through its minute perforation, and the pulsations under the diminished pressure are recorded without interruption. This manipulation is repeated until the pulsations begin to diminish in size. A series of records upon a straight line will thus be obtained at various pressures. They will show a distinct maximum. The pressure at which this maximum record was obtained is the minimum or diastolic pressure in the artery.

A NEW FORM OF ERGOGRAPH.

By WINFIELD S. HALL.

The principal objection raised to the classic form of the Mosso ergograph is that the muscle soon becomes so far fatigued that it cannot lift the weight at all, and, therefore, is not credited with accomplishing work, yet it is exerting not a small amount of unmeasured energy. If a spring were used a small amount of work would be shown by a small extension of the spring. Again, if the observer wishes to study the degree and variations of tension in the "isometric" muscular contractions, the spring ergographs seem necessary. Two other objections have been urged against the weight ergograph: (1) The highest tension required in moving a weight is that which overcomes its inertia and starts the weight with the other movable parts of the apparatus into motion. Once it is started, a sudden stopping of the

motive force (the finger) is not followed by an instantaneous stopping of the weight, pulley, and cord. Thus the graphic record may be distorted. (2) All work with the weight ergograph shows only "isotonic" contractions, while it is possible with a spring ergograph to study both isotonic and isometric contractions.

The writer has attempted to construct a weight ergograph in which the various imperfections of the classic ergograph are, in a measure at least, remedied. The principal feature of this ergograph is that *the muscle works only during contraction.* This is accomplished through the device of a differential pulley. The arrangement accomplishes the same thing physiologically as Fick's Arbeitsammler.

Next in importance is the fact that the weight moves so slowly that quick movements of the finger never result in the inertia of the weight affecting the curve.

The objection that the nearly exhausted finger cannot start the weight, and will, therefore, make no record and do no mechanical work, is not a valid objection from the standpoint of everyday work. Every object that the laborer lifts requires a greater expenditure of energy to start it moving than to keep it moving.

DEMONSTRATION OF APPARATUS.

By WARREN P. LOMBARD.

1. *Ergograph for the Index-finger* reported for Thomas A. Storey, Assistant in Physiology, Leland Stanford Jr. University. The instrument was devised by Mr. Storey in the summer of 1900, in the Physiological Laboratory of the University of Michigan. It permits of an accurate record of the angular movement about the metacarpophalangeal joint of the index finger. It may be employed both by voluntary and by electrical excitation of the abductor indicis. It enables the muscle to work against a weight or spring, and isotonically or isometrically. It permits movements of the finger to be recorded directly on a horizontal or vertical drum, or indirectly by means of a distant lever or other appropriate device. It allows the use of various forms of "Arbeitsammler" in connection with it. It permits the attachment of weights so that the strain on the finger shall (except for inertia effects) be constant, and the effect of the throw of the weight be minimized.

The essential element of the device is a lever, bearing on its axis

pulleys of various diameters for the attachment of cords to be connected with the weights or springs to be moved, or the apparatus employed to record the movement of the finger. A direct record of the movement of the lever can be taken from a celluloid pointer at the extremity. The centre of the metacarpo-phalangeal joint of the index finger is brought into line with the axis of rotation of the lever, and the end of the first phalange is fastened in the clamp at the end of the lever arm. The rest of the hand and thumb are fixed on suitable supports.

A photograph and working drawings of the instrument, together with curves obtained by it, were shown, and a working model, differing slightly from the original, was exhibited and its use demonstrated.

2. The following apparatus intended for the use of students were demonstrated: A platinum mercury key; an upright rheocord; a graphite rheostat — to demonstrate the effect of rate of change of strength of a battery current upon excitation of nerve; a short contact key — to demonstrate the effects of duration of a battery current upon the excitation of nerve; a modified form of Fitz's belt pneumograph; a ball and socket joint to connect the disk on a tambour with a lever; a celluloid lever; a spring support for a glass slide; plaster of Paris electrodes and a method of moistening the skin for excitation of human nerve and muscle.

DEMONSTRATION OF APPARATUS.

By W. T. PORTER.

The following pieces of the Harvard Physiological Apparatus were shown: Adjustable plate, aortic cannula, circulation scheme, femur clamp, platinum electrodes, capillary electrometer, artificial eye, frog board and clips, inductorium, rocking key, short-circuiting key, simple key, optical lantern, light muscle lever, heavy muscle lever, moist chamber, square rheochord, signal magnet, sphygmograph tambour.

The Mechanism of Fibrillar Contraction of the Heart. By W. T. Porter.

Further Experiments on the Importance of Sodium for the Heart-beat. By D. J. Lingle.

By invitation.

On the Prolongation of the Life of Unfertilized Eggs of the Sea-Urchin by Potassium Cyanide. By J. Loeb and W. H. Lewis.

This journal, 1902, vi, pp. 305–317.

The Physiological Effects of the Electrical Charge of Ions and the Electrical Character of Life Phenomena. By J. Loeb.

Compare this journal, 1902, vi, pp. 411–433.

The Nature of Nerve Stimulation and Alterations of Irritability. By A. P. Mathews.

Effects of Potassium Cyanide and of Lack of Oxygen on the Development of Sea-urchin Eggs. By E. P. Lyon.

By invitation.

The Formula for Determining the Weight of the Central Nervous System in Frogs of Different Sizes. By H. H. Donaldson.

The Chemical Analysis of the Brain. By W. Koch.

By invitation.

The Mode of Action of Certain Substances on the Colored Blood-corpuscles, with Special Reference to the Relation between so-called Vital Processes and the Physico-chemical Structure of Cells. By G. N. Stewart.

A Convenient Rabbit-Holder. By G. Lusk.

On the Surface Action of Metals. By F. G. Novy.

An Arterial Cannula and other new Physiological Apparatus. By G. P. Dreyer.

Glycocoll in Gelatoses. By P. A. Levene.

Read by title.

A Contribution to the Physiology of the Thyroid Gland. By L. Breisacher.

Read by title.

On the Nucleic Acid of the Suprarenal. By W. Jones.

Read by title.

On Gluco-phosphoric Acid. By P. A. Levene.

Read by title.

Embryochemical Studies. II. — The Presence of Mono-amido-acids in the Developing Egg. By P. A. Levene.

Read by title.

The Frontal Lobes (cerebral) and the Formation and Retention of Associations. By S. I. Franz.

Read by title.

The Movements of the Intestines Studied by Means of the Röntgen Rays. By W. B. Cannon.

This journal, 1892, vi, pp. 251–277.

Read by title.

The Relation of the Parathyroid to the Thyroid Gland. By W. S. Carter.

Read by title.

THE COMPOSITION AND CHEMICAL QUALITIES OF THE ALBUMOID IN BONE.

By P. B. Hawk and William J. Gies (reported by W. J. Gies).

(Read by title.)

In the first report to this society of the discovery of osseomucoid attention was drawn to the fact that the method used for the preparation of the glucoproteid would also favor a study of the albuminoid constituents of osseous tissue. The collaginous residue remaining after extraction of osseomucoid from ossein yields an insoluble, elastin-like substance on boiling in water. This substance is neither the elastin of Smith nor the keratin of Brösicke, but appears to be almost or quite identical with Mörner's chondroalbumoid. Although our product is digestible in pepsin-hydrochloric acid, it appears to be somewhat more soluble in dilute acid and alkali than chondroalbumoid. Unlike the latter body, however, it does not contain loosely bound sulphur.

We have prepared a number of samples of osseoalbumoid from ossein by the method Mörner used for the preparation of the albumoid substance in cartilage. The chief difficulty in this work has been the removal of phosphates and the preparation of ash-free products. Our analyses thus far indicate the average elementary composition given in the summary below, where comparison is also made with keratin and elastin.

	C	H	N	S	O
Osseoalbumoid . . .	50.03	6 85	15.93	0.55	26.64
Ligament elastin . .	54.08	7.20	16.85	0 30	21.57
Hair keratin	50.65	6.36	17.14	5.00	20.85

Osseoalbumoid does not contain phosphorus. Unfortunately, analytic comparisons with chondroalbumoid are not now possible, as Mörner made no analyses of that body, although he found that the nitrogen content (three determinations) of albuminates made from it varied between 15 and 16 per cent. We have obtained larger proportions of this residual substance from bone than from cartilage. It is our purpose to study chondroalbumoid in this connection also.

A COMPARATIVE STUDY OF THE REACTIONS OF VARIOUS MUCOIDS.

By L. D. MEAD and WILLIAM J. GIES (reported by W. J. GIES).

(Read by title.)

Comparative studies of many of the precipitation reactions of osseomucoid, chondromucoid and tendomucoid have shown thus far a very striking sameness in result. Each of these glucoproteids also is digested in pepsin-hydrochloric acid, with a formation of proteoses and peptones and the separation of nitrogen-containing substance rich in reducing material, probably chondroitin sulphuric acid or essentially the same body in each case. The microscopic appearance of the phenylosazone bodies obtained from each is the same as that of dextrosazone, indicating glucosamine among the products of acid hydration.

All these compound proteids contain sulphur obtainable as sulphate and as sulphide. They are acid to litmus, neutralize alkali, have essentially the same elementary composition and yield practically the same amount of heat on combustion. In physical appearance the substances whether dry, freshly precipitated, or in solution, are practically identical. Attempts to obtain crystalline mucoid, by the methods which recently have given such fruitful results in other connections, have thus far been without success. When the electric current is passed through neutral or alkaline mucoid solutions (consisting of sodium or calcium salts of mucoids) turbidity results within a short time, and flocks eventually form and can be filtered off.

Our studies in this general connection have not been completed. We are convinced, however, that the connective tissue mucoids are practically identical substances.

ARE PROTEIDS WHICH ARE PREPARED BY THE USUAL METHODS COMBINED WITH FAT OR FATTY ACID?

By E. R. POSNER and WILLIAM J. GIES (reported by W. J. GIES).

(Read by title.)

Chemical analysis of the glucoproteids has resulted in wide variations in the figures for elementary composition, not only for bodies from different sources, but for products of similar origin. Such variation has been attributed to admixture of impurities, particularly of non-nitrogenous character. Nerking's recent experiments with mucins, ovomucoid, and various simple animal and vegetable proteids indicate that possibly the mucin substances, and other proteids as they are commonly prepared, are admixed or combined with fat or fatty acid.

In order thoroughly to test this matter we have analyzed numerous samples of "chemically pure" connective tissue mucoids and albuminoids. Using Dormeyer's method on quantities of proteid from 2 to 13 grams in weight, and following Nerking's procedure, our extractive results were always entirely negative.

We are convinced, therefore, that the mucoids and albuminoids as they are prepared to-day are not "fat-proteid compounds."

ON THE TOXICOLOGY OF SELENIUM AND ITS COMPOUNDS.

By I. O. WOODRUFF and WILLIAM J. GIES (reported by W. J. GIES).

(Read by title.)

The researches of Tunnicliffe and Rosenheim indicate that the numerous cases of "arsenical poisoning" in England recently may have been due in part to selenium. Through the kindness of Prof. Victor Lenher our studies are being made with absolutely chemically pure preparations. Thus far our results on dogs confirm most of the general observations of Rabuteau, and of Czapek and Weil. We are unable, however, to discover Rabuteau's crystals in the blood of the heart after death, or to agree with him that death results from mechanical interference with the circulation.

Selenium is very much more toxic than tellurium, although its poisonous effects are qualitatively much the same. The expired

methyl compound of selenium is produced in much less quantity than that of tellurium under similar conditions. Injection of four milligrams of selenite or selenate per kilo under the skin of dogs usually results in death in a few minutes. Speedy death follows the introduction of like amounts per os or rectum. Four grams of the finely powdered metal, when taken into the stomach, manifested no toxicity whatever, and passed out in the fæces. The introduction of soluble salts is quickly followed by elimination of selenium in the urine and the breath. After subcutaneous injections, the distribution of selenium to the organs is similar to that found by us recently for tellurium. Selenium, although chemically related to sulphur, is very much like arsenic in its toxic properties.

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A STUDY OF THE METABOLISM IN DOGS WITH SHORTENED SMALL INTESTINES.

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CONTENTS.

Introduction.

THE effect of excision of various parts of the gastro-intestinal canal upon metabolism and upon the nutritive value of the food-stuffs has been a subject of considerable study by numerous observers. Such studies possess a double interest. On the one hand the physiologist looks to this method of excision for the light that it may throw on the normal function of the various parts of the intestinal tract, and, on the other hand, the operation is of considerable practical interest to the physician and to the surgeon. For the surgeon it points out the limits, or, more correctly, enlarges the field of his encroachment on the gastro-intestinal canal; and for the physician it serves as a guide to the proper nutrition of patients when parts of the gastro-intestinal canal have been removed from functional activity either by the hand of the surgeon or by the inroad of disease.

The effect of excision of the stomach has been more or less carefully studied in a few cases, which have been collected by Deganello.[1] They include three cases in man and two series of experiments on dogs. In a few of these the nutritive value of the food-stuffs was investigated. The disturbances in absorption were but slight. In general, nitrogen absorption was affected shortly after the operation, but after the lapse of a certain interval of time, four or more months, digestion seemed to proceed almost normally. The ratio of the ethereal to the alkaline sulphates was increased in Deganello's case, but even this disturbance tended to disappear in the course of time. It is interesting to note in connection with Deganello's case that although the ratio above mentioned was increased there was no absolute increase in the amount of ethereal sulphates eliminated in the urine.

The effect of throwing the pylorus out of function has been made the subject of careful work by Rosenberg.[2] In a certain number of dogs upon which the operation of gastro-enterostomy had been performed, he found that more than the normal amount of nitrogen, fat, and carbohydrates escaped absorption. He believes that the explanation of these disturbances lies in the altered relative action of the digestive juices. Under normal conditions the chyme passes into the intestine in small portions. When the controlling action of the pylorus is removed large quantities of acid gastric juice are poured into the intestine at short intervals and the inorganic acid in excess probably alters the action of the intestinal juices.

A considerable part of the large intestine has been excised in man by Treves.[3] The patient recovered rapidly from the effects of the operation. The composition of the fæces was not studied. A similar operation has been performed on dogs by Harley.[4] He subjected his animals to a thorough series of feeding experiments and this makes his work in many ways more complete than any of this nature that has heretofore been done in the same field. As we shall often refer to his observations throughout this paper, an account of his results would here be out of place.

Complete excision of the small intestine has never been a success, so far as we are aware. The difficulties of such an operation are prac-

[1] Deganello: Archives italiennes de biologie, 1900, xxxiii, p. 118.
[2] Rosenberg: Archiv für die gesammte Physiologie, 1898, lxiii, p. 403.
[3] Treves: Lancet, London, 1898, No. 1, p. 276.
[4] Harley: Proceedings of the Royal Society, London, 1899, lxiv, p. 255.

tically insurmountable. However, in dogs a very large part of the small intestine has been removed successfully. Senn,[1] as a result of his work on dogs and cats, believed that the removal of more than one third of the small intestine results fatally sooner or later. Trzebicky[2] believes that the removal of one half of the ileum of dogs is fatal, and that the danger increases the nearer to the stomach the excision is made. Monari[3] states that he has successfully removed from a dog seven eighths of the small intestine. Monari's dog is of especial interest to us because so far as we know it is the only dog upon which metabolism experiments have been performed after excision of the small intestine. At the autopsy on this dog only 28 cm. of small intestine were found. De Filippi,[4] who made the metabolism experiments, found that the animal was capable of nourishing itself almost normally. The only change referable to the operation seemed to be an incomplete absorption of fat (19 per cent appeared in the fæces). Carbohydrates were completely used and no more nitrogen escaped absorption than in a normal dog used for comparison.

Records of extensive resection of the small intestine of man are not numerous. In no case does the relative length of bowel resected approach that of Monari's dog nor that of the dogs that have been the subjects of our experiments. Dreesmann,[5] in 1899, was able to collect twenty-six operations on men in which more than one metre was removed. Of those that survived the operation only four had symptoms clearly referable to the intestine and these symptoms consisted only of a tendency to mild diarrhœa. In one of the four cases three metres and in the others somewhat more than two metres of bowel were resected.[6] The largest resection was 330 cm. It is very interesting to note that this patient showed no subjective symptoms referable to the operation. The ability to absorb food-stuffs was not investigated. To these cases of Dreesmann we are able to add one reported by Schlatter,[7] and one unreported case of Mitchell. Mitchell's patient died as a result of the operation, but the case is

[1] Senn: Experimentaler Beitrag zur Darmchirurgie, cited by Dreesmann; Berliner klinische Wochenschrift, 1899, xxxvi. p. 337.

[2] Trzebicky: Archiv für klinische Chirurgie, 1894, xlviii, p. 54.

[3] Monari: Beiträge zur klinischen Chirurgie, 1896, xvi, p. 479.

[4] De Filippi: Archives italiennes de biologie, 1894, xxi, p. 445.

[5] Dreesmann: Berliner klinische Wochenschrift, 1899, xxxvi, p. 337.

[6] Fantino: Gazetta medica di Torino, 1896, xlvii, p. 181; abstract in Centralblatt für Chirurgie, 1896, xxiii, p. 614.

[7] Schlatter: Correspondenzblatt für schweizerische Aerzte, 1899, xxix, p. 417.

interesting from the fact that a larger proportion of bowel was removed than in any other operation on record. Approximately ten feet of small intestine were removed on account of gangrene from infarction. A fæcal fistula was established by suturing both ends of the small intestine to the abdominal wall. The patient gradually sank without any apparent cause and died on the tenth day. At the autopsy it was found that all of the small intestine had been removed except about six inches of the ileum in the neighborhood of the ileo-cæcal valve and about one foot of intestine adjacent to the stomach.

In only three cases has the metabolism of these patients been studied. An investigation of the metabolism of Schlatter's patient was made by Dr. Plaut one month after the operation. The patient, aged twenty-three, was allowed to eat *ad libitum*. Great quantities of food were consumed, the average for nine days containing 31.8 grams of nitrogen and 109 grams of fat per day. 10.47 per cent of the nitrogen ingested and 13.91 per cent of the fat appeared in the fæces. The nitrogen loss was at the upper limit of normal, the loss of fat considerably above the normal under such circumstances (4 to 6 per cent). Although the patient did not suffer from diarrhœa he never regained his former activity, and eight months after the operation he could walk but slowly and with rests, and could eat only bouillon, soups, and meat. Riva Rocci[1] studied Fantino's patient, from whom 310 cm. of small intestine had been removed. The stools were more frequent than normal and contained a larger percentage of fats. On a diet containing 14.6 grams of nitrogen and 36 grams of fat, 29 per cent of the nitrogen and 23 per cent of the fat appeared in the fæces. As can be seen from our results these losses are the more remarkable because the diet was so restricted. The patient, although sixty years old, prevented loss of weight by taking a very ample diet. Giovanni Sagini[2] investigated the exchange of materials in Ruggi's patient. The motor function of the intestine was normal. In one series of experiments the loss of nitrogen was 5.9 per cent of that ingested, the loss of fat 12.1 per cent. In a second series of experiments the nitrogen loss was 13.2 per cent of the ingesta, while of the fat 15.3 per cent escaped absorption.

[1] Riva Rocci: Gazetta medica di Torino, 1896. xlvii, p. 121.
[2] Ruggi: Il policlinico, sezione chirurgica, 1896, iii, p. 49.

General Remarks on Dogs with Shortened Intestines.

In the early part of 1900 Flint and Rand[1] conducted a series of experiments in the Anatomical Laboratory of the Johns Hopkins University to determine the limits to which intestinal resection could be carried in dogs and to study the anatomical results of such resections. Of the dogs operated upon by them three were alive in the autumn of 1900, and through the kindness of Mr. Flint and Mr. Rand the authors of this paper were allowed to use these dogs in the metabolism experiments which will be described below.

For convenience we shall call these dogs No. 1, No. 2, and No. 3, which numbers correspond to No. 2, No. 12, and No. 11 respectively of Flint and Rand's series. All the measurements of length of intestine were made at the time of operation before the resection was begun. In dog No. 1 two separate operations were performed. The total length of the ileum and jejunum as measured at the first operation was 232 cm. Of this 80 cm. were removed at the first operation and 84 cm. at the second, making a total of 164 cm. Thus, about 70 per cent of the combined jejunum and ileum were removed. However, this percentage may be considered as only approximately correct, for it was found at the second operation that the remaining small intestine had lengthened somewhat after the first operation. This dog was studied by us in December 1900, eight months after the second operation. In dog No. 2, 238 cm. were removed at one operation from a total of 289.6 cm. of combined jejunum and ileum, this being 82 per cent of the movable[2] small intestine. This dog was studied by us in November 1900 about seven months after the operation. In dog No. 3, 298 cm. were removed at one operation from a total of 357.5 cm., being an exsection of 83 per cent of the movable small intestine. Dogs from which larger amounts of intestine were removed died.

Following the operation each of the dogs developed diarrhœa and lost weight. In dog No. 1, and dog No. 2, the nutrition gradually improved, the lost weight was recovered, and at the time of our experiments they appeared to be well nourished. They showed, however, a marked tendency to diarrhœa. This diarrhœa mani-

[1] Flint and Rand: results not yet published.

[2] By movable small intestine is meant that portion which is supplied with mesentery.

fested itself when the dogs were placed on the ordinary diet of scraps of meat from the kitchen which food contained much indigestible matter. When placed on the easily digestible diet used in our experiments the diarrhœa ceased.

Dog No. 3 differed from the two other dogs in that it never regained its former state of nutrition after the operation. It looked lean, and was constantly below its normal weight. It had a voracious appetite and at the same time had an almost constant diarrhœa. The malnutrition appeared to be due simply to a failure to absorb a sufficient quantity of nourishment from its intestines. This dog was the only one of the three that seemed much affected by the extensive resection of small intestine. Unfortunately, we were prevented from making exact feeding experiments upon this dog. We began our work with it and in our inexperience placed it upon a diet which was insufficient to keep up its nutrition. The dog became emaciated rapidly. Finding that its condition was becoming serious we returned it to the former diet of refuse from the kitchen. The dog then developed a most severe diarrhœa and despite a voracious appetite and unquenchable thirst continued to lose rapidly in weight. Four days after the return to this diet the animal died. At autopsy the intestinal wound was found perfectly healed and no cause for death other than malnutrition was discovered. Thus, we were unable to get any exact results from this dog which seemed to be suffering most as a result of the exsection of small intestine.

In conjunction with the work on these dogs with shortened intestines a similar series of experiments was performed upon a normal dog to be used for comparison. This normal dog can best be compared with dog No. 2, for both were of the same breed, of the same size and approximately of the same weight.

Diet and Methods.

General remarks. — In our study of the effects of exsection of the small intestine upon dogs No. 1 and No. 2 and our normal dog we have followed closely the work of Harley.[1] We have often used his results as a standard of comparison with our own. In preparing our diagrams we have also used his figures in the construction of some of the curves, believing that we could thus show more strik-

[1] Harley: *Loc. cit.*

ingly the differences in the results following the removal of small intestine as compared with those following the removal of the large intestine.

Diet. — The diets used in the following determinations consisted of 150 grams of lean beef and 100 grams of dry soda biscuit to which a varying amount of olive oil was added. In order to secure uniformity in the meat moderately large quantities were well ground and mixed, then weighed out into 150 gram lots and placed in flasks. The flasks were sterilized in the autoclave for fifteen minutes at 120° C. and from each lot of meat the contents of two flasks were analyzed separately for nitrogen and ether extract and the results were averaged. The biscuits were weighed into lots of 100 grams, which were then wrapped and used as required. Two packages were analyzed for nitrogen and ether extract. The first diet for each dog consisted of 150 grams of the beef and 100 grams of soda biscuit. For convenience we shall call this diet A. The second, diet B, contained 150 grams of beef, 100 grams of biscuit, and 25 c.c. of olive oil (specific gravity 0.912). Diet C consisted of 150 grams of beef, 100 grams of biscuit, and 100 c.c. of olive oil. No limit was placed on the amount of water taken. Some was given in the food and in addition the dogs were allowed to drink as much as they desired about twice a day. Before beginning the analyses the dogs were kept at least three days on the diet to be used in order to approximate a condition of equilibrium. No difficulty was experienced in obtaining a complete consumption of the food in the case of dog No 2 and of our normal animal. Dog No. 1, however, refused to eat all of its food while on the last diet containing 100 c.c. of olive oil. In order not to lose this series the nitrogen and ether extract was determined in the residue. This being subtracted from the amounts of nitrogen and fat in the total food, the remainder represents the total taken by the animal.

Methods. — During the experiments the dogs were kept in zinc-lined cages. The urine was obtained from dog No. 2 and from our control normal dog by means of catheterization so far as this was possible. This operation is not difficult in the case of sluts but we were disappointed as to its value; for even though we catheterized four times a day the animals would void into the cage at times. The method had the value, however, of sharply separating one day's urine from the next. Dog No. 1 was allowed to void into the cage entirely, for he could not be catheterized nor could he be trained to void when

taken from the cage. It will be noticed that the amount of nitrogen in the urine of this dog is somewhat below that voided by our other dogs. Such a loss is inevitable when special methods of collecting the urine cannot be employed. But as not less than four days were used in computing the average, these averages are probably relatively correct, although the absolute values are only approximate. The fæces were taken from the floor of the cages. In order to determine accurately the fæces belonging to the period of experiment the dogs were given lampblack on the day preceding the period and again on the last day of the period. We then began with the fæces first appearing after the blackened fæces and ended with the last of the second lot of blackened fæces. In this way the total amount of fæces obtained was correctly marked off, although the amount obtained from day to day varied considerably.

The total nitrogen was determined by the Kjeldahl method as modified by Argutinsky.[1]

The amount of water in the fæces was determined by drying at 100° to 110° C. to constant weight.

The ether extract was obtained by the method described by E. Voit.[2] It consists of a twenty-four hour extraction of the acidified and dried material in a Soxhlet apparatus with ethyl ether, evaporation of the ether, re-extraction with petroleum ether, and weighing. As is done in most metabolism experiments we shall call this ethereal extract, fat.

The alkaline and ethereal sulphates were determined by the gravimetric method.[3]

The determination of carbohydrates in the fæces was not systematically carried out during the course of the experiments because we found that they were entirely absent in dog No. 2 and in our normal dog when tested for by the method described by Hoppe-Seyler.[4] During the final series of experiments on dog No. 1 Pavy's method of testing for carbohydrates[5] was applied to the fæces after the fat had been extracted. About 10 grams of fæces were used. A very well

[1] Argutinsky: Archiv für die gesammte Physiologie, 1890, xlvi, p. 581.

[2] E. Voit: Zeitschrift für Biologie, 1897, xxxv, p. 555.

[3] Salkowski: Virchow's Archiv für pathologische Anatomie, 1880, lxxix, p. 551.

[4] Hoppe-Seyler: Handbuch der physiologisch- und pathologisch-chemischen Analyse, Berlin, 1883, 5te Auflage, p. 504.

[5] Pavy: Physiology of the carbohydrates, London, 1894, p. 61.

marked reduction of Fehling's solution resulted after boiling with sulphuric acid, although when tested for by the method of Hoppe-Seyler carbohydrates were apparently completely absent. The reducing substances thus indicated must have been formed during the treatment of the original substance with the reagents employed in Pavy's method. They may have been derived either from the normal mucin of the fæces,[1] from the proteids or from the cellulose and allied substances (hemi-cellulose and dextrane). A small amount of the proteids [2] and of the cellulose may have come from the food-stuffs directly. But a larger amount may have come from the large number of bacteria present in the fæces.[3] It is quite impossible in our present state of knowledge to decide these questions. This same uncertainty is reflected in the literature. While it is generally agreed that no starch granules can be found in the fæces of an animal placed on an easily absorbable diet,[4] statements differ as to the chemical findings. For instance Harley [5] found that when his dogs were on a mixed, easily absorbable diet the fæces contained no carbohydrates even after resection of the large bowel. He does not state his method. On the other hand, Tsuboi [6] experimenting on dogs with practically the same amount of carbohydrates found 0.57 gram per day in the fæces. Tsuboi's method of determining the carbohydrates consisted in treating the fæces with dilute acid and then applying Allihn's method. It is interesting to note in this connection that in the fæces of starving animals Tsuboi found no substances capable of reducing copper sulphate.

The Urine of Dogs with Shortened Small Intestine.

Amount.— In the interpretation of the following figures only the most obvious results will be noticed. Such a series of experiments contains so many sources of error that finer deductions from the figures hardly seem to be justified.

The urine of dogs deprived of a large part of the small intestine

[1] Von Jaksch: Klinische Diagnostik, 1896, 5te Auflage, p. 277; Pfeiffer: Journ. Landw. 1885, xxxiii, p. 535, cited by Atwater and Langworthy: Bulletin 45, United States Department of Agriculture, 1898, p. 381.

[2] Kermauner: Zeitschrift für Biologie, 1897, xxxv, p. 316.

[3] Sahli: Lehrbuch der klinischen Untersuchungsmethoden, 1899, p. 463.

[4] Moeller: Zeitschrift für Biologie, 1897, xxxv, p. 291.

[5] Harley: *Loc. cit.*

[6] Tsuboi: Zeitschrift für Biologie, 1897, xxxv, p. 68.

differs but little from that of the normal animal. Harley,[1] Pugliese[2] and others have shown that the amount of urine in normal dogs tends to diminish as fat is added to the diet, and that with this diminution in amount there is a corresponding rise in specific gravity. This, however, is not an invariable result of the addition of fat to the diet.[3] Harley further showed that the same holds true for dogs after removal of the entire large intestine. We have found it true also for dogs after extensive resection of small intestine (Table I).

TABLE I.

Showing the excretion of water by way of the urine and fæces.

Diet.	Normal Dog.			Dog No. I.			Dog No. II.		
	Urine.		Water in fæces. Gms.	Urine.		Water in fæces. Gms.	Urine.		Water in fæces. Gms.
	Amt. c.c.	Sp. gr.		Amt. c.c.	Sp. gr.		Amt. c.c.	Sp. gr.	
A. (10–13 gms. fat)	268	1.040	24.3	183	1.038	15.0	268	1.031	33.0
B. (33–36 gms. fat)	175	1.040	41.6	151	1.039	25.0	241	1.043	36.6
C. (83–104 gms. fat)	173	1.046	42.3	99	1.042	36.2	130	1.048	93.7

As the dogs were allowed to drink freely the coincident increase in the total amount of water in the fæces could hardly be said to be the cause of the diminution in the urine. As may be seen by the accompanying table the increased amount of water in the fæces could not account for the much larger diminution in the quantity of urine. It must be attributed to some effect of the oil in altering the metabolism of the body, an effect probably associated with a diminution in the amount of nitrogen excreted.

Nitrogen.—The amount of nitrogen excreted by way of the urine in dogs deprived of small intestine shows no great deviation from that excreted by normal dogs. Harley showed that as fat was added to the diet the amount of nitrogen in the urine diminished both in normal dogs and in those deprived of the large intestine. And the same fact has been demonstrated in normal sheep by Wicke and Weiske.[4] We

[1] Harley: *Loc. cit.* [2] Pugliese: Archiv für Physiologie, 1897, p. 473.

[3] Laas: Zeitschrift für physiologische Chemie, 1895, xx, p. 233.

[4] Wicke and Weiske: Zeitschrift für physiologische Chemie, 1895. xxi, p 42; 1896. xxii, p. 137.

have found it true as well in dogs deprived of large amounts of the small intestine.

In normal dogs this fall in the amount of nitrogen in the urine is not usually accompanied by an increase in the amount of nitrogen in the fæces. Harley's normal dogs, however, show such an increase. In this observation his results do not agree with those of Wicke and Weiske, Rubner,[1] nor Laas,[2] a point which seems to have escaped

TABLE II.

Showing in grams the excretion of nitrogen by way of the urine and fæces with the nitrogen balance.

	Urine.	Fæces.	Total.	Food.	Balance.
	Normal Dog.				
Diet A (10–13 grams fat)	5.256	0.817	6.073	6.511	+0.438
Diet B (33–36 grams fat)	4.669	0.769	5.438	6.332	+0.894
Diet C (83–104 grams fat)	4.688	0.817	5.505	6.332	+0.827
	Dog No. I.				
Diet A (10–13 grams fat)	4.408	0.624	5.032	6.332	+1.300?
Diet B (33–36 grams fat)	3.513	0.894	4.407	6.332	+1.925?
Diet C (83–104 grams fat)	2.522	1.203	3.725	4.929	+1.204?
	Dog No. II.				
Diet A (10–13 grams fat)	5.096	0.913	6.009	6.511	+0.502
Diet B (33–36 grams fat)	4.937	1.085	6.022	6.511	+0.489
Diet C (83–104 grams fat)	4.653	1.596	6.258	6.511	+0.253

Harley's attention in mentioning the work of the first named authors. Nor do they agree with the results obtained from our normal dog (Table No. II). There is therefore a diminution in the total excretion of nitrogen by way of the urine and fæces when fat is added to the diet of normal dogs. This is an example of the nitrogen-sparing power of fats.[3] After exsection of the large intestine Harley found

[1] Rubner: Zeitschrift für Biologie, 1879, xv, p. 115. [2] Laas: *Loc. cit.*

[3] Wicke and Weiske: *Loc. cit.*; Laas: *Loc. cit.*

that this nitrogen-sparing action of fat was still present. Our results for dogs deprived of much small intestine would seem to indicate that in them the so-called nitrogen-sparing action of fats is absent. Dog No. 1 does not show this change as well as dog No. 2. Yet we place less reliance on the figures for dog No. 1 owing to the fact that this dog was allowed to void into the cage and to the fact that it was on a diet containing constantly diminishing quantities of nitrogen. Dog No. 2, however, instead of showing a diminution in the combined excretion of nitrogen by the fæces and urine as fat was added to the diet shows an actual increase in this excretion. A probable explanation of this fact is given below. The nitrogen of the urine does diminish, but this may be due to the diminished absorption from the intestines. There is no laying on of nitrogen as occurs in normal animals (Table II).

Sulphates.— It is most interesting to study the sulphates of the urine after extensive intestine resections owing to the light that this throws upon intestinal putrefactive processes. Intestinal putrefaction may be estimated, first, by the absolute amount of ethereal sulphates in the urine [1] and second, by the ratio of the ethereal to the alkaline sulphates in the urine. The weight of opinion inclines to the first method as the more valuable, although on the same diet the latter is perhaps equally good.[2]

The average daily amount of ethereal sulphates in the urine of our normal dog was 0.048 gram. The average ratio was 1 : 7.5. In Harley's normal dogs the average amount of ethereal sulphates on a diet poor in nitrogen was in one animal 0.062 gram per day; in the other, on a rich nitrogenous diet 0.075 gram, the ratios being 1 : 8.5 and 1 : 6.7 respectively. After total removal of the large intestine, Harley found a marked diminution in the amount of ethereal sulphates, the averages being 0.031 gram in one case and 0.042 gram in the other. The ratios in these cases varied unaccountably, the averages being respectively 1 : 18 and 1 : 6.8. However, the reduction in ethereal sulphates may be taken as indicating a diminution in intestinal putrefaction after removal of the large intestine.

After extensive resection of small intestine, both of our dogs showed a constant high ratio of conjugated sulphates to the alkaline

[1] Salkowski: Zeitschrift für physiologische Chemie, 1888, xii, p. 222; Müller: Zeitschrift für klinische Medicin, 1887, xiii, p. 62.

[2] Kunkel: Archiv für die gesammte Physiologie, 1877, xiv, p. 344; Harnack and Kleine: Zeitschrift für Biologie, 1899. xxxvii, p. 417.

sulphates and at the same time when the diarrhœa caused by the large amount of fat in the diet did not cause a reduction of the sulphates[1] the total quantity of ethereal sulphates was increased. Thus, in dog No. 1 with 70 per cent of the small intestine removed, the average ratio of ten determinations was 1 : 4.9. During the same period the average of the ethereal sulphates excreted was 0.056 gram. During the last series of experiments (diet C) this dog had slight

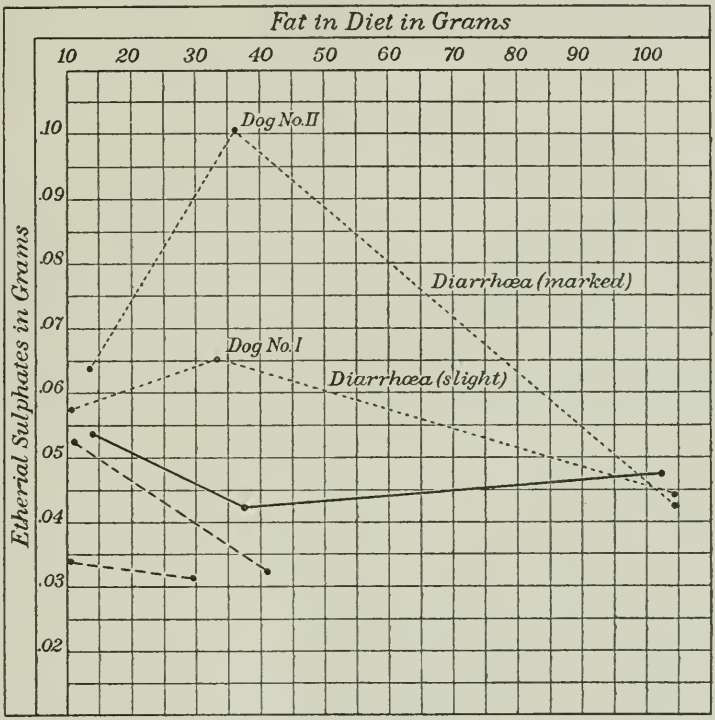

FIGURE 1. — Showing the amount of ethereal sulphates in the urine of : —

1. ——— A normal dog.
2. – – – – Dogs after exsection of large intestine (Harley).
3. ------ Dogs after resection of small intestine.

diarrhœa, at least two stools per day and the nitrogen in its food was considerably diminished. If we omit from our calculations this last series of experiments, the ratio of the sulphates becomes 1 : 5 and the average of the ethereal sulphates excreted per day 0.062 gram, a figure considerably above that for our normal dog, although

[1] SALKOWSKI : *Loc. cit.*

not so high as for one of Harley's dogs. In dog No. 2 with 83 per cent of the movable intestine exsected the average of the ratios of the ethereal sulphates to the alkaline sulphates was 1 : 5.5. The average amount of ethereal sulphates eliminated during this period was 0.0698 gram. In the case of this dog the diarrhœa which resulted from the large amount of fat was marked. If we discard this period the ratio of the ethereal to the alkaline sulphates becomes 1 : 5, while the average of ethereal sulphates increases to 0.0831 gram per day, a very high figure.

TABLE III.

Showing in grams the excretion of total and ethereal sulphates and the ratio of ethereal to the alkaline sulphates.

Diet. Grams.	Normal Dog.			Dog No. I.			Dog No. II.		
	Total sulphates.	Ethereal sulphates.	Ratio of ethereal to alkaline.	Total sulphates.	Ethereal sulphates.	Ratio of ethereal to alkaline.	Total sulphates.	Ethereal sulphates.	Ratio of ethereal to alkaline.
A (10–13 fat)	0.4484	0.0535	1 : 7.4	0.3697	0.0577	1 : 5.7	0.3970	0.0629	1 : 5.4
B (33–36 fat)	0.3824	0.0426	1 : 7.9	0.3275	0.0665	1 : 4.3	0.5686	0.1034	1 : 4.6
C (83–104 fat)	0.3825	0.0472	1 : 7.4	0.2614	0.0440	1 : 4.8	0.3249	0.0432	1 : 6.5
Average	0.4044	0.0477	1 : 7.6	0.3195	0.0557	1 : 4.9	0.4302	0.0698	1 : 5.2

There is, therefore, an increase in intestinal putrefactive processes after the removal of a large part of the small intestine. This may be explained on the supposition that more food material reaches the large intestine and that it resides longer in the large intestine than in the normal dog. Consequently, the putrefactive changes there are greater in amount than is normal.

In normal animals it has been noted by Laas (*loc. cit.*) that the addition of fat to the diet is practically without constant result; that the addition of fat to the diet does not increase or diminish the amount of intestinal putrefaction. But the addition of fat to the diet of dogs with resected large intestine showed a decided tendency to diminish putrefaction. In our observations the diarrhœa caused by adding large quantities of fat to the diet obscures this point. If we consider only the results obtained when our animals were on diets A and B containing relatively small amounts of fat, a tendency of fat

to increase the total amount of ethereal sulphates eliminated becomes evident. (Fig. 1.) It is not unreasonable to suppose that this is brought about by a tendency of the fat either to hurry the food through the shortened small intestine which is already on the border of functional insufficiency or to retard the absorptive processes in the small intestine. The effect would be to permit bacteria of the large intestine to act on the larger amount of unabsorbed food material. This fact probably explains in part the effect of an increase of fat in the diet of dogs with shortened intestine in increasing rather than in diminishing the total elimination of nitrogen. (See Table III.)

The Fæces.

The fæces in our dogs after large intestinal resections varied greatly in character. When on a mixed diet largely made up of kitchen refuse dog No. 3 had an almost constant severe diarrhœa, with yellowish fluid stools. The other dogs also suffered to a lesser extent from diarrhœa on this diet. When, however, dog No. 3 was placed on an easily absorbable and, as was afterwards seen, insufficient diet, the diarrhœa was quickly checked so that the dog had only four stools in nine days and these were very hard. Likewise, when dogs No. 1 and No. 2 were placed on an easily absorbable diet the diarrhœa was quickly checked and the dogs showed some tendency to constipation. When a larger amount of oil was added to the food the fæces became softer and the stools more frequent. Dog No. 1 had a single, somewhat firm, well formed stool each day when on diets A and B. Diet C made the stools very soft and two or three per day were not at all unusual. Dog No. 2 had profuse fluid stools after being given a large amount of oil. The fluidity of these stools, as the analyses show, was not due to the high percentage of water present but rather to the large percentage of oil, which amounted to one fourth of the weight of the dry fæces. The fæces possessed a characteristic glistening silky appearance described as due to the presence of fat. The fæces in the control dog changed but little in character as fat was added to the diet. They became somewhat more copious and also somewhat softer but presented none of the marked changes seen in the fæces of the animals that had been operated upon.

Microscopical examination. — Microscopically, there seems to be very little qualitative difference between the stools of normal dogs

and those of dogs with shortened intestines. In both when on the same diet and on different diets muscle fibres in various stages of disintegration, fat globules and fatty acid crystals occur. We examined simultaneously the fæces of our normal dog when on diet A, and the fæces of dog No. 2 when on diet B. Both contained the same elements as mentioned above and it was difficult to decide that these elements were more abundant in the one than in the other.

Reaction. — The reaction of the fæces to litmus was not constant in dogs with large resections of small intestines. It was more frequently acid than alkaline, but the acidity or alkalinity was never marked. The reaction of the fæces seemed to be entirely without significance.

Amount of water.—The percentage of water present in the stools varied considerably at different times in the experiment. The averages of our dogs with intestines removed did not differ materially from the averages of normal dogs. It may be noticed in the following table (Table IV), that the percentage of water falls somewhat as the fat in the diet is increased, although owing to the increased weight of the fæces the absolute amount of water present is increased. (See Table I.)

TABLE IV.

Showing the percentage of water in the fæces of a normal dog and of dogs with shortened small intestines.

Diet.	Normal Dog.	Dog No. I.	Dog No. II.
	Per cent.		
A (10–13 grams fat)	64	64	75
B (33–36 grams fat)	80	64	69
C (83–104 grams fat)	71	61	70
Average	72	63	71

The greater variations in the normal dogs are due possibly to the few days taken to make up some of the averages. The average of figures for Harley's control animals is 65.8 per cent. It is interesting to compare with these results the average percentage of water in the fæces of dogs whose large intestines have been removed. Harley's figures are uniformly high, being 77.9 per cent for one dog

and 81.3 per cent for the other, a marked increase in the amount of water in the stools. The accompanying diagram (Fig. 2) shows the curves for the percentages of water in the fæces on varying diets. On account of its irregularity, the curve from our control animal is omitted and the curves for Harley's normal dogs have been used instead. These figures show the importance of the large intestine in the absorption of water in the fæces. When the large intestine

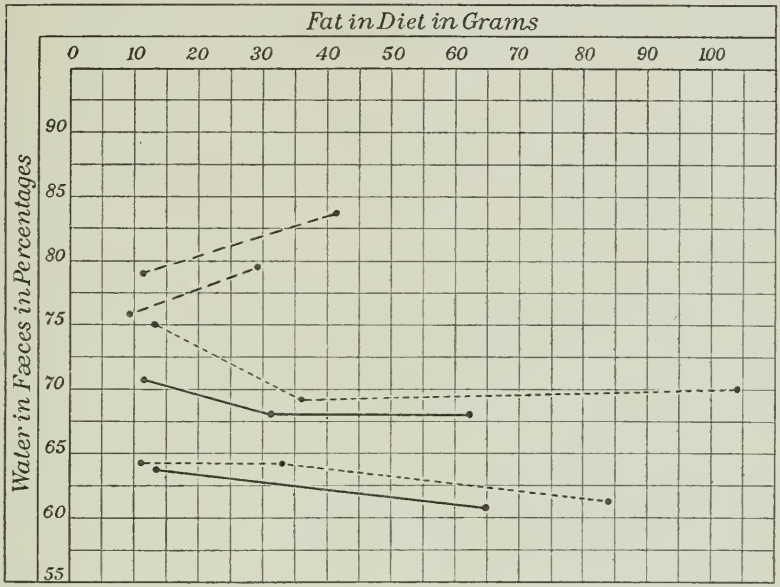

FIGURE 2. — Showing the percentages of water in the fæces: —
——— of normal dogs (Harley).
– – – – of dogs after removal of large intestine (Harley).
------ of dogs after extensive resection of small intestine (upper is Dog No. II; lower is Dog No. I).

is removed the water in the fæces is increased, but when present, the percentage of the water in the fæces varies between 60 per cent and 75 per cent, even though a large amount of the small intestine has been removed. The removal of the large intestine, therefore, allows more water to pass into the fæces than the removal of a much longer piece of small intestine.

Absorption of fat. — It is generally held that most of the fat in the diet is absorbed in the small intestine.[1] The recent work of Ham-

[1] HARLEY: *Loc. cit.*: CZERNY: Virchow's Archiv für pathologische Anatomie, 1874, lix, p. 161; DEUCHER: Deutsches Archiv für klinische Medicin, 1896, lviii, p. 210.

burger,[1] however, has shown that the large intestine is capable of absorbing fat with a degree of rapidity and completeness not exceeded in the small intestine. Of especial interest, therefore, is the question of the behavior of animals with a large amount of small intestine removed toward increasing quantities of fat in their diet. De Filippi[2] states that there was some increase in the fat of the fæces of his dog after removal of seven eighths of the small intestine. Plaut found in Schlatter's patient[3] (192 cm. of small intestine removed) on a diet containing on an average 31.8 grams of nitrogen and 100.9 grams of fat, 13.91 per cent of the fat ingested in the fæces. In a normal man only about 4–6 per cent should appear in the fæces on this diet. Riva Rocci working on Fantino's patient[4] (310 cm. removed) with a diet containing 14.6 grams of nitrogen and 36 grams of fat found that 23 per cent of the fat appeared in the fæces. Ruggi's patient failed to absorb from 12 to 15 per cent of the fat ingested.

Before considering the amount of fat in the fæces of dogs from which a large percentage of the small intestine has been resected it seems best to consider the effect which increasing the amount of fat in the diet has upon the fat in the stools of a normal animal. Harley[5] and Laas[6] have shown in dogs and Kayser[7] in men that when the fat in the diet is increased the absolute amount of fat in the stools may increase slightly, but that there is a greater relative absorption of fat by the intestines, or, in other words, that a smaller percentage of the fat taken appears in the fæces. A curve for one of Harley's dogs is represented in Fig. 3, which shows the downward direction of the curve for the percentage of unabsorbed fat when the amount of fat in the diet is increased. The figures for our normal dog correspond closely to Harley's figures. Upon the addition of a moderate amount of fat to the diet, there was a marked drop in the percentage of unabsorbed fat. When, however, there was a very large amount of fat in the diet the percentage of unabsorbed fat rose very slightly above the percentage obtained upon a moderate addition of fat to the diet. Thus, on diet A (13.4 grams fat) 8.9 per cent of the fat taken was unabsorbed. On diet B (33.8 grams fat) 3.4 per cent was unab-

[1] Hamburger: Archiv für Physiologie, 1900, p. 433.
[2] De Filippi: *Loc. cit.*
[3] Schlatter: *Loc. cit.*
[4] Fantino: *Loc. cit.*
[5] Harley: *Loc. cit.*
[6] Laas: *Loc. cit.*
[7] Kayser: Archiv für Physiologie, 1893, p. 371.

sorbed and on diet C (102.3 grams of fat) 4.4 per cent was unabsorbed. The curve obtained from these figures is shown in Fig. 3. Where the large intestine has been removed the fat absorption does not differ materially from that in the normal dog. The direction of the curve is still downward and the percentages absorbed are approximately the same.

This apparent diminution of fat escaping absorption is probably the result of the fact that a certain amount of the ether extract in the fæces is derived from an excretion into the intestinal canal.[1] Thus the ether extract of the fæces of starvation may amount to 0.67 gram per day[2] and Harley[3] has shown that after extirpation of the pancreas more fat is found in the small intestine than was ingested. It is impossible to say how much of the fat in the fæces of an animal on a mixed diet is excretory and how much has been derived from unabsorbed fat. The amount of fat in starvation fæces is probably relatively greater than that in normal fæces, just as the nitrogen of starvation fæces is relatively greater.[4] The same argument that we shall offer to explain the event of an increase of nitrogen in the fæces of animals with shortened small intestine applies to the fat in the fæces. Should the amount of fat in the fæces of such an animal be in excess of the normal amount, there is every reason for believing that such an excess represents fat that has escaped absorption.

After removal of the small intestine, our dogs showed an inability to absorb fat normally if a large quantity of it was present in the diet. Dog No. 1 with 70 per cent of the movable small intestine removed gave a curve of fat non-absorption which nearly approached the curve in the normal dog (Fig. 3). With a very small amount of fat in the diet the amount of fat in the fæces was approximately equal to that in the normal dog, about 8.5 per cent (Table V). When a moderate amount of fat was added to the diet there was a drop in the percentage of fat eliminated in the fæces similar to the drop which occurs in normal dogs. The fall, however, was not so pronounced, the percentage eliminated being 5.6 per cent as compared with 3.4 per cent for our normal animal. When the fat in the diet was still further increased, the percentage eliminated in the fæces rose rapidly

[1] Fr. Voit: Zeitschrift für Biologie, 1892, xxix, p. 325; Prausnitz: Zeitschrift für Biologie, 1897, xxxv. p. 335.

[2] Tsuboi: *Loc. cit.*

[3] Harley: Journal of physiology, 1895, xviii. p. 1.

[4] C. Voit: Hermann's Lehrbuch der Physiologie, 1881.

and with an average of 83.4 grams of fat in the diet 15.9 per cent passed through the intestine unabsorbed, while our control animal on a larger fat diet absorbed all except 4.4 per cent of the fat ingested. Dog No. 2 with 82 per cent of the movable small intestine removed showed an inability to absorb fat normally even when very small amounts were present in the diet. On a diet containing 13.4 grams

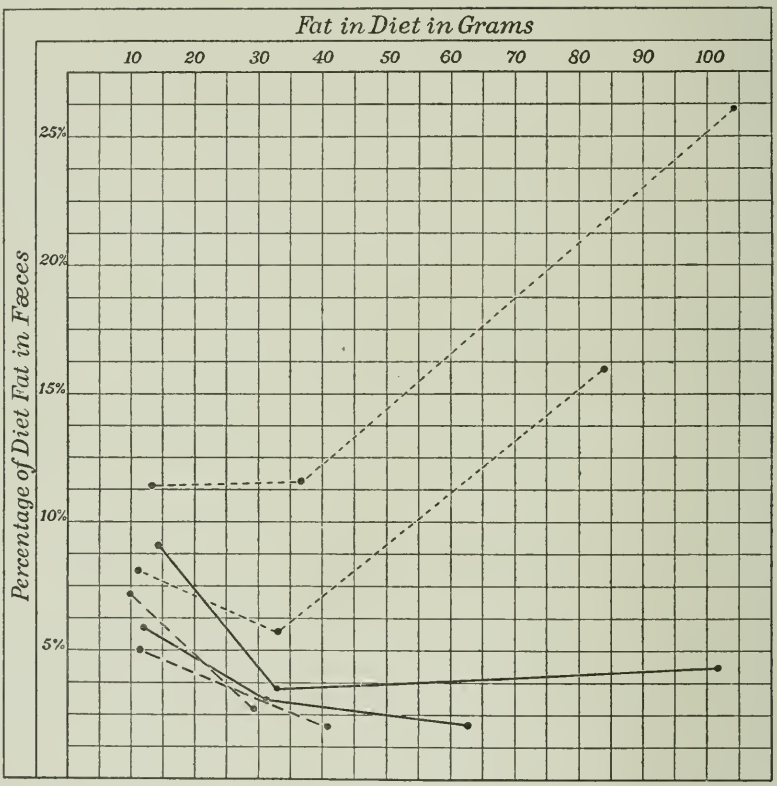

FIGURE 3. — Showing the percentages of the fat in the diet which appears in the fæces of: —

1. ——— Normal dogs (upper, ours; lower, Harley's).
2. – – – – Dogs after removal of large intestine.
3. ------ Dogs after extensive resection of small intestine.

of fat 11.3 per cent was unabsorbed in dog No. 2 as compared with 8.9 per cent in the control animal. With a moderate amount of fat in the diet this inability to absorb is shown more clearly. Instead of the percentage of unabsorbed fat sinking as it does in a normal animal (3.4 per cent) there is a slight rise to 11.5 per cent (Fig. 3). When the fat was increased still further to 104.6 grams the amount

which was not absorbed by dog No. 2 made up 26 per cent of the amount ingested, whereas in the normal animal, only 4.4 per cent passed through unabsorbed. We may say, therefore, that in a normal dog practically all the fat in the diet is absorbed, even though very large amounts be ingested. After resection of from 70 to 83 per cent of the movable small intestine, however, a dog absorbs fat properly only when the fat is present in very small amounts in the diet. When large amounts of fat are present, from one sixth to one fourth of the amount ingested passes through unabsorbed (Table V).

TABLE V.

Showing the amount of fat in fæces and the percentage of fat not absorbed.

Diet.	Normal Dog.		Dog No. 1.		Dog No. 2.	
	Amount. Grams.	Not absorb'd. Per cent.	Amount. Grams.	Not absorb'd. Per cent.	Amount. Grams.	Not absorb'd. Per cent.
A (10–13 grams fat)	1.193	8.9	0.915	8.4	1.515	11.3
B (33–36 grams fat)	1.137	3.4	1.907	5.6	4.176	11.5
C (83–104 grams fat)	4.539	4.4	13.342	15.9	27.207	26.0

To recapitulate, dogs after removal of the large intestine absorb just as much fat as normal dogs. Increasing the fat in the diet of normal dogs and of dogs deprived of large intestine diminishes the percentage of fat which appears in the fæces. Small amounts of fat are absorbed by dogs with shortened small intestine in nearly normal percentages. When large amounts of fat are added to the food, abnormally large percentages of fat escape absorption, even though the fæces be evacuated but once per day. When we consider that the length of the small intestine removed was 164 to 298 cm., while the length of the large intestine was only about 60 cm.,[1] we are not justified in saying from our figures that a given length of small intestine absorbs fat more completely than the same length of large intestine. On the other hand, looking at the small and large intestines as wholes, we can say that the small intestine is necessary for proper fat absorption, whereas, the large intestine is not necessary for fat absorption, although under exceptional circumstances such as in our dogs it doubtless assists in the absorption of fat.

[1] Ellenberger and Baum: Anatomie des Hundes, 1891.

Absorption of proteids. — The percentage of the nitrogenous material of the diet which appears in the fæces of normal dogs may be taken as from 7 to 13. We shall speak of the nitrogenous contents of the fæces as consisting of the unabsorbed nitrogen of the food and in the percentages shall calculate it as such. As is well known [1] a large amount if not all,[2] of the nitrogen in the fæces resulting from a mixed easily absorbable diet consists of material excreted into the intestinal tract. When, however, we find the nitrogenous contents of the fæces increased as a result of removal of intestine such an increase, in the absence of an inflammation of the mucous membrane, comes in large part, we believe, from the unabsorbed nitrogen of the food. We must, however, take into consideration in this connection that the bacteria have an indirect influence on the utilization of food in that the material that the bacteria incorporate into themselves is lost to absorption, the bacteria being, so to speak, filtered off by the intestinal mucosa.[3] Therefore with the thrift of the micro-organisms there ought to be a corresponding increase in the amount of nitrogen in the fæces. If the constant corresponding to the nitrogen excreted could be eliminated, the amount of nitrogen lost by our operated dogs as compared with that lost by our normal dog would be relatively greater than that ratio as we have calculated it. To take an example, if on a certain diet a normal dog eliminated 0.8 gram of nitrogen in the fæces as compared with 1.6 grams in another dog with a large amount of small intestine removed, the latter would lose twice as much as the former. If, however, 0.4 gram (a low estimate) in each case had been excreted by the intestines the amounts of diet nitrogen lost would be 0.4 gram and 1.2 grams respectively, that is, in the dog after operation the loss in diet nitrogen is three times that lost by the normal dog. As we have no means of computing what proportion of the nitrogen is excretory we shall disregard this factor altogether. At the same time it is necessary to recognize that our figures err on the side of making the nitrogen losses in operated dogs relatively small when compared with those in normal dogs.[4] As we have already said, precisely this same reasoning holds good in regard to fat losses.

[1] Tsuboi: *Loc. cit.*

[2] Prausnitz: Zeitschrift für Biologie, 1897, xxxv, p. 335.

[3] Prausnitz: *Loc. cit.*

[4] In this connection it is of interest to examine the tables obtained from our dog No. 3 from which 83 per cent of the small intestine had been removed. This

As we have said, the percentage of the nitrogenous material in the diet which appears in the fæces of normal dogs may be taken as from 7 to 13 per cent. As the fat in the diet is increased, the nitrogen of the diet remaining constant, the percentage of nitrogenous material absorbed remains approximately constant (Fig. 4).

When the large intestine has been removed, the percentage of nitrogenous material eliminated in the fæces by Harley's dogs is high, 15.5 to 16.5 per cent on a diet containing but little fat. As the fat was increased Harley's two dogs varied somewhat. In one there

dog was on a small, in fact, starvation diet consisting of 100 grams of lean beef and 50 grams of corn-meal and containing 4.45 grams of nitrogen and 5.24 grams of ether extract.

The average daily elimination of nitrogen in the fæces was 0.494 gram, which amount possibly approximates the amount of nitrogen excreted from the intestines of our operated dogs. The rise in the daily excretion of nitrogen by way of the urine which was very marked toward the end is to be interpreted as a premortal increase (SCHULTZ: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 381). It is, however, more marked and extends over a longer period than is usual in such a rise. The dog died four days after returning to a sufficient diet. The urine was not examined for sugar.

DOG No. III.

Eighty-three per cent of movable small intestine removed. Diet insufficient to preserve equilibrium.

Day.	Weight.	Diet.		Excretion of N.	
	Kgms.	N. Grams.	Fat. Grams.	Fæces. Grams.	Urine. Grams.
9	11.22	4.45	5.24	none	6.652
10	....	4.45	5.24	1.745	6.896
11	....	4.45	5.24	0.944	7.769
12	....	4.45	5.24	none	lost
13	....	4.45	5.24	none	lost
14	....	4.45	5.24	none	8.868
15	....	4.45	5.24	none	9.160
16	....	4.45	5.24	1.089	10.050
17	9.76	4.45	5.24	0.664	13.712
Averages . .		4.45	5.24	0.494	——

was a marked drop in the percentage eliminated in the fæces, while in the other the percentage remained persistently high (Fig. 4).

The amount of nitrogen in the fæces after excision of a large part of the small intestine in man has been studied by Plaut,[1] by Riva Rocci,[2] and by Giovanni Sagini.[3] De Filippi working on dogs found no change in the amount of nitrogen excreted. Plaut in Schlatter's case found that the fæces contained 10.47 per cent of the nitrogen ingested. Riva Rocci found that the fæces of Fantini's patient contained 29 per cent of the nitrogen ingested. Ruggi's patient showed in two series of experiments 5.9 per cent and 12.1 per cent of the nitrogen ingested in his fæces. In our dogs after resection of a large

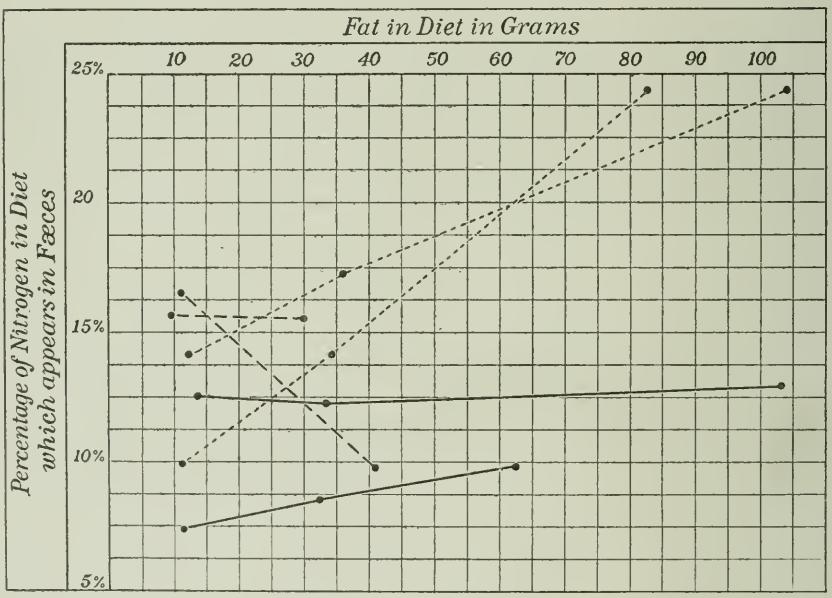

FIGURE 4. — Showing the percentages of the nitrogen in the diet which appear in the fæces: —

——— of normal dogs (upper, our dog; lower, Harley's dog).
– – – – of dogs after exsection of large intestine (Harley).
------ of dogs with shortened small intestine.

part of the small intestine there was not a great change in the percentage of nitrogen eliminated in the fæces provided the dog was on a diet low in fat. In dog No. 1 with 70 per cent of the movable small intestine removed 9.9 per cent of the nitrogenous material

[1] PLAUT: *Loc. cit.* [2] RIVA ROCCI: *Loc. cit.* [3] RUGGI: *Loc. cit.*

ingested appeared in the fæces. In dog No. 2 with 82 per cent of movable small intestine removed 14 per cent of the diet nitrogen appeared in the fæces, which is above the percentage in the normal animal and yet not so high as in dogs whose large intestines have been removed. As fat is added to the diet the dog with a shortened small intestine shows its inability to absorb food properly, for not only the fat itself, but the nitrogenous material as well, passes through the alimentary tract in larger amounts than in normal animals. Thus, on a diet containing a moderate amount of fat the percentage of nitrogen eliminated by our normal dog was 12 per cent, while of our other dogs, dog No. 1 eliminated 14 per cent and dog No. 2 17 per cent. On a diet containing a very large amount of fat 12.9 per cent of the nitrogen ingested was eliminated by our normal dog, 24.5 per cent by dog No. 1 and 24.4 per cent by dog No. 2. The curves obtained from the above figures show well the effects of excision of a large portion of the small intestine upon the absorption of nitrogenous material. (Fig. 4.) When there is a small amount of fat in the diet the amount of nitrogen in the fæces is about equal for normal dogs and for dogs with small intestines removed, but it is high for dogs with large intestines removed. As fat is added to the diet the amount of nitrogen in the fæces remains about the same or even grows less in the case of normal dogs and of dogs with large intestines removed. In dogs with the small intestines removed, however, there is an increase in the amount of nitrogen in the fæces proportionate to the increase of fat in the diet. With large amounts of fat in the diet one fourth of the nitrogen ingested passes out unabsorbed, a loss twice as great as in the normal animal.

Summary.

A dog with a large amount of small intestine removed behaves very much like a normal dog so long as it is on an easily absorbable diet which contains only a small amount of fat.

The elimination of fat and nitrogenous material in the fæces may not exceed the elimination in the normal dog (dog No. 1) or it may do so to a slight extent (dog No. 2). When, however, the fat in the diet is increased the insufficiency of the shortened intestine as an organ for absorption becomes very apparent. The amount of fat in the fæces may be 25 per cent of that ingested, while the nitrogen in the fæces may also amount to 25 per cent of the diet nitrogen. The

loss of fat is in itself of slight importance, for a large amount of fat is still absorbed and as a matter of fact our dogs increased in weight on a diet rich in fat. The diminution in fat absorption and especially the loss of nitrogenous material expresses an interference in the power for absorption in such an animal. This lack may manifest itself under other conditions of diet. For example, dog No. 2 after its feeding experiments were over was placed on an unlimited diet of kitchen refuse. It developed a severe diarrhœa, became emaciated and only recovered when great care was taken with its further diet. The whole appearance of dog No. 3 was that of an animal suffering from malnutrition in consequence of its inability to absorb nourishment properly. When living on a diet of kitchen refuse it ate ravenously, had profuse diarrhœa and remained emaciated. It seems to us that the inert non-digestible material in such a diet might easily increase peristalsis so as to carry through a large proportion of the unabsorbable food into the large intestine. This may be quickly evacuated with a consequent loss of absorbable material to the animal or it may reside for a longer time in the large intestine exposed to the wasteful action of bacteria. Such an animal might starve to death, owing to its inability to cope with the indigestible material.

Conclusions.

(1) Dogs from which from 70 to 83 per cent of the combined jejunum and ileum have been removed may live indefinitely after recovery from the operation. Their nutrition may appear to be perfectly normal, or it may be so poor that even when eating ravenously they do not seem to be able to keep well nourished.

(2) Such dogs are peculiarly liable to be affected with diarrhœa which may be caused by a diet too rich in fat or one containing too much inert non-digestible material. This diarrhœa is of very serious moment to such a dog and may cause its death.

(3) The urine of such dogs shows no great variation in quantity, specific gravity or nitrogenous contents from that of normal animals.

(4) The conjugated sulphates in the urine are increased absolutely and relatively to the alkaline sulphates indicating an excess of intestinal putrefaction.

(5) The quantity of fæces varied in our two animals. In the dog from which 70 per cent of the movable small intestine had been removed there was no marked increase in the amount of fæces; in

the dog from which 82 per cent had been removed the amount of fæces was increased.

(6) The percentage of water in the fæces of dogs deprived of large amounts of small intestine may equal or only slightly exceed the percentage of water in the fæces of normal dogs. This is in contrast with the increased percentage of water in the fæces of animals deprived of the large intestine.

(7) On a diet poor in fat the dog with a shortened small intestine absorbs the fat as well or almost as well as a normal dog. As the fat in the diet is increased the fall in the percentage eliminated in the fæces which occurs in the normal animal may either occur to a lesser extent or may not occur at all in dogs deprived of small intestine. With large amounts of fat in the diet 25 per cent of that ingested may appear in the fæces, whereas, in our normal dog only about 4.5 per cent appeared.

(8) The addition of fat to the diet of a normal dog does not greatly affect the amount of nitrogenous material eliminated by the fæces. The addition of fat to the diet of dogs deprived of small intestine causes an increased elimination of nitrogenous material in the fæces. On a diet rich in fat the amount of nitrogen eliminated in the fæces may be double that eliminated by a normal dog, although on a diet poor in fat there is no great difference between the two.

NORMAL DOG.

Day.	Weight. Kgms.	Diet.		Fæces.						Urine.					
						N.		Ether extract.					Sulphates.		
		N. Grams.	Ether extract. Grams.	Amt. Grms.	Water. Per cent.	Amt. Grms.	Not absorbed. Per cent.	Amt. Grms.	Not absorbed. Per cent.	Amt. c.c.	Sp.gr.	N. Grms.	Total Gram.	Ethereal. Gram.	Ratio of ethereal to alkaline.
5	10.50	6.511	13.407	24.0	62	0.573	....	0.927	....	163	1.052	5.325	0.4701	0.0560	1 : 7.4
6	10.47	....	....	18.0	55	0.443	....	0 928	....	260	1.040	5.224	0.4902	0.0582	1 : 7.4
7	....	....	....	72.0	75	1.435	....	1.724	....	380	1.028	5.219	0.3850	0.0462	1 : 7.4
		6.511	13.407	38.0	64	0.817	12.5	1.193	8.9	268	1.040	5.256	0.4484	0.0535	1 : 7.4
15	9.90	6.332	33.762	57.0	78	0.843	....	0.848	....	270	1.029	4.819	0.3674	0.0430	1 : 7.6
16	....	....	....	48.0	83	0.694	....	1.415	....	140	1.045	4.498	0.3127	0.0347	1 : 8 0
17	9.87	....	....	....	..	....	....	....	....	115	1.046	4.689	0.4672	0.0502	1 : 8 3
		6.332	33.762	52.0	80	0.769	12.1	1.137	3.4	175	1.040	4.669	0.3824	0.0426	1 : 7.9
21	10.05	6.332	102.265	80.6	78	0.951	....	1.008	....	214	1.039	4.729	0.4521	0.0533	1 : 7.5
22	10.13	....	....	56.3	74	0.706	....	7.029	....	132	1.054	4.320	0.3297	0.0406	1 : 7.1
23	10.16	....	....	42.0	62	0.793	....	5.581	....	[600]	[1.015]	5.014	0.3657	0.0477	1 : 7.7
		6.332	102.265	59.6	71	0.817	12.9	4.539	4.4	173	1.046	4.688	0.3825	0.0472	1 : 7.4

70 per cent of movable small intestines removed.

Day.	Weight. Kgms.	Diet.		Fæces.						Urine.					
						N.		Ether extract.					Sulphates.		
		N. Grams.	Ether extract. Grams.	Amt. Grms.	Water. Per cent.	Amt. Grms.	Not absorbed. Per cent.	Amt. Grms.	Not absorbed. Per cent.	Amt. c.c.	Sp.gr.	N. Grms.	Total. Gram.	Ethereal. Gram.	Ratio of ethereal to alkaline.
6	7.55	6.332	10.965	46.4	66	1.135	....	1.514	....	255	1.026	4.266	0.3655	0.0481	1 : 6.6
7	....	....	....	24.4	78	0.449	....	0.631	....	152	1.041	3.960	0.2993	0 0424	1 : 6.1
8	....	....	....	10.0	..	0.293	....	0.631	....	180	1.035	4.433	0.4442	0.0827	1 : 4.4
9	7.43	....	....	14.0	49	0.617	....	0.883	....	145	1.049	4.974	....	....	....
		6.332	10.965	23.7	64	0.624	9.9	0.915	8.4	183	1.038	4.408	0.3697	0.0577	1 : 5.7
13	7.46	6.332	33.790	10 6	48	0.230	....	0.989	....	102	1.057	3.980	0 3809	0.0721	1 : 4.3
14	....	....	....	55.8	73	1.201	....	3.202	....	220	1.027	3.942	0.3962	0.0786	1 : 4.0
15	....	....	....	36.7	74	0 769	....	1.261	....	148	1.036	3.167	0.2713	0.0487	1 : 4.6
16	7.46	...	....	43.3	60	1.374	....	2.178	....	134	1 035	2.961	0.2618	....	....
		6 332	33.790	39.1	64	0.894	14.1	1.907	5.6	151	1.039	3.513	0.3275	0.0665	1 : 4.3
20	7.52	6.332	102.265	98 3	72	1.846	....	17.007	....	80	1.054	2.345	0.3313	0.0483	1 : 5.8
21	7.58	4.044	66.951	30.5	63	0.513	....	4.056	....	130	1.040	3.086	0 3475	0 0552	1 : 5.2
22	....	5.432	85.753	28.3	52	0.544	....	10.899	....	78	1.038	1.999	0.1710	0.0324	1 : 4.2
23	7.55	3.900	79.789	80.6	58	1.910	....	21.408	....	110	1.037	2.658	0.1959	0.0403	1 : 3.9
		4.929	83.439	59.4	61	1.203	24.4	13.342	15.9	99	1.042	2.522	0.2614	0.0440	1 : 4.8

DOG NO. II.

82 per cent of movable small intestines removed.

Day.	Weight. Kgms.	Diet.		Fæces.						Urine.					
						N.		Ether extract.					Sulphates.		
		N. Grams.	Ether extract. Grams.	Amt. Grms.	Water. Per cent.	Amt. Grms.	Not absorbed. Per cent.	Amt. Grms.	Not absorbed. Per cent.	Amt. c.c.	Sp.gr.	N. Grms.	Total Gram.	Ethereal. Gram.	Ratio of ethereal to alkaline.
6	9.47	6.511	13.407	43.0	75	0.949	....	1.305	....	260	1.031	5.255	0.410	0.0572	1 : 6.1
7	....	....	....	40.0	71	0.963	....	1.611	....	270	1.028	4.760	0.338	0.0500	1 : 5.8
8	9.59	....	....	53.0	77	1.093	....	1.793	....	238	1.037	5.505	0.443	0.0776	1 : 4.7
9	....	....	....	15.0	..	0.299	....	0.550	....	250	1.036	4.857	0.396	0.0670	1 : 4.9
10	9.47	....	....	67.0	78	1.263	....	2.318	....	320	1.022	4.925	....	....	....
		6.511	13.407	44.0	75	0.913	14.0	1.515	11.3	268	1.031	5.096	0.397	0.0629	1 : 5.4
15	9.31	6.511	36.207	75.0	66	1.424	....	4.787	....	425	1.030	4.702	0.6955	0.1428	1 : 3.8
16	....	....	....	30.0	68	0.628	....	2.382	....	175	1.046	5.060	0.5807	0.0937	1 : 5.2
17	....	....	....	35.8	70	0.794	....	4.083	....	146	1.055	5.290	0.5558	0.1165	1 : 3.8
18	9.54	....	....	75.0	74	1.371	. ..	5.802	....	125	1.048	4.720	0.4391	0.0843	1 : 4.2
19	9.42	....	....	50.0	69	1.197	....	3.826	....	335	1.038	4.915	0.5719	0.0796	1 : 6.2
		6.511	36.207	53.0	69	1.085	16.7	4.176	11.5	241	1.043	4.937	0.5686	0.1034	1 : 4.6
25	....	6.511	104.607	118.0	67	1.388	....	24.759	....	140	1.049	4.770	0.3616	0.0482	1 : 6.5
26	9.85	....	....	149.7	72	1.804	....	29.655	....	120	1.048	4.535	0.2882	0.0382	1 : 6.5
		6.511	104.607	133.9	70	1.596	24.5	27.207	26.0	130	1.048	4.653	0.3249	0.0432	1 : 6.5

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS.—VII. THE MANNER IN WHICH BACTERIA REACT TO STIMULI, ESPECIALLY TO CHEMICAL STIMULI.

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By H. S. JENNINGS and J. H. CROSBY.

IN earlier numbers of this series of studies the manner in which ciliate and flagellate infusoria react to various stimuli has been described. It has been shown, especially in the second,[1] fourth,[2] and fifth[3] of these papers, that the so-called tactic phenomena of these organisms are not as a rule due to an orientation, or direct turning of the organism to or from the source of stimulus, as has often been assumed to be the case. On the contrary, the phenomena are due to a definite movement or reflex action, produced by the stimulating agent, and always taking place in essentially the same manner. The organisms when stimulated by a chemical, by heat, by cold, by mechanical shock or other similar agent, swim backward and turn toward a structurally defined side. To this simple reaction is due the collecting of the organisms in certain regions and their apparent avoidance of other regions, — the so-called positive and negative chemotaxis, thermotaxis, etc. (The reaction to the electric current is complicated by other factors).

Early in the progress of the work, it was incidentally observed in a number of cases that the bacteria have an analogous method of reaction. At that time opposition had developed in certain quarters to the account given in these studies of the method by which the so-called tactic phenomena take place in the infusoria. In view of this fact, and the further fact that Pfeffer in his classical studies on the reactions of unicellular organisms had distinctly asserted[4] that the

[1] This journal, 1899, ii, pp. 311–341.

[2] *Ibid.*, pp. 355–379.

[3] *Ibid.*, 1900, iii, pp. 229–260.

[4] "Dass die Ansammlungen nicht etwa nur zu Stande kommen, weil die zufällig einschwärmenden Organismen beim Versuch des Entfliehens zurückschrecken, lehrt die direkte Beobachtung."—Pfeffer, Untersuchungen aus dem botanischen Institut, Tübingen, 1888, ii, 648, note. See also ibid., 1884, i, 464.

reactions of the bacteria were not of the nature which our observations showed them to be, it was not deemed worth while to publish an account of these reactions of the Bacteria until the description given of the reactions of the infusoria had been confirmed. This description has now been confirmed and extended to other organisms by various observers. A careful study of the movements and reactions of certain bacteria was therefore undertaken during the past winter, with the intention of publishing the results of the investigation.

This work was entirely finished when the valuable paper of Rothert,[1] dealing partly with the same subject, appeared. This paper, so far as it covers the same ground, agrees throughout with our own observations and clearly establishes the fact that the reaction method of the bacteria to chemicals is not by an orientation, but is analogous to that of the infusoria, as described in these studies.

As the subject is of much interest, and as our work was done from a different standpoint from that of Rothert, with different methods and, to a certain extent, different organisms, a brief account of our own observations will not be superfluous even after the publication of Rothert's paper. In a field where so much uncertainty and disagreement exists, the mutual confirmation of two investigators working independently is of importance. For a discussion of the literature and of the general bearing of the results, reference should be made to the paper of Rothert.

The bacteria studied by us were those occurring in cultures of hay and of aquatic plants decaying in water. Two species of Spirillum, apparently Spirillum volutans and S. undula, were selected for special investigation.

The gross features of the reactions of bacteria to chemicals, as usually shown, are well known through the work of Engelmann,[2] Massart,[3] Verworn,[4] and others. Particularly striking is the reaction to oxygen. The bacteria, mounted on a slide and covered with a cover glass, collect (1) about bubbles of air; (2) about the edge of the cover glass, next to the air; (3) about green plant cells, diatoms, desmids, etc., which are giving off oxygen through the action of

[1] Rothert: Flora, 1901, lxxxviii, pp. 371–421.

[2] Engelmann: Archiv für die gesammte Physiologie, 1881, xxv, pp. 285–292.

[3] Massart, J.: Bulletin de l'Académie royale de médecine de Belgique, 1891 (3), xxii, pp. 148–167.

[4] Verworn: Psychophysiologische Protistenstudien, 1889, pp. 103–106.

the chlorophyll. (See in Verworn's General Physiology, Figs. 211 and 214, and Davenport's Experimental Morphology, Part 1, Figs. 3–5*a*.) Such collections may be observed in any preparation containing the ordinary bacteria of decay.

How are these collections formed? Do the bacteria turn and direct their course toward the centre of diffusion of the oxygen, — proceeding directly toward the region of greatest oxygen density? Or are the collections brought about more indirectly, in a manner similar to that by which Paramecium collects in regions containing an acid?

Before describing the observations by which this question is answered, it will be necessary to give a brief account of the form and usual movements of the organism Spirillum.

Spirillum volutans forms an elongated rod, very slender, with a length of from 15 to 50 μ. It is curved into the shape of a spiral of from two to six turns, so that it resembles a corkscrew in form. At each end of the spiral are found one or two flagella. There is no observable difference between the two ends of the organism, nor is there any marked differentiation of two sides, such as distinguishes oral and aboral sides in Paramecium, for example.

Movements.— Spirillum swims in the direction of the long axis of the spiral by means of its flagella. At the same time it revolves, the revolution following the direction of the spiral, and being therefore (usually at least) from left over to right, if one faces in the direction in which the organism is swimming. At intervals the movement is reversed, the organism swimming then with the opposite end in advance, and revolving in the opposite direction. As a rule, neither end seems to be preferred as the anterior one, Spirillum swimming indifferently in either direction. Given individuals are observed, however, at times to swim for long periods with a certain end in advance, the reversals lasting but a moment.

Occasionally an individual may be seen revolving on its long axis without progressing in either direction, while in other cases there is a rapid whirling on a tranverse axis. But these methods of movement are rare.

"**Chemotaxis.**" —If the Spirilla are mounted on a slide beneath a cover-glass, in company with some desmids or other green algal cells, after a time they will be observed to have formed collections about the algæ, as illustrated in the figures referred to above. How does this occur?

Careful observation shows the course of events to be as follows: —

At the beginning the bacteria are scattered uniformly throughout the preparation. They are swimming rapidly in all directions. At first they pass close to the green plant cells without any reaction whatever. The algæ begin, in the light, to give off oxygen, so that after some time each desmid or other alga must be conceived as surrounded by a zone of water impregnated with oxygen.

Now begins the collection of the Spirilla about the algæ. The bacteria surrounding the algæ do not change their direction of motion and swim toward the centre of diffusion of the oxygen. On the contrary, all continue to swim in the same direction as before. A Spirillum passing close to the alga into the oxygenated zone does not at first change its movement in the least. It swims across the zone till it reaches the other side. It is here that the reaction occurs; the organism reverses its movement and swims in the opposite direction till it reaches the opposite boundary of the oxygenated area. It then reverses again, and this is continued, — the direction of movement being reversed as often as the organism comes to the boundary of the zone of oxygen. The Spirillum therefore remains within the area, which thus acts like a trap. Other Spirilla, swimming at random, enter the area in the same way, react at the outer edges in the same manner, and remain. In the course of time therefore the zone of oxygen swarms with Spirilla.

There is thus no orientation shown either by the organisms within the area or by those outside. *Within*, the Spirilla are swimming in all directions, crossing each other's paths at every angle, and agreeing only in the fact that the movement is reversed on coming to the boundary of the zone of oxygen. A single individual may be seen to oscillate back and forth from one side of the area to the other an indefinite number of times. *Without*, movements are occurring absolutely without relation to the position of the alga and its oxygen zone. Many Spirilla pass close to the edge of the zone, but do not enter unless their original course carries them directly into it. Many of the bacteria therefore remain scattered throughout the preparation, not gathering about the algæ, no matter how long the slide is allowed to stand. But through their continued movement in all directions, dense groups are soon formed about the algal cells.

It is evident therefore that the collections of bacteria arise through the agency of a "motor reflex" essentially similar in character to that of the infusoria, described in previous numbers of these studies.

This motor reflex consists in the bacteria in a reversal of the direction of movement, upon stimulation. The direct cause of the reaction is a change in the nature of the surrounding medium. In the cases already described, it is the change from water containing *much* oxygen to water containing *little* oxygen. The "boundary" of the oxygen zone, above spoken of, is of course merely the region where the change in oxygen density is sufficiently great to cause the reaction.

The bacteria collect in exactly the same manner about air bubbles, and about the edge of the cover-glass, next to the air. In these cases the bacteria usually collect in a narrow zone a short distance from the air surface. If their movements be observed here, it will be found that the reversal of motion is brought about in two different regions. (1) The passage from the optimum zone of oxygen to a region having *less* oxygen pressure causes the reaction. (2) Passage from the optimum into a region having *greater* oxygen pressure, — next to the air surface, — causes the reaction with even greater precision than the opposite change. The Spirilla therefore remain in the narrow optimum zone a short distance from the bubble or the edge of the cover-glass.

The above phenomena are cases of what has been spoken of as "positive chemotaxis." "Negative chemotaxis," or the avoidance of regions containing certain chemicals, takes place in the same manner, save that the reaction occurs when the organism comes, from the outside, against the outer boundary of the area in question. Thus, if a drop of a $\frac{1}{5}$ per cent solution of sodium chloride be introduced beneath the cover-glass by means of a capillary pipette, the following phenomena will be observed. The bacteria do not orient themselves and move in radial lines away from the centre of diffusion of the salt solution. On the contrary, all move in random directions, as before. But on coming against the outer boundary of the salt solution, the organism reacts by reversing the direction of its movement. Hence it does not enter the drop. As every Spirillum that comes in contact with the drop reacts in the same way, the drop remains empty.

Solutions of most acids, alkalies, and salts act in the same manner, so that a drop of any of them (of sufficient concentration) remains empty when introduced beneath the cover-glass.

In addition to the observations on Spirilla, the reactions of a number of the other bacteria found in decaying vegetable matter were studied. In every case the reactions took place in the manner above described for Spirillum, so that there can be no doubt that this

method is of general occurrence among the bacteria. In this respect our results agree throughout with those of Rothert.[1]

The same method of reaction often occurs when the bacteria strike against a solid obstacle. The movement is reversed, the organism swimming in the opposite direction.

This manner of reaction was first observed by Engelmann in the reaction of Bacterium (Chromatium) photometricum to light.[2] If a small circumscribed area on the slide is lighted from beneath, the bacteria, swimming at random, pass into this area in the same manner as described above for an area of oxygen. On attempting to pass from this light area into the dark, this organism, according to Engelmann, suddenly reverses its movement and swims backward, — thus remaining in the lighted area. This reversal lasts in the case of Bacterium photometricum but a short time, the organism beginning soon to swim forward again. This is due to the fact that this bacterium has flagella only at one end and normally swims with that end in advance. The reversal of the movement is therefore soon followed by a return to the original direction. This reaction was called by Engelmann a "Schreckbewegung;" it is clearly identical with the "motor reflex" described in these studies.

Bacteria thus react to chemicals, to mechanical obstacles, and to light (or darkness) in the same way, — by a "motor reflex," comparable to that of the ciliate infusoria.

This method of reaction is denominated by Rothert *apobatic* taxis, in contradistinction to *strophic* taxis, which consists in a turning of the organism toward or from the source of stimulus, so as to bring the axis of the body into a definite orientation with respect to the stimulus. The bacteria would thus show apobatic chemotaxis and apobatic phototaxis, using this method of denomination. The really fundamental phenomenon in these cases is the definite reflex action produced by the stimulus; whether aggregation or scattering of the organisms occurs and where it occurs, depend merely on what agencies produce this reflex.

The "motor reflex" of the bacteria differs from that of the infusoria in the same way that the form and structure of the body differ in the two cases. In such bacteria as Spirillum there is no differentiation as between the two ends, or between the two sides of the organism. In correlation therewith, movement takes place indifferently in the

[1] *Loc. cit.*

[2] ENGELMANN: Archiv für die gesammte Physiologie, 1883, xxx, 95–124.

direction of either end, and the motor reflex consists merely of a reversal of the direction of the movement, — without subsequent return to the original direction except as a response to a new stimulus. In the infusoria there is a differentiation both between the ends and between the sides of the animal. The movements reflect these differentiations. The organism swims normally with a certain end in advance, and usually swerves toward a certain side. The motor reflex consists in a reversal of the direction of movement, so as to swim toward the opposite end, together with a turning toward a definite side, and this is always followed soon by a return to the original motion with the anterior end in front. In the case of Engelmann's Bacterium photometricum we have an interesting intermediate condition. Here there is a differentiation between the two ends of the organism, only one bearing flagella, while apparently all sides are alike. The reaction to a stimulus consists in a reversal of the direction of movement, as in the other bacteria, but without any turning toward a certain structurally defined side, such as occurs in the infusoria. But the reaction is followed, as it is in the infusoria, by a return to the original direction of movement. The reactions thus give throughout, in their simplicity or complexity, a faithful reflection of the structure.

THE FORMATION OF ALLANTOÏN FROM URIC ACID IN THE ANIMAL BODY.

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DESPITE the numerous investigations of recent years which have dealt with the synthesis and transformation of uric acid in the animal body, much remains to be ascertained before we can reach a clear conception of the processes determining the excretion of this substance. Any comprehensive consideration of the extent of uric acid production calls for knowledge regarding the fate of uric acid when introduced as such into the organism. The decomposition products obtainable in the laboratory include such familiar compounds as urea, oxalic acid, and allantoïn. The latter is readily formed by the gentle oxidation of uric acid.[1]

Wöhler and Frerichs[2] conducted the earliest feeding experiments with uric acid. They searched in the urine for those products which Wöhler had obtained by oxidation of uric acid with lead peroxide, viz., urea, oxalic acid, and allantoïn. The latter substance could not be isolated from the urine of man, the dog, or the rabbit, after introduction of uric acid into the system ; the investigators concluded that it was broken down further into compounds unknown to them, and thus escaped detection. Neubauer,[3] later, also failed to find allantoïn in the urine of rabbits after feeding uric acid. A large increase in the urea output was observed, however.

In 1876, Salkowski[4] first demonstrated allantoïn excretion after feeding uric acid to dogs. Four grams were given to one animal on two days in succession ; 1 42 grams of allantoïn were separated from the urine by crystallization, and identified by analysis. This result has been confirmed by Minkowski[5] in two cases. From one dog he

[1] Claus : Berichte der deutschen chemischen Gesellschaft, 1874, vii, p. 226.

[2] Wöhler and Frerichs : Annalen der Chemie, 1848, lxv, p. 335.

[3] Neubauer : Annalen der Chemie, 1856, xcix, p. 217.

[4] Salkowski : Berichte der deutschen chemischen Gesellschaft, 1876, ix, p. 719.

[5] Minkowski : Archiv für experimentelle Pathologie und Pharmakologie, 1898, xli, p. 398 (foot-note 3).

obtained 0.763 gram of allantoïn and 0.161 gram of uric acid after administration of five grams of uric acid with the diet. Mendel and Brown[1] likewise obtained a considerable yield of allantoïn after feeding uric acid to cats.

Recently, however, Poduschka[2] has failed to obtain any allantoïn after feeding uric acid to dogs. It is easy to understand the failure of the earlier investigators in view of the lack of any satisfactory method for the quantitative estimation and isolation of allantoïn.[3] But Poduschka's results were obtained after the application of a new and accurate quantitative method which will be discussed later. His analytical data may be briefly summarized here[4]: —

I. One gram of sodium urate, dissolved in water, was given to a dog with his ordinary diet. The large quantities of water required increased largely the subsequent excretion of urine. The output of urea considerably exceeded that corresponding to the nitrogen of the ingested urate. There was an insignificant increase in the allantoïn excretion.

II. A dog of 9.8 kilos received on the second day of fasting 100 c.c. of 0.8 per cent sodium sulphate solution subcutaneously, and 140 c.c. of water per os. On the following morning the urine (70 c.c.) was removed by catheterization, and estimations of various constituents made. The animal then received two doses of one gram of sodium urate each. The 78 c.c. of urine obtained by catheterization during the following twenty-four hours were analyzed. The increase in urea-nitrogen demonstrated the absorption and decomposition of the uric acid. No noticeable production of allantoïn was observed. The quantity of uric acid fed in the last experiment was sufficient to have yielded 1.03 grams of allantoïn.

From these data Poduschka concludes that the decomposition of uric acid in the dog does not involve the formation of allantoïn. The experiments with which the present paper deals have been undertaken to explain the negative results recorded.

Methods of allantoïn estimation. — Before proceeding with a quantitative study of allantoïn excretion it became necessary to consider the methods available for its estimation. The older methods of Meissner, which will be found described in various text-books, are

[1] Mendel and Brown: This journal, 1900, iii, p. 267.

[2] Poduschka: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 65.

[3] Cf. Minkowski: *Loc. cit.*, p. 396.

[4] Poduschka: *Loc. cit.*, p. 65.

somewhat unsatisfactory. Moscatelli[1] has outlined a method which the writer has applied in slightly modified form with excellent results. The fluid to be examined is treated with a solution of mercuric nitrate, which precipitates the allantoïn completely, together with other substances. The precipitate is washed with cold water and decomposed with hydrogen sulphide. The filtrate is evaporated to a small volume after being made alkaline with ammonia, and is again precipitated with an ammoniacal solution of silver nitrate. The silver precipitate is filtered off after twenty-four hours and decomposed with hydrogen sulphide. From the solution thus obtained the allantoïn may be crystallized out. For analytical purposes it is sufficient to determine the nitrogen in the silver precipitate, from which the content of allantoïn can be calculated. The most serious criticism of this method applies to the solubility of allantoïn-silver in an excess of ammonia. This fact has been recognized in the method recently devised by Loewi.[2] It likewise depends upon the quantitative precipitation of allantoïn with alkaline silver nitrate solution. The excess of ammonia is avoided by the substitution of magnesium oxide which may be added in excess without interfering with the complete precipitation of the allantoïn-silver.

In Loewi's method, the urine is first treated with mercurous nitrate which removes the other nitrogenous compounds without precipitating allantoïn. The mercury is removed from the filtrate by the use of hydrogen sulphide, and the solution is then treated with magnesium oxide and silver nitrate. The silver precipitate contains no nitrogenous compounds except allantoïn. The latter may be estimated by determining the nitrogen in the washed precipitate, or by decomposition, reprecipitation with mercuric nitrate, the subsequent removal of the mercury with hydrogen sulphide, and the weighing of the allantoïn after evaporation of the filtrate to dryness. Like Loewi, the writer has obtained very good results with this method. Great care must be taken, however, not to have the solution of mercurous nitrate too strongly acid, and to prevent the formation of mercuric nitrate.

Poduschka[3] has lately recommended the following method. Fifty or one hundred cubic centimetres of urine are precipitated with

[1] Moscatelli: Zeitschrift für physiologische Chemie, 1889, xiii, p. 203.

[2] Loewi: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 20.

[3] Poduschka: *Loc. cit.*, p. 61.

the necessary amount of basic lead acetate, and the excess of lead is removed from a definite volume of the filtrate by means of concentrated sodium sulphate solution. To a definite portion of the second filtrate, 5–10 per cent silver nitrate solution is added, the precipitate filtered off and rejected, and to the new filtrate dilute ammonia (1 per cent) added drop by drop. The allantoïn-silver is precipitated, and can be washed thoroughly with one per cent sodium sulphate solution until free from ammonia. The allantoïn can then be estimated by a Kjeldahl determination of nitrogen in the precipitate. With this method Poduschka recovered from 93 to 95 per cent of the allantoïn added to urine.

Some results obtained in the course of a study of the relative accuracy of the methods outlined are detailed below.

I. Meissner's method. — 0.332 gram of allantoïn was dissolved in 500 c.c. of normal dog's urine which contained no allantoïn when examined by Loewi's method.

Allantoïn crystals recovered = 0.3012 gram.

The crystals were somewhat discolored, therefore a Kjeldahl nitrogen determination was made.

Total nitrogen found = 0.0906 gram = 0.2982 gram allantoïn = 89.8 per cent.

II. Loewi's method. — (a) 0.3621 gram of allantoïn was dissolved in 500 c.c. of normal dog's urine as above, and an estimation of allantoïn made.

Allantoïn crystals recovered = 0.3524 gram = 97.3 per cent.

(b) 0.2891 gram of allantoïn was dissolved in 500 c.c. of normal dog's urine, and an estimation made by determining the total nitrogen in the first precipitate of allantoïn-silver.

Total nitrogen found = 0.0861 gram = 0.2834 gram allantoïn = 98.0 per cent.

III. Poduschka's method. — 0.3082 gram of allantoïn was dissolved in 500 c.c. of normal dog's urine.

Total nitrogen found in the allantoïn-silver precipitate = 0.0899 gram = 0.2961 gram allantoïn = 96.0 per cent.

Experimental methods. — The present feeding experiments were made upon two dogs: one a large bitch of 17 kilos; the other a very small young terrier of 5 kilos. Animals of such widely different size were purposely selected in order to demonstrate any possible difference in urine acid metabolism which might be attributable to this factor. They were confined in suitable metabolism cages, and

in the case of the larger animal the urine was collected twice daily by catheterization. The diet consisted of casein (freshly precipitated from skimmed milk), lard, and cracker-dust. Casein was selected as a suitable proteid foodstuff instead of meat or similar products, since Salkowski[1] has demonstrated that allantoïn excretion may occur in the dog after a meat diet. In the earlier experiments the daily diet was made up as follows:

Food.	Large dog.	Small dog.
Casein	200 grams.	150 grams.
Cracker-dust	50 "	50 "
Lard	50 "	25 "

LARGE DOG (17 kilos).

Experiment.	Day.	Body-weight. Kilos.	Urine volume. c.c.	Uric acid fed Grams.	Allantoïn in urine. Gram.
I	1	16.9	140	1.0	None.
	2	17.0	200	....	
	3	17.1	280	....	
II	1	17.2	108	4.0	0.430
	2	17.2	201	....	
	3	17.1	345	....	
III	1	17.2	180	5.0	0.590
	2	17.2	260	....	
	3	17.1	280	....	
IV	1	17.3	200	6.0	0.620
	2	17.2	240	....	
	3	17.2	190	....	

[1] Salkowski: Berichte der deutschen chemischen Gesellschaft, 1878, xi, p. 500.

The uric acid was administered as sodium urate with the daily diet. Water was given *ad libitum*. The allantoïn was estimated by Loewi's method in all the experiments, during a period of three days after the uric acid feeding. Experience showed that the allantoïn formed is entirely eliminated by the end of this time. The fæces obtained were usually examined roughly for uric acid by extraction with dilute sodium hydroxide solution, and subsequent acidification with hydrochloric acid. See protocols on pages 42 and 43.

SMALL DOG (5 kilos).

Experiment.	Day.	Body-weight. Kilos.	Urine volume. c.c.	Uric acid fed. Grams.	Allantoïn in urine. Gram.
V	1	5.0	140	1.0	
	2	4.9	200	....	0.240
	3	5.0	95	....	
VI	1	5.2	160	4.0	0.620
	2	5.3	110	....	0.140 } 0.780
	3	5.2	85	....	0.020
VII	1	4.9	145	5.0	
	2	4.8	210	....	0.935
	3	4.9	85	....	

Since the preceding experiments demonstrate that only a relatively small portion of the uric acid ingested reappears in the form of allantoïn, it seemed desirable to obtain more definite quantitative data. The larger dog was therefore fed upon a diet which maintained nitrogenous equilibrium in this animal. After a preliminary period of three days, three grams of uric acid (in the form of sodium urate) were daily added to the food for three days; this period was followed by an after-period of three days. The diet was carefully analyzed for nitrogen. The nitrogen content of the urine, fæces, and hair was ascertained, allantoïn was estimated in the urine by Loewi's method, oxalic acid by Baldwin's modification of Dunlop's method,[1] and uric acid by Hopkins' method. The diet consisted of —

[1] BALDWIN: Journal of experimental medicine, 1900, v, p. 30.

300 grams of casein containing	9.9	grams of nitrogen.
50 grams of cracker-dust containing . .	0.8	grams of nitrogen.
50 grams of lard containing	0.0	grams of nitrogen.
Total nitrogen of diet . . .	10.7	grams of nitrogen.

The results of this experiment are given in tabular form.

EXPERIMENT VIII.

Date. 1901. March.	Body Weight.	Food.		Urine.					Fæces and Hair. Nitrogen.
		Dietary. N.	Uric acid.	Vol.	Nitrogen.	Uric acid.	Oxalic acid.	Allantoïn.	
	Kilos.	Grams.		c.c.	Grams.				
7	16.3	10.70	3.0	500	10.53	0.106	0.0070	0.310	0.830
8	16.3	10.70	3.0	350	9.85	0.080	0.0053	0.308	1.800
9	16.2	10.70	3.0	356	11.98	0.062	0.0064	0.260	0 000
10	16.3	10.70	0.0	410	12.26	trace	0.0032	0.088	0.112
11	16.2	10.70	0.0	230	9.40	trace	0.0021	0.021	1.261
12	16.2	10.70	0.0	360	11.26	....	....	....	0.260
Totals		64.20	9.0 = 3.0 grams N.		65.28	....	....	0.987	4.263

From these results it appears that the yield of allantoïn excreted under these circumstances is far smaller than the quantity theoretically obtainable from the amounts of uric acid fed. Thus from nine grams less than one gram of allantoïn was recovered. Although the output of oxalic acid was slightly increased during the uric acid period, the increase is far too small to account for much of the metabolized material. The significance of oxalic acid as a metabolic product derived from purin groups such as are contained in nucleins has recently been emphasized by Salkowski[1] and his co-workers.

The yield of allantoïn obtained in the metabolism of varying quantities of uric acid is summarized in the following table: —

[1] Salkowski: Berliner klinische Wochenschrift, 1900, p. 494.

SUMMARY OF ALLANTOÏN EXCRETION.

Uric acid fed. Grams.	Allantoïn excreted.	
	Large dog.	Small dog.
	Gram.	
1	none	0.240
4	0.430	0.780
5	0.590	0.935
6	0.620	....
9[1]	0.987	....

The difference in the allantoïn output of the two animals is more striking when the effects are expressed in terms of units of body-weight, as indicated below:—

Uric acid fed per kilo body-weight.	Allantoïn output per kilo body-weight.	
	Large dog.	Small dog.
	Milligrams.	
59	none	....
200	....	48
235	25	....
294	35	....
353	37	....
529	58	....
800	....	156
1000	....	187

In attempting to explain these differences, we recall the failure of various investigators[2] to demonstrate allantoïn production in man

[1] This was fed in the course of three days.

[2] Cohn: Zeitschrift für physiologische Chemie, 1898, xxv, p. 509; Minkowski: *Loc. cit.*, p. 398; Loewi: *Loc. cit.*, p. 22.

after ingestion of uric acid-yielding food, such as thymus. Allantoïn may be looked upon as an intermediate product in uric acid metabolism in the body. Ordinarily, where the conditions are favorable, uric acid and its antecedents are more completely oxidized in the system, and the nitrogen of these compounds presumably reappears in large part as urea. This might be looked upon as representing the ordinary fate of uric acid when introduced into the human organism in such doses as are permissible. Furthermore, allantoïn itself may be oxidized almost completely in the body.[1] In the dog, also, comparable quantities of uric acid may apparently be metabolized beyond the stage where allantoïn appears as an end-product — as Experiment I above and Poduschka's experiments show. When larger quantities (per kilo of body-weight) are fed, however, the system is apparently unable to bring about so complete a decomposition of the purin radical; and under these conditions allantoïn may appear as an end-product of the transformation of a part of the ingested uric acid precisely as it has repeatedly been shown to arise after nuclein,[2] or even allantoïn[3] feeding. The differences shown between man and other animals (dog and cat) thus appear as due to variations in the extent of metabolism in the organs involved, such as the liver, rather than to specific peculiarities of different animals. In regard to the importance of the liver, reference may be made to the occurrence of allantoïn in cases of cirrhosis of the liver[4] and in diamid poisoning.[5] For the latter case, Borissow has already pointed out that allantoïn occurs in the urine, presumably owing to the inhibition of normal processes of metabolism; and Poduschka's experiments also suggest that hepatic changes may play an important rôle.

Summary.

The experiments demonstrate, in agreement with the observations of Salkowski and Minkowski, that allantoïn is excreted by the dog

[1] Minkowski: *Loc. cit.*, p. 399; Poduschka: *Loc. cit.*, p. 64.

[2] Cohn: *Loc. cit.*, p. 507; Minkowski: *Loc. cit.*, p. 393; Salkowski: Centralblatt für die medicinische Wissenschaften, 1898, p. 929; Mendel and Brown: This journal, 1900, iii, p. 265 (Cat).

[3] Minkowski: *Loc. cit.*, p. 399; Poduschka: *Loc. cit.*, p. 64.

[4] Moscatelli: *Loc. cit.*, p. 202.

[5] Borissow: Zeitschrift für physiologische Chemie, 1894, xix, p. 499: Poduschka: *Loc. cit.*, p. 59.

after ingestion of uric acid. Considerable quantities of this substance may, however, be burned up beyond the allantoïn stage.

After uric acid feeding the output of uric acid in the urine is only slightly increased.

In conclusion, the writer acknowledges his obligation to Professor Lafayette B. Mendel, not only for the suggestion of the subject of this investigation, but also for valuable assistance and criticism.

SOME DECOMPOSITION PRODUCTS OF THE CRYSTALLIZED VEGETABLE PROTEID EDESTIN.

By P. A. LEVENE and LAFAYETTE B. MENDEL.

MORE than two years ago we undertook a study of the decomposition products of various albuminous substances and began the investigation by endeavoring to determine the proportion of nitrogen split off in different groups by the action of acids. Phosphotungstic acid was used to precipitate the basic compounds, and the estimations were made in somewhat the same way as those by Hausmann [1] in his research on the proteids. It was very soon found, however, that concordant and reliable results could not be obtained, and the method was given up as unsatisfactory. Similar experience led Henderson [2] and Chittenden and Eustis [3] to announce that Hausmann's method of determining the distribution of nitrogen in the proteid molecule is unreliable for quantitative purposes, and that results obtained by this method must be accepted with caution. Quite recently Kutscher [4] has arrived at a similar conclusion, apparently without being aware of the observations just referred to.

We have made a qualitative study of the hexon bases arising from the decomposition of the crystalline vegetable proteid edestin, and the earliest analyses together with demonstrations of the products separated were presented to the American Physiological Society in 1899.[5] Edestin was selected for study at that time, because it could be obtained in large quantities as a relatively pure crystalline compound; furthermore, the proteid is unusually rich in nitrogen (18.7

[1] HAUSMANN: Zeitschrift für physiologische Chemie, 1899, xxvii, p. 95; 1900, xxix, p. 136.

[2] HENDERSON: Proceedings of the American Physiological Society, 1899; This journal, 1900, iii, p. xxx. Also, Zeitschrift für physiologische Chemie, 1899, xxix, p. 47.

[3] CHITTENDEN and EUSTIS: Proceedings of the American Physiological Society, 1899; This journal, 1900, iii, p. xxxi.

[4] KUTSCHER: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 215.

[5] LEVENE and MENDEL: Proceedings of the American Physiological Society, 1899; This journal, 1900, iii, p. iv.

per cent), and the experience of Schulze [1] with the proteid from the seeds of Picea excelsa suggested the possibility that the edestin from the hemp-seed might likewise yield the hexon bases in abundance.

Kossel and Kutscher [2] have lately published the results of an extensive investigation of the nitrogenous groups — especially the hexon bases — in various proteids. Edestin or similar crystalline vegetable proteids are not included in their research; and since no reference is made to our earlier observations we have concluded to present a very brief account of these experiments. Additional interest is now attached to them in view of the specific differences which Kossel and Kutscher found among the proteids of wheat and corn (maize) which they studied. Thus the alcohol-soluble proteids, zein, gliadin, etc., yielded no lysin whatever, although it was readily obtained from the so-called gluten-casein of wheat.

The edestin used in the present investigation was obtained by Osborne's method of extracting hemp-seed with warm 5 per cent sodium chloride solution.[3] Large quantities of the carefully prepared and washed proteid were decomposed by heating with 20 per cent hydrochloric acid and stannous chloride for seventy-two hours. The tin was then removed from the diluted fluid with hydrogen sulphide; and after driving off the excess of this gas most of the acid was removed by treatment with freshly precipitated lead hydroxide. The filtrate was again treated with hydrogen sulphide to remove the lead in solution, and the fluid concentrated. It reacted slightly acid and still contained some hydrochloric acid. Nitric acid was added, and enough silver nitrate to combine with all the bases present. The quantity necessary for this purpose was ascertained by diluting 10 c.c. of the concentrated liquid to 25 c.c., and adding 5 per cent silver nitrate solution from a burette until a drop of the mixed liquids formed a brownish yellow trace when added to a solution of barium hydrate on a watch-glass. The calculated quantity of silver nitrate solution was then added to the entire acid solution, and the precipitate of silver chloride which formed was removed by filtration. From the resulting filtrate the

[1] SCHULZE: Zeitschrift für physiologische Chemie, 1898, xxiv, p. 276.

[2] KOSSEL and KUTSCHER: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 165.

[3] For the properties, etc., of edestin, cf. OSBORNE: American chemical journal, 1893, xiv, p. 38; CHITTENDEN and MENDEL: Journal of physiology, 1894, xvii, p. 48.

three hexon bases were finally separated and identified by the methods of Kossel,[1] as follows: —

Histidin. — To remove the histidin, the acid fluid was neutralized with barium hydrate solution. A slightly yellowish precipitate was formed and filtered off by suction. It was washed with half-saturated barium hydrate solution, then taken up with water and rendered slightly acid with sulphuric acid. The silver was next removed by means of hydrogen sulphide, the sulphuric acid with barium hydroxide, and the excess of the latter by means of carbonic acid. The filtrate from the barium carbonate was concentrated, made acid with hydrochloric acid, decolorized with charcoal, again concentrated with addition of more acid until crystallization began. This process was allowed to continue in a vacuum desiccator until crystals of histidin dichloride — some 10 mm. long and about 6 mm. in thickness — were obtained. The mother liquor yielded more of the same crystals on further similar treatment. The material was washed with alcohol and ether, dried over sulphuric acid and analyzed.

0.1578 gram yielded 0.1995 gram AgCl = 31.22 per cent Cl.

0.1423 gram yielded by the Dumas method, 25 c.c. of N at 30° C. and 747 mm. Hg pressure = 18.62 per cent N.

$C_6H_9N_3O_2 \cdot 2HCl$

	Calculated.	Found.
N	18.42 per cent	18.62 per cent
Cl	31.14 per cent	31.22 per cent

Arginin. — The filtrate from the histidin-silver compound was saturated with powdered barium hydrate. The white precipitate which formed rapidly turned brown. It was filtered off by suction, washed with concentrated barium hydrate solution, taken up with water and acidulated with sulphuric acid. The silver was next removed by means of hydrogen sulphide, the sulphuric acid with barium hydroxide, and the excess of the latter with carbon dioxide. The filtrate from the barium carbonate was concentrated to a thick syrup and transferred to a vacuum desiccator. A partly crystalline mass of arginin carbonate was gradually formed. The crystalline material was easily separated from the colored mother liquor and finally a white mass obtained which was washed with alcohol and ether.

[1] For references to the most recent methods, see KOSSEL and KUTSCHER: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 165.

The bulk of the arginin remained in the mother liquor and washings, from which more of it could be isolated. Only the white purified carbonate was used to obtain the nitrate and silver salts. The acid silver salt was analyzed.[1]

0.250 gram yielded 0.06715 gram Ag = 26.86 per cent Ag.

0.100 gram yielded by the Dumas method 19.5 c.c. of N at 32° C. and 756 mm. Hg pressure = 20.74 per cent N.

$\frown\!\!\!-\!\!\!-$ $C_6H_{14}N_4O_2 \cdot HNO_3 + AgNO_3$ $-\!\!\!-\!\!\!\frown$

	Calculated.	Found.
Ag	26.54 per cent	26.86 per cent
N	20.64 per cent	20.74 per cent

Lysin. — The filtrate from the arginin-silver was treated with sulphuric acid to remove the barium, then with hydrogen sulphide to eliminate the silver. After driving off the excess of gas by heat, the acid fluid was treated with phosphotungstic acid, and the precipitate formed was decomposed in the usual manner with barium hydrate.[2] To the final solution containing the carbonate of lysin, and probably other bases, an alcoholic solution of picric acid was added and the material allowed to stand for twenty-four hours. The lysin picrate formed was redissolved several times in hot water and reprecipitated. It was dried *in vacuo*, then at 120° C. and analyzed.

0.1645 gram yielded, according to an analysis by the Kjeldahl-Gunning[3] method, 18.55 per cent N.

0.2153 gram yielded on combustion, 0.3025 gram CO_2 = 38.30 per cent C, and 0.1025 gram H_2O = 5.28 per cent H.

$\frown\!\!\!-\!\!\!-$ $C_6H_{14}N_2O_2 \cdot C_6H_3N_3O_7$ $-\!\!\!-\!\!\!\frown$

	Calculated.	Found.
C	38.40 per cent	38.30 per cent
H	4.53 per cent	5.28 per cent
N	18.67 per cent	18.55 per cent

In view of the high content of hydrogen found, the preparation was recrystallized and analyses obtained as follows: —

I.	C . . .	38.38 per cent
	H . . .	5.50 per cent
II.	C . . .	39.01 per cent
	H . . .	6.65 per cent

[1] Cf. Hedin: Zeitschrift für physiologische Chemie, 1894, xx, p. 189.

[2] Cf. Kossel: Zeitschrift für physiologische Chemie, 1899, xxvi, p. 586.

[3] This analysis was made before the publication of Henderson's criticism of the

These results suggested that a partial decomposition of the salt had taken place during the successive recrystallizations. The picrate was therefore converted into the chloride by Kossel's method.[1] The material thus obtained was too small in quantity to furnish a satisfactory analysis. We believe, however, that the first analysis taken in connection with the mode of preparation and properties of the compound leaves little doubt as to the identity of the latter.

The foregoing experiments have demonstrated that the crystallized vegetable proteid edestin resembles the ordinary animal proteids in yielding the three known hexon bases, arginin, lysin, and histidin, and differs from the alcohol soluble proteids of vegetable origin which fail to yield lysin among their decomposition products.[2]

N-estimation in lysin by the Kjeldahl method: Zeitschrift für physiologische Chemie, 1900, xxix, p. 322.

[1] KOSSEL: Zeitschrift für physiologische Chemie, 1899, xxvi, p. 587.

[2] KOSSEL and KUTSCHER: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 212.

DO SPERMATOZOA CONTAIN ENZYME HAVING THE POWER OF CAUSING DEVELOPMENT OF MATURE OVA?

By WILLIAM J. GIES.

[*From the Department of Physiology in the Marine Biological Laboratory at Wood's Holl, Mass.*[1]]

CONTENTS.

OUR knowledge of the chemical properties of enzymes is very slight, and our understanding of the part they play in zymolysis anything but clear. Nevertheless, the great importance in biological events of these energy-transforming substances is generally recognized. The lack of precise information regarding the essential qualities of enzymes no doubt accounts for the current tendency to attribute indefinitely to ferment influence various processes of morphological or chemical character which are not satisfactorily comprehended through ordinary experimental means, or which, in some cases, have not even been subjected to such investigation.

A fundamental biological question has lately been put into this category. The process of segmentation in the fertilized egg has been ascribed in part, at least, to enzyme influence.

With the advice and many helpful suggestions of Professor Loeb, I have attempted to ascertain whether any experimental justification can be found for recent statements that the spermatozoön carries substance into the ovum which effects proliferation by zymolysis.

[1] I am indebted to the kindness of Professor Curtis for the use of the investigator's room at Wood's Holl, reserved for the Department of Physiology of Columbia University.

Historical.

Piéri,[1] after some observations on *Strongylocentrotus lividus* and *Echinus esculentus* in the Marine Laboratory at Roscoff, in August, 1897, reported that he had extracted soluble sperm enzyme having power to bring about segmentation of the ovum. "Ovulase," as he called it, was obtained by merely shaking the spermatozoa of these Echinoderms for a quarter of an hour in a flask with sea-water, or with distilled water. Microscopic examination of the filtrates showed that the spermatozoa which passed through the paper were without tails and immobile; "that is to say, dead."

The fresh mature ova, well washed in sea-water, were placed in shallow dishes (size not stated) with the extract, immediately, or within ten hours, after its preparation. Segmentation proceeded slowly and reached the morula stage in about ten hours, with the usual phenomena of karyokinesis. Microscopic examination showed that there had been no penetration by spermatozoa. The "ovulase" in distilled water was less effective than that obtained in sea-water; it produced only a few segmentations (greatest number not mentioned).

At the end of his paper Piéri himself mentions two "objections" to his conclusions which it appears to the present writer destroy their force: (1) Only the spermatozoa in the distilled water (which extract he has distinctly indicated possessed the lesser, if any, segmental power) were always killed by the shaking process. He suggests that the spermatozoa might be eliminated, and pure "ovulase" obtained with the aid of the centrifuge or porcelain filter. (2) Some of the main supply of eggs in sea-water, from which those tested were taken, segmented (to what stage is not stated), "in spite of the precautions taken."

Piéri gives few details of his work, and no direct judgment can be passed on his methods. What proportion of the eggs developed? The few divisions caused by the distilled water extract can hardly be emphasized, for Piéri found that distilled water alone caused control eggs to become clear and fragmentary. Is it possible, in microscopic examination of myriads of such minute bodies as spermatozoa, to be certain that each individual can be seen? Is the apparent lack of

[1] Piéri: Archives de zoologie expérimentale et générale, 1899, vii; Notes et revue, ix, p. xxix.

motility in those actually observed conclusive evidence of the death of all? Besides, not all of the fluid in use can be examined by means of the microscope. Further, what effect did boiling have on "ovulase"? Was it destroyed at that temperature, as all ferments are? What means were taken to kill the spermatozoa which may have been present in the sea-water used to wash the eggs? These important points Piéri has not considered.

Shortly after Piéri's communication, Dubois[1] presented a brief note of a similar character. Dubois arrived at the conclusion that natural fertilization comes about through the action of a fecundative ferment. He claims that he was able to separate such a body, "d'une zymase fécundante," from the testicles of *Echinus esculentus*, but no experiments showing its qualities were reported by him. Dubois named the ferment (?) "spermase" and credited it with the power of modifying a hypothetical substance pre-existent in the ovum, which he called "ovulose." As long as experimental evidence of the truth of such a conclusion is wanting, it must continue to remain an unsatisfying speculation.

Winkler's[2] experiments were made on *Sphaerechinus granularis* and *Arbacia pustulosa*. Every precaution was taken to prevent the action of live spermatozoa. Winkler made extracts of spermatozoa by shaking them for about half an hour with distilled water (quantities not stated). In order to prevent destructive action on the part of the distilled water, a precaution Piéri had not observed, Winkler added to the extract, before using it on the test ova, a sufficient quantity of evaporated sea-water to make the concentration of the extract the same as that of sea-water ("ca. 4%"). Another kind of extract of sperm was made in the fluid obtained by evaporating 400 c.c. of sea-water to one fourth its volume.[3] The filtered extract was finally treated with enough distilled water to lower its concentration to that of normal sea-water.

[1] Dubois: Comptes rendus hebdomadaire des séances de la Société de Biologie, 1900, lii, p. 197. The author has not had access to the original paper and relies upon the review made of it by Winkler. (Ref. below.)

[2] Winkler: Nachrichten von der königliche Gesellschaft der Wissenschaften zu Göttingen. Mathematisch-physikalische Klasse, 1900, p. 187.

[3] Winkler states that the sea-water he used contained "ca. 4%" of saline matter and that by evaporating 400 c.c. to 100 c.c. he obtained a solution of "ca. 20%." The author fails to see how anything but a 16% solution was obtained if the process was conducted as described. Loeb's experiments have shown how necessary exact knowledge of concentration is in such work.

In both kinds of extract the eggs showed some tendency to segment, but only a few divided.[1] Sometimes with the same extract the eggs of one individual "reacted," while the eggs of another did not. Finally, it is decidedly significant that the proliferation went at most only to the 4-cell stage, and that then separation of the cells occurred from the absence of retaining membrane, and "abnormal" forms resulted. In the control experiments these manifestations were not apparent.

Winkler does not claim that the slight changes he observed were due to an enzyme. He states that he did not determine the effect of heat on the power of his extracts. The nature of the active substance, he says, is completely unknown. It might be reasonable to assume that dissolved nucleoproteid had stimulated proliferation, but it seems much more probable that the initial segmentations Winkler observed were really due to increased concentration and the consequent osmotic conditions, not to ferment action or extractive influences. Errors in making up the saline solutions might of themselves have accounted for all that was observed. A concentration very little above that of normal sea-water would produce the results.[2] Further, it is well known that the eggs of sea-urchins are prone to divide into a few cells if they are allowed to remain undisturbed in normal sea-water for about a day.[3]

Winkler's results are hardly positive enough, therefore, to permit of the deduction he draws; they might, in fact, be used to show how unwarranted were Piéri's conclusions.

Experimental.

General methods of procedure. — The investigations recently done under Professor Loeb's supervision in this connection were conducted with *Arbacia punctulata*. In a few experiments, as will be pointed out, the testes of *Strongylocentrotus purpuratus* were used. Males and females were kept together in a tank in running sea-water until they were needed. Immediately before they were used all extraneous matter was carefully washed off in an abundance of *fresh* water, which killed any adherent spermatozoa. The various instruments employed in the work were repeatedly washed in the same way.

[1] "Nur ein nicht sehr grosser Theil."

[2] Loeb: This journal, 1900, iii, pp. 436 and 437.

[3] Loeb: *Loc. cit.*

The sea-water in these experiments was collected in a large stoppered bottle on one day for use upon the next. This insured the use of the same water for each set of experiments and the corresponding controls. Gemmill[1] has shown experimentally that if free spermatozoa are kept in sea-water (in "*dilute* mixture") for five hours they lose their ability to impregnate the ovum. Consequently our method rendered inert any spermatozoa which may have been alive in the water at the time of collection and made boiling unnecessary. Moreover, Loeb[2] has lately called attention to the fact that sea-urchins have practically died out in the immediate neighborhood of Wood's Holl, and that for this reason, even at the height of the spawning season, there is little or no danger that the supply of sea-water used in this laboratory contains any live spermatozoa of this animal.

In procuring testes or ovaries the oral surface of the animal was cut away and the alimentary and vascular membranes carefully torn out. After thorough flushing in sea-water to eliminate body fluid and dissolved matter such as digestive enzyme, etc., the glands were transferred to perfectly clean vessels for appropriate treatment without delay.

The ovaries, from which the eggs used as indicators were taken, were transferred directly to a shallow dish with just enough sea-water to cover them. In most cases the eggs from one animal were sufficient for a connected series of observations. As a rule the ovaries were full of eggs and mere shaking sufficed to liberate the latter into the surrounding fluid, where a comparatively thick layer quickly formed. A few drops of this sediment, containing thousands of eggs, were sufficient for each individual test. The ovaries were never taken from the animal until all other preparations had been completed, so that the eggs were perfectly fresh when employed.

Only such unfertilized eggs as were found to be normal and mature were used. In each of the series of experiments to be described some of the ova were either fertilized directly with spermatozoa or were first subjected for an hour or two to the influence of solutions of higher osmotic pressure than sea-water (mixtures of 88 c.c. sea-water + 12 c.c. $\frac{20}{8}$ *n* KCl were usually made up for the purpose) and then were placed in sea-water to test their capacity for parthenogenetic division. In many experiments both methods were used.

[1] Gemmill: Journal of anatomy and physiology, 1900, xxxiv, p. 170.

[2] Loeb: *Loc. cit.*, p. 450.

Under these test conditions the eggs employed were always found to develop into swimming larvæ within twenty-four hours. These facts are not specially noted in the records given below because of their uniformity throughout. The "control" tests mentioned with each series refer to the eggs which had been placed only in normal sea-water for comparison with ova treated by special processes.

In each of the following series of experiments the volume of sea-water in each test was, as a rule, 100 c.c. (Note exceptions farther on.) It was increased only by the addition of portions of extract as specified under each series and by the few drops of sea-water carrying the eggs, in pipette, from the main supply. The sea-water was contained in small bowls of uniform size, making the depth of the fluid (about an inch) practically the same for all of the experiments. Throughout each series the bowls were kept covered with glass plates. The air space above the fluid was about an inch in depth, thus insuring abundant supply of oxygen. Occasionally, as will be noted, eggs were placed in quantities of the extract alone, held in smaller vessels. These were also kept covered. The temperature of the room varied between 18–20° C. The amount of evaporation, as indicated by sensible condensation on the under side of the cover-plates, was comparatively slight during twenty-four hours, so that no material concentration occurred during the interval.

The extracts of the spermatozoa were made directly from the testes. It was not thought necessary to attempt separation of the non-spermatic tissue elements. The testes were always thoroughly ground to a thick paste in a mortar with dry sand which had been heated above 100° C. for from fifteen to twenty minutes. Water and saline extracts were used within a few hours. Fluids containing preservatives, however, were given more time for extraction, as will be noted below. The extractions were made in bottles to permit of frequent and vigorous shaking. Clear filtrates were obtained in each case without special difficulty.

In each series of experiments carefully measured quantities of extract were added to sea-water, and the mixtures stirred to prevent inequalities of concentration. The eggs were distributed after the mixtures of sea-water and extract had been made. The experiments were begun in the morning. At intervals of an hour or two until late at night, samples of eggs were quickly removed with pipettes from the bowls to watch glasses for observation under the microscope. Hundreds were examined carefully each time. None were

ever returned to the main supplies. The eggs in each series were always under observation for from at least twenty to twenty-four hours, seldom longer than that, and unless otherwise stated the "results" recorded below are for periods of that length.

Experimental Data.

Our experiments are described here briefly, though in some detail, so that whatever value they may possess may be accurately estimated. The first series of extracts were made with spring water.

Fresh water extract. — *Fresh testes.*— I. The glands from one animal were extracted in 15 c.c. H_2O for 1 hr., 30 mins. Three tests were made as follows: —

(1) Control (2) Extract — 4 c.c. (3) Fresh H_2O — 4 c.c.[1]

Result: No segmentation.

II. The glands from one animal were extracted in 15 c.c. H_2O for 3 hrs.

(1) Control (2) Extract — 2 c.c. (3) Fresh H_2O — 2 c.c.

Result: No segmentation.

III. Glands from two animals in 10 c.c. H_2O for 4 hrs.

A. Control. B. Extract: (a) 1 c.c. (unfiltered), (b) 4 c.c., (c) 0.05 c.c., (d) eggs in 3 c.c. + equal volume of $\frac{1}{8}^{0}$ *n* NaCl. C. Some of (d) into sea-water after 2 hrs.

Result: Irregular parthenogenetic forms in a very small proportion of (a), (b), and (c) after 4 hrs. A few groups of 8 and one or two of 16 cells from individual eggs, in 24 hrs., in (b). None beyond the 4-cell stage in (a) and (c). A few parthenogenetic in C as far as the 8-cell stage. No morulæ in any. No segmentations in the control.

The results of the third series encouraged the belief that enzyme action was demonstrable, although we did not lose sight of the fact that perhaps increased concentrations, induced by unobserved circumstances, or other unknown conditions, would account for the proliferations noted. In the fourth and fifth series the effects of fresh were compared with those of boiled extract.

IV. Five sets of testes extracted in 60 c.c. H_2O for 3 hrs. One half was boiled in an Erlenmeyer flask 10–15 mins. An appreciable concentra-

[1] It will be understood from what was stated on page 58 that this abbreviated reference to the three tests means that besides being under normal conditions (in 100 c.c. sea-water alone), eggs were subjected to the influence of both 4 c.c. of extract in 100 c.c. of sea water and 4 c.c. of fresh H_2O in the same large quantity of sea-water. This system will be adopted throughout for brevity's sake.

tion resulted, but of course no approximation to the specific gravity of sea-water was effected.

A. Control. B. Fresh extract: (a) 10 c.c., (b) eggs in 8 c.c. extract alone. C. Boiled extract: (c) 10 c.c., (d) eggs in 8 c.c. extract alone. D. Samples of B and C in 100 c.c. sea-water after 1 hr., 30 mins.

Result: During the first 12 hrs. there was no segmentation in any of B and C. An occasional kidney-shaped cell was found in the control and D after 5 hrs. At the end of 24 hrs. there were a few 4 to 8 cell divisions in the eggs of (a) and (c) which had been transferred to sea-water. Only a few 2 to 4 cell groups were found in the control at the end of the same period.

V. Testicles from 15 animals extracted in 85 c.c. H_2O for 3 hrs. One half was boiled as in the preceding series.

A. Control. B. Fresh extract: (a) 20 c.c., (b) 10 c.c., (c) eggs in 10 c.c. extract alone. C. Boiled extract: (d) 10 c.c., (e) 8 c.c. D. Eggs in B and C transferred to normal sea-water after 1 hr.

Result: Not a single segmentation could be detected. A very few of the eggs of (d) and (e) which had been transferred to sea-water were kidney-shaped as though in an initial parthenogenetic stage.

The results of the first five series were indecisive, but, where positive, they strongly suggested initial osmotic parthenogenesis, caused probably by conditions beyond control, rather than zymolytic influences. On the assumption that the concentration of the extracts was somewhat lower than sea-water in spite of the salts and proteids dissolved from the testes, and that variations in effects occurred as a consequence, the sixth series was arranged to overcome this difficulty.

VI. Fourteen sets of glands were extracted in 35 c.c. H_2O for 3 hrs. Just before the filtered extract was used it was mixed with an equal volume of normal NaCl, making approximately a $\frac{4}{5}\,n$ NaCl mixture (sea-water is equivalent to about $\frac{5}{6}\,n$ NaCl).

A. Control. B. Extract: 20 c.c., 10 c.c., 1 c.c., eggs in 10 c.c. extract alone. C. Eggs in each of B transferred to 100 c.c. sea-water at the end of 2 hrs.

Result: No divisions or irregular forms.

The generally negative results of the preceding experiments made it seem desirable to resort to other means before abandoning the study of fresh water extracts. Various enzymes are more easily extracted after the containing cells have been dried and thoroughly broken up. This expedient was tried, therefore.

Dry testes. — The glands from each animal were macerated and spread out separately in a thin layer on watch glasses. These were placed in desiccators over concentrated sulphuric acid or calcium chloride. Drying was accomplished within eighteen hours. When desired for use the dry substance was scraped into a mortar, and ground up thoroughly with sand and extracted as in the previous experiments.

VII. The dry substance of four sets of glands was extracted in 30 c.c. H_2O for 3 hrs.

A. Control. B. Extract: (a) 5 c.c. (unfiltered), (b) 10 c.c., (c) 5 c.c., (d) 1 c.c., (e) eggs in extract + equal volume $\frac{1}{8}^0$ *n* NaCl.

Result: Within 12 hrs. no change. At the end of 24 hrs. a very few were in initial parthenogenetic stages, 2 to 4 cell groups, in all except (a). They could be found only after careful search and there were as many in the control as in any of the others.

VIII. Eight sets of dried testes in 25 c.c. H_2O for 4 hrs. Filtrate mixed with an equal quantity of $\frac{1}{8}^0$ *n* NaCl before using.

A. Control. B. Extract: 7 c.c., eggs in extract alone. C. Some of the eggs in B were transferred to 100 c.c. sea-water after 1 hr., 45 mins.

Result: No segmentations or parthenogenetic forms in any.

It seemed necessary to conclude at this point that fresh water extracts of spermatozoa do not contain substance of zymolytic power or else that the conditions attending their use are unfavorable to such manifestation. Enzymes which are soluble in water are also soluble in solutions of electrolytes, so that attempts were next made with the latter.

Salt water extract. — A common method of extracting enzymes includes treatment of the tissue with ordinary salt solution. Sea-water itself furnishes such a dilute solution, but is not so favorable to rapid destruction of spermatozoa as fresh water or stronger salt solution. Since spermatozoa pass through ordinary filter paper, however often they may be subjected to filtration, it was necessary in using fresh testes to give particular attention to killing the spermatozoa by mechanical means. Prolonged grinding in a mortar with fine sand, as had been done previously, followed by continuous shaking for several hours, accomplished this.

Fresh testes. IX. Twelve sets of glands were extracted in 50 c.c. sea-water for 4 hrs.

A. Controls (2). B. Extract: 20 c.c., 10 c.c., 5 c.c., 1 c.c., 0.25 c.c.
Result: Not a single division could be found.

The very greatest care is necessary, in this connection, in the use of solutions of electrolytes, because of the ready osmo-parthenogenetic response the eggs make to slightly increased concentration. There is little reason for believing that an enzyme is present in spermatozoa which is insoluble in dilute, but soluble in strong salt solution. Therefore it seemed unnecessary to try the effect of more concentrated extractive. The tenth series shows the result of an effort to make the best of saline extraction of fresh testes, however, in a way somewhat different than that of the preceding.

X. Eight sets of testes in 40 c.c. $\frac{5}{8}$ *n* NaCl for 2 hrs. One half was warmed to 35–40 C. 15–20 minutes.
A. Controls (2). B. Extract (unwarmed): (a) 5 c.c., (b) eggs in 5 c.c. extract alone. C. Extract (warmed): (c) 5 c.c., (d) eggs in 5 c.c. extract alone. D. Some eggs of B and C in 100 c.c. normal sea-water after 2 hrs.
Result: No segmentation within 6 hrs. In 12–24 hrs. a very few 2-cell groups were found with difficulty in (a), (b), and (c) and in one of the controls.

Dry testes. The preliminary process of drying was also resorted to in this connection.

XI. Dry material from three animals was extracted in 5 c.c. sea-water for 2 hrs.
A. Control. B. Extract: 2 c.c. (unfiltered), 1 c.c., 0.25 c.c.
Result: Not a sign of segmentation.

Do the extracts possess poisonous qualities? — One condition that may appear to be against the action of an enzyme in the extracts used in these experiments is the possible presence of poisonous substances in the extract. This question now required a definite answer. We had varied the quantities of extract considerably, between all reasonable extremes, in the belief that the most favorable amount might be indicated, but it will be observed from the foregoing account of results that no such relation was suggested. The eggs which had been subjected to the extracts alone, and those placed in sea-water with the greater proportions of extract, usually showed abnormalities after a few hours, such as the development of enclosing membrane or transparent periphery (thicker and not comparable to the "vitelline" membrane after fertilization), swelling, disintegration, discol-

oration, agglomeration of pigment, etc., but none of these changes were constant so far as their relation to observed conditions could be determined. The sperm extracts contained salts and dissolved proteids, of course, and it would be reasonable to assume that these bodies were present in larger proportion, in some of these experiments at least, than they ever are under normal conditions of fecundation.

This important matter was definitely tested several times. The following results of two experiments are cited to show the facts in the case:

A. Five sets of fresh testes were ground in the usual way and extracted for 2 hrs. in 30 c.c. fresh water. An equal quantity of $\frac{1}{8}^{0}$ *n* NaCl was added to the filtrate. The eggs were placed in this mixture and samples transferred at intervals of an hour to 100 c.c. sea-water, to which fresh spermatozoa had been added. Results of examination at the end of 24 hours, the numerals indicating the number of hours the eggs were kept in the extract: (1) Swimming gastrulæ. (2) Blastulæ (none alive). (3) A few dead blastulæ, mostly morulæ. (4) Many unsegmented, none beyond the 32-cell stage. (5) About the same as those after the 4-hr. treatment. (6) Very few went so far as the 32-cell stage, many were in the 4 to 8 cell groups. There were no segmentations in the eggs kept for 24 hrs. in the extract.

B. Six sets of fresh glands were extracted in 30 c.c. sea-water, 3 hrs. Eggs from one animal were placed in the filtered extract and also into an equal quantity of sea-water (as control). At intervals eggs were withdrawn from each supply and transferred to 100 c.c. sea-water containing perfectly fresh spermatozoa. Results at the end of 36 hours from the time of the first transferral, the numerals again indicating the number of hours the eggs were under the direct influence of the extract or the normal sea-water: (1) Plutei in each. (2) Advanced gastrulæ in each. (3) Gastrulæ in each. (4) Many gastrulæ in the control; hardly any live ones, mostly morulæ, among those treated with the extract. (7) A large number of blastulæ were present in the control, but no divisions beyond the 32-cell stage could be found among the eggs which had been in the extract; most of the ova were unsegmented. There were no proliferations in the eggs retained in the extract itself. In the earlier tests the proportion of unsegmented cells was uniformly greater in the control than in the other series, whereas the living larvæ were relatively more numerous in the latter. The extract seemed at first to stimulate, and later to inhibit karyokinesis. Possibly, however, the accumulation of bacteria in the bowls containing extract was responsible for the latter effect.

It is clear, from the foregoing, that the dissolved substances of our extracts have not prevented the eggs from segmenting. From this

we may safely conclude that they doubtless would not interfere with zymolysis if such were demonstrable.

The results of all the preceding series seemed to point in the same general direction and to indicate no mitotic action. Before accepting this negative conclusion, however, we proceeded to employ various other familiar methods for the separation of enzymes in the hope of eventually extracting and demonstrating the presence of such a substance.

Extract of spermatozoa which had been treated with, and preserved in alcohol. — Enzymes may readily be extracted from tissues hardened in alcohol. In fact they are frequently isolated by such preliminary treatment, which brings about disintegration of the cellular protoplasm as well as coagulation of soluble proteid, and thus diminishes the proportion of undesirable extraneous material in the final extract. Through the kindness of Professor Loeb, I was enabled to make extracts of the spermatozoa of *Strongylocentrotus purpuratus*, which had been preserved in an excess of 95% alcohol. The testes were taken from animals collected on the Pacific Coast about a year ago, while Professor Loeb was engaged there in his classical researches on artificial parthenogenesis.

In these experiments, with Arbacia as well as Strongylocentrotus, the alcoholic sperm mixture was filtered. Both the solid and fluid portions were transferred to shallow dishes and dried in the air. The liquid soon evaporated and left an oily residue which dissolved to a milky fluid when mixed with water.

Strongylocentrotus purpuratus. XII. Three grams of the dry sperm residue were thoroughly ground with sand and 30 c.c. fresh H_2O. After an hour an equal volume of $\frac{1}{8}$ *n* NaCl was added. Extraction in this mixture was continued an hour.

A. Control. B. Extract: 17 c.c., 7 c.c., and eggs in 8 c.c. of extract alone. C. Some of the eggs in each of B were transferred to 100 c.c. sea-water after 3 hrs.

Result: Not the slightest trace of segmentation.

XIII. Two grams of the finely divided dry substance were extracted in 40 c.c. sea-water for 3 hrs.

A. Control. B. Extract: (a) 12 c.c., (b) eggs in 10 c.c. extract alone. C. Eggs from B transferred to 100 c.c. normal sea-water after 2 hrs., 15 mins.

Result: Only a few forms in initial parthenogenesis in the control and in (a). These were found only after very careful search. Entirely negative results in the others.

It did not seem very likely that the alcoholic filtrate would contain a mitotic enzyme, if such a substance could not be extracted from the portion insoluble in alcohol. Yet, since some enzymes are soluble in diluted alcohol, the following experiments were made in order to ascertain definitely.

XIV. Half the residue of evaporated alcoholic extract was dissolved in 40 c.c. sea-water and filtered.

A. Control. B. Extract: (a) 15 c.c., (b) 5 c.c., (c) eggs in 20 c.c. of the extract alone. C. Eggs from (c) were transferred to 100 c.c. sea-water after 1 hr., 30 mins.

Result: Within 6 hrs. no perceptible effect. At the end of 18 hrs. a number of irregular parthenogenetic forms and some groups of 4 and 8 cells in C. No traces of segmentation in any of the others.

XV. The result in the preceding series seemed to be due to increased concentration caused by the accumulated salts of the original alcoholic extract. If this assumption were correct, dilution of the extract should prevent the effect noticed above. Only a fourth of the residue was next dissolved in 50 c.c. sea-water.

A. Control. B. Extract: (a) 20 c.c., (b) eggs in 20 c.c. extract alone. C. Samples of B were transferred to 100 c.c. sea-water after 2 hrs.

Result: Only a very few irregular shapes in the control and the transferred eggs of (b). One 4-cell group was found among thousands in the control; none among the others even after prolonged search.

XVI. A third experiment was made with the alcoholic residue. The solution was made more concentrated again. The remaining portion (one fourth) of the evaporated extract was dissolved in 15 c.c. sea-water.

A. Control. B. Extract: (a) 8 c.c., (b) eggs in 5 c.c. extract alone. C. Samples of each of B transferred to 100 c.c. sea-water after 3 hrs.

Result: Parthenogenetic groups of small cells in the transferred eggs of (b), but nothing of the sort in any other.

The results of the last three series emphasize the necessity of preventing material change in the composition of the sea-water and suggest how easy it might be, in cases of slightly increased concentration to mistake ion parthenogenesis for enzyme proliferation.

Arbacia. Twenty-one sets of testes were treated with 500 c.c. 95% alcohol. After remaining in contact with the latter for two days the solid substance was collected on a filter.

XVII. The dry solid matter was thoroughly extracted in 100 c.c. sea-water for 12 hrs.

A. Control. B. Extract: (a) 25 c.c., (b) 15 c.c., (c) 10 c.c., (d) 5 c.c., (e) 1 c.c., (f) 0.5 c.c. C. Samples of B transferred to 100 c.c. sea-water after 2 hrs.

Result: A very small percentage of 2-cell groups was found in the control, in (b) and among those of (d) which had been transferred to normal sea-water. One 2-cell segmentation had been found among the normal eggs immediately after they had been taken from the ovaries.

XVIII. In 24 hours the alcoholic filtrate (500 c.c.) had evaporated to 30 c.c. Practically all the alcohol had disappeared. The residue was made up to 100 c.c. with sea-water and filtered.

A. Control. B. Extract: 25 c.c., 15 c.c., 10 c.c., 5 c.c., 1 c.c. C. Samples of B transferred to 100 c.c. sea-water after 2 hrs.

Result: An occasional 2 to 4 cell group in practically all including the control — less than 2 per 100.

Glycerine extract. — Glycerine in water seems to be one of the best of enzyme extractors. Extracts of fresh Arbacia sperm were made by the previous general process in mixtures of equal parts of glycerine and water. It has been assumed, of course, that the glycerine in such extracts would exert specific deleterious effects and naturally careful control experiments were made to ascertain its influence in the quantities used in this series. These preliminary control tests determined the influence of glycerine under three general conditions: (*a*) its direct effect on the eggs, (*b*) its influence on normal fecundation, (*c*) its action on artificial parthenogenesis.

An abundant supply of equal parts of glycerine and sea-water was made for use in all these experiments. Normal eggs were found to remain unsegmented in all proportions of this glycerine solution with sea-water, although a few irregular parthenogenetic forms were produced by 15 c.c. in 100 c.c. normal sea-water. Quantities of this glycerine solution greater than 5 c.c. in 100 c.c. of sea-water prevented the normal segmentation by spermatozoa, but many swimming larvæ formed in the presence of 2 c.c. of the glycerine solution per 100 c.c. sea-water. Even 15 c.c. of the glycerine solution in 100 c.c. of sea-water did not, however, entirely prevent proliferation in ova which had previously been kept for 2 hrs. in 88 c.c. sea-water + 12 c.c. $\frac{3}{8}$ *n* KCl, yet none of the segmentations under these conditions went beyond the 8 to 16 cell stage. With smaller quantities, swimming larvæ were obtained.

With these facts established the result of the following experiments are not without significance.

XIX. Seventeen sets of testes in 75 c.c. of the above glycerine solution for 48 hrs.

A. Control. B. Extract: (a) 15 c.c., (b) 5 c.c., (c) 2 c.c. C. Samples of each of B transferred to 100 c.c. sea-water after 1 hr.

Result: Here and there a kidney-shaped cell was found among those of (a) which had been transferred to normal sea-water. No distinct segmentations.

XX. Same glycerine extract after having been shaken with the tissue 24 hrs. longer.

A. Controls (2). B. Extract: 5 c.c., 2 c.c., 0.5 c.c., 0.25 c.c. C. Some of each of B transferred to 100 c.c. sea-water after 1 hr.

Result: Not the slightest suggestion of segmentation.

XXI. Twenty sets of testes were extracted in 80 c.c. of the glycerine solution four days. The filtrate was poured into a litre of 95% alcohol. A bulky, though light, white flocculent precipitate formed at once. After 24 hrs. this precipitate was filtered off, treated with 25 c.c. of sea-water for several hours and the filtrate used in the following experiment:

A. Control. B. Extract: (a) 10 c.c., (b) 5 c.c., (c) 2 c.c., (d) 1 c.c., (e) 0.25 c.c. C. Samples of each lot of B transferred to normal sea-water after 2 hrs.

Result: One or two irregular parthenogenetic forms were found in (c) and among those of (a) which had been transferred to normal sea-water. The number of such was less than 5 per 1000.

Ether extract. — Substances which cause the death of the cell or which appreciably lessen its vitality are known to favor solution of enzyme into the surrounding medium. Small quantities of alcohol or ether effect such results. Mathews[1] has recently shown that exposure of the unfertilized eggs of Arbacia to a saturated solution of ether in sea-water for ten to fifteen minutes leads to karyokinetic division of nearly all the eggs. In the use of ether in these experiments the greatest care was taken, therefore, to ascertain the influence of ether in the small quantities employed.

A solution for general use in this connection was made by mixing sea-water and ether in the proportion of 100 c.c. of the former and 7 c.c. of the latter. This amount seemed sufficient for any extractive usefulness ether might possess here. Intimate solution resulted. The odor of ether from the solution was still quite distinct at the conclusion of the experiments, though not strong at

[1] Mathews: This journal, 1900, iv, p. 345.

any time. In three control experiments, similar to those outlined under the head of glycerine extract, it was found that as much as 15 c.c. of this ether solution failed to effect parthenogenesis, although after eighteen hours a few 2-cell groups and irregular forms suggesting an initial stage of mitosis were found. As these were also present in the control, however, no importance could be attached to the result. After the usual treatment with sea-water plus $\frac{2}{8}$ *n* KCl, swimming larvæ developed when the eggs were transferred to 100 c.c. of sea-water containing as much as 25 c.c. of the ether solution. The same result was obtained, with as much ether solution present, when spermatozoa were added to the eggs in 100 c.c. of sea-water.

XXII. Ten sets of fresh testes were extracted in 60 c.c. of the ether solution for 3 days.

A. Control. B. Extract: 25 c.c., 15 c.c., 5 c.c., 1 c.c., 0.25 c.c. C. Some of each lot of eggs in B transferred to 100 c.c. normal sea-water after 2 hrs.

Result: During the first 12 hrs. no changes were manifested. At the end of 24 hrs., however, all, including the control, had a few 2 to 4 cell groups. The effect was not at all striking; it required careful search to find any signs of proliferation.

XXIII. The same extract, after having been 24 hrs. longer in contact with the tissue, was again employed.

A. Control. B. Extract: 4 c.c., 2 c.c., 0.5 c.c. C. Eggs from each of B placed in 100 c.c. normal sea-water after 1 hr., 30 mins.

Result: No sign of segmentation.

Alcohol extract.— Mathews[1] has also shown that alcohol affects Arbacia eggs much as ether does. He found that when the ova are placed in sea-water containing 4 to 5 parts of alcohol and are left there for from ten to fifteen minutes, they segment into several cells when they are replaced in sea-water. In these experiments, care was taken, therefore, to determine precisely the influence of the smaller quantities of alcohol employed.

A general supply of 10% alcohol in sea-water was kept for the experiments. Quantities not over 25 c.c. of this dilute alcohol, added to 100 c.c. of sea-water, were without mitotic influence. As much as 15 c.c. in 100 c.c. of sea-water interfered to no appreciable extent either with normal fertilization or osmotic parthenogenesis, as swimming larvæ developed within the usual period in both cases.

XXIV. Testes from 12 animals in 60 c.c dilute alcohol solution 48 hrs.

A. Controls (2). B. Extract: (a) 25 c.c., (b) 15 c.c., (c) 5 c.c.,

[1] Mathews: *Loc. cit.*, p. 346.

(d) 2 c.c., (e) 0.5 c.c. C. Some of each of B in 100 c.c. normal sea-water after 1 hr., 30 mins.

Result: No appreciable effect in any during the first 12 hrs. At the end of 24 hrs., however, several 2, 3 and 4 cell groups were found in both controls and also in each of those transferred to sea-water. The eggs of (d) which had been put into sea-water had a relatively larger proportion that showed initial division, although the actual number was in reality small — less than 10 in 1,000.

XXV. Some of the filtrate used in the preceding series was taken to repeat a part of the experiment just described.

A. Control. B. Extract: 2 c.c. C. Eggs from B into 100 c.c. sea-water after 1 hr., 30 mins.

Result: No divisions at any time within 24 hrs.

XXVI. Seven sets of testes in 10% alcohol 4 days.

A. Control. B. Extract: (a) 15 c.c., (b) 8 c.c., (c) 2 c.c. C. Some of the eggs of each of B in 100 c.c. normal sea-water after 2 hrs.

Result: Negative during the first twelve hours. At the end of 24 hrs. there were a very few 2 and 4 cell groups in the control and among those of (a) which had been transferred. No effect in any of the others.

Alkaline extract. — Many enzymes show their greatest activity in media which are either acid or alkaline. Fluids of either reaction are also especially efficient in transforming zymogens into enzymes. If the latter cannot be extracted from spermatozoa, as the preceding results may be taken to indicate, might not zymogens be detected?

Loeb[1] found, in his experiments on Echinoderms and Annelids that the addition of a small quantity of acid or alkali caused the unfertilized eggs to segment much more quickly than when they were left in normal sea-water. NaOH seemed less effective than KOH, but some development occurred in the presence of as little as 2 c.c. $\frac{n}{10}$ NaOH in 100 c.c. sea-water. Great care had to be exercised here, therefore. Proportionately smaller amounts were used as a safeguard.

A saline solution was made for this series containing 8 c.c. of $\frac{n}{10}$ NaOH for every 100 c.c. $\frac{3}{8}$ *n* NaCl. This solution was faintly though distinctly alkaline and could hardly be considered destructive to any enzymes in the cells. In control experiments similar to those conducted previously to ascertain the influence of foreign substances it was found that as much as 25 c.c. of this

[1] Loeb: This journal, 1901, iv, p. 438; also *Ibid.*, 1900, iii, p. 136.

solution when added to eggs in 100 c.c. of sea-water caused only a few initial segmentations and that comparatively slight influence was exerted either on osmotic parthenogenesis or spermatic proliferation by the same quantity.

XXVII. Twenty sets of testes in 100 c.c. alkaline solution 24 hrs.

A. Controls (2). B. Extract: 25 c.c., 10 c.c., 5 c.c, 1 c.c.

C. Some of each of B in 100 c.c. normal sea-water after 1 hr.

Result: Not a single division.

Extract made in fluid of alternate reaction. — XXVIII. With a view of aiding still further the transformation of any zymogen not affected by previous extractions, twelve sets of testes were macerated in the usual way and allowed to remain in the mortar, covered with a glass plate, for 12 hours. The normal alkaline reaction of the fresh tissue became faintly acid to litmus during that interval. 25 c.c. of fresh water was added, the mixture neutralized and then made faintly alkaline with $\frac{n}{10}$ NaOH and repeatedly shaken up in this mixture for about 6 hours. Finally it was neutralized with very dilute HCl and the filtrate mixed with one-third its volume of 2 *n* NaCl to bring the concentration of the extract close to that of ordinary sea-water.

A. Controls (2). B. Extract: (a) 20 c.c., (b) 10 c.c., (c) 1 c.c.

C. Samples of B in 100 c.c. normal sea-water after 1 hr., 30 mins.

Result: No effect during the first twelve hours. At the end of 24 hrs. only an occasional 2-cell division could be found in (c) and among those of (a) which had been transferred.

The persistently negative results of the preceding experiments, in which the existence of neither an enzyme nor a zymogen could be indicated, gradually developed the idea that possibly an enzyme is formed from material in the egg, or in the sperm, or in both, on contact of the two living elements. If such were really the case it would seem that extracts of the eggs which had been normally fertilized might, under appropriate conditions, possess the power of inducing segmentation in unfertilized ova.

Extracts of fertilized eggs.—The general experimental procedure by which this matter was investigated was essentially the same in some respects as for the preceding series. The fresh full ovaries were broken up in sea-water in shallow dishes. Only sufficient ova were kept in each dish to form a single layer at the bottom. The glandular tissue, with such eggs as remained entangled in it, was withdrawn. A minute quantity of fresh spermatic fluid was thrown into 100 c.c. of sea-water and a few drops of this mixture transferred to the dishes containing the eggs. Within a few hours practically all of the eggs were developing and some spermatozoa in excess were in active motion among them.

When the eggs were desired for extraction the fluid containing them was

thrown into a large funnel, the outlet of which was closed with a stopper. The eggs quickly converged to the neck and soon settled to the bottom of the tube in a thick layer, with a clear supernatant fluid. Practically all of this could be eliminated by decantation, leaving a thick mass of eggs in only a small quantity of fluid. The whole process of collection could be completed in two hours. The segmented eggs were finally thoroughly ground with sand and appropriately extracted.

Glycerine extract. — XXIX. Eggs from 15 females, many of which had developed to the 16-cell stage, were ground, in small quantities, with 30 c.c. sea-water and 30 c.c. pure glycerine. They were repeatedly shaken in this mixture. At the end of 24 hours the eggs were considerably swelled and distorted, but were little disintegrated, in spite of the grinding. The latter process was repeated. More of the eggs were broken up, but many were held intact by the fertilization membrane. The extraction process was continued 36 hours longer, by which time at least half of the eggs were still unbroken, though distended. A clear filtrate was obtained.

A. Controls (2). B. Extract: (a) 12 c.c., (b) 8 c.c., (c) 4 c.c., (d) 1 c.c., (e) 0.25 c.c. C. Some eggs in each of B were transferred to 100 c.c. normal sea-water after 2 hours.

Result: No segmented cells were found in any except (d). After 12 hours 3 or 4 irregular 2 to 4 cell groups could be found among thousands after diligent search.[1]

Saline extract. — XXX. Eggs from 20 females. Development was allowed to continue until the more advanced had reached the morula stage, when only a very few remained unsegmented and the majority were at or beyond the 8-cell proliferation. They were ground up in 40 c.c. of fresh water, to which 40 c.c. of $\frac{1}{8}^{0}$ *n* NaCl was added later. Extraction was continued 36 hours. At the end of that time many groups of cells remained tightly held together in the enclosing membrane; thorough grinding had not sufficed to disintegrate them as completely as was desired.

A. Controls (2). B. Extract: (a) 35 c.c., (b) 20 c.c., (c) 10 c.c., (d) 5 c.c., (e) 1 c.c. C. Some of the eggs of each of B transferred to 100 c.c. of sea-water after 2 hrs.

Result: Negative at first. After 12 hrs. occasional irregular forms in initial cleavage were found among thousands in one of the controls, in (b), (c), (d), and among those of (a), (b), (c), and (e), which had been transferred to normal sea-water — just such forms as are sometimes found among normal unfertilized Arbacia eggs which have been kept undisturbed in sea-water for about 24 hours.

Alcoholic extract. — XXXI. Eggs from 18 sets of ovaries, after segmenta-

[1] The extracts of the fertilized eggs were no more destructive to the test-eggs than the sperm extracts had been. See page 63.

tion had proceeded in many to the blastula stage, were ground in 20 c.c. of sea-water and extracted in this fluid plus 20 c.c. of 20% alcohol. Extraction was continued for 48 hours. The alcohol favored complete disintegration, for before 24 hours practically all of the cells were reduced to granules.

A. Controls (2). B. Extract: (a) 15 c.c., (b) 8 c.c., (c) 5 c.c., (d) 1 c.c. C. Some of each of B transferred to 100 c.c. normal sea-water after 2 hrs.

Result: After 12 hrs. a small number of cells in irregular initial segmentation were found among those of one of the controls, also in (d) and among those of (a) which had been transferred to sea-water. The number was less than 10 in 1,000.

Discussion of Results.

The chief feature of the results we have obtained is their negative character. Occasionally segmentations were noted, but these were few and rarely went beyond the 2-cell stage. Further, when the test-eggs segmented those of the controls did also. These few divisions could not have been due to spermatozoa, since not a single group was surrounded with the fertilization or so-called "vitelline" membrane, whose absence, Loeb[1] has indicated, practically proves non-spermatic influence. Thousands of eggs in the control and extract series were carefully examined in each experiment and yet only a trifling proportion showed initial segmentation; excepting very few, none of these went as far as the 8-cell stage; and no morula or swimming larva was ever seen.

The conditions of the experiments were made as nearly normal as possible and every precaution was taken to guard against evaporation. Special ion parthenogenesis was entirely excluded, therefore. All of the eggs were ascertained to be ripe and susceptible to segmentation influences. Sufficient variety of extraction process was employed to guard against failures in withdrawal method and the many experiments excluded accidental sources of error. It seems necessary to conclude, therefore, that the occasional segmentations in initial stages that were observed were only such as have repeatedly been seen in ripe unfertilized Arbacia eggs which have been exposed to sea-water for from twelve to twenty-four hours.[2]

I have not exhausted the means commonly used for enzyme extraction. The time at my disposal for this work, and the facilities of

[1] Loeb: This journal, 1901, iv, p. 454.

[2] Loeb: *Ibid.*, 1899, iii, p. 136; 1900, iii, pp. 436 and 437.

this laboratory, have not favored the trial of every known method nor attempts to devise new ones. It may be that sperm enzyme is as intimately connected with the structural elements of the cell, and as resistant to extraction processes, as Fischer has found the inverting ferment of *Monilia candida* to be. Buchner's experience with zymase has not been overlooked, nor the suggestions it offers ignored. However, unless the hypothetical sperm enzyme were very different from most of the others, the numerous methods employed would have succeeded in bringing it to light, if any enzyme action can be exerted by substance in fluids surrounding the ova.

It should be recalled in this connection that Loeb[1] has recently made a series of experiments with various foreign enzymes to determine proliferative power on unfertilized Arbacia eggs, but with negative results. He states that "the only enzyme that caused the egg to segment at all was papain," but he could not be certain that this was not due to some accidental constituent of the sample of enzyme used. "The other enzymes were absolutely without effect." Two years ago Mathews, in some unpublished experiments cited by Loeb,[2] tried the effect of rennin on unfertilized eggs of the sea-urchin. The eggs were placed in sea-water solutions of rennet tablets for a while and then transferred to normal sea-water, when segmentation into a comparatively small number of cells resulted. The effect closely resembled those previously described by Morgan,[3] and Mathews concluded that the results noted had been produced not by the enzyme, but by the salts in the tablets increasing the concentration of the water.

Negative results rarely justify sweeping deductions. The outcome of these experiments, negative in detail, rather emphasizes possibilities which have not yet been specially considered. It may be that either too much extract was employed in each series for positive results to occur or else possibly not enough was taken. Such possibility led to the wide variations of quantity and condition in these experiments, but as no differences were noted between the effects of the largest as contrasted with the smallest proportions of extract, the results afford no conclusive answer in this connection.

Again, since enzymes are indiffusible, or, at most, are only very

[1] Loeb: This journal, 1901, iv, p. 456.

[2] Loeb: *Ibid.*, 1900, iii, p. 437.

[3] Morgan: Archiv für Entwickelungsmechanik der Organismen, 1899, viii, p. 448.

slightly diffusible, it is possible that, in experiments of the kind conducted by Loeb, Mathews, Piéri, Winkler, and myself, enzyme which may be contained in the extract does not or cannot enter the substance of the ovum. It might be assumed that mere contact with enzyme in such solution would not cause segmentation and that, even if the peripheral portions of the cytoplasm should be directly affected by such immersion, the general effect would be entirely different if contact, or diffusion, occurred within the substance farther toward the nucleus. Further, may not the morphological character of the spermatozoön, specially adapted as it is for great motility and penetration, imply that segmentation by indiffusible enzyme, contained in fluid surrounding the ovum, is no more possible in artificial than in normal fecundation. If it be ever found that enzymes, or zymogens, are causative influences in natural fertilization, I venture to predict, in view of the results of these experiments, that their action will also be shown to depend on their direct delivery to points *within* the ovum.

The results of this work do not warrant any additions to current speculations on the mechanism of fertilization, but a recent suggestion may seem to be connected with these results and therefore should be considered here.

Loeb,[1] referring to his experiments with Echinoderms and Annelids, has expressed the view that "the spermatozoön can no longer be considered *the cause* or *the stimulus* for the process of development, but merely an agency *which accelerates a process that is able to start without it*, only much more slowly." Accordingly it may be assumed that "the spermatozoön carries a catalytic substance into the egg." Loeb considered that enzymes and ions may be among these "catalytic substances."

If ions are to be reckoned among the agents of proliferation, why it may be asked, did they not make active the sperm extracts used in these experiments? But what is the proportion of dissociated electrolyte in the spermatozoön and in such extracts, it may be inquired in return? The composition of the ash does not furnish an accurate idea of the amount in the spermatozoön of salts pre-existent as salts and *dissociable* in extracts. Arbacia spermatozoa have not been analyzed in this connection nor the amount of *dissociated* electrolytes in these extracts determined. We know little of the relative proportions of the various constituents of spermatozoa and ova. As

[1] Loeb: This journal, 1901, iv, p. 456.

we have no knowledge of the absolute or relative quantity of free *ions* entering or acting within the ovum, we therefore know nothing of the influence or sufficiency in this connection of the methods used in these experiments. Further, the ions which become active in the ovum may be originally a part of the molecules of the proteid compounds of the ovum or of the sperm, or of both, until the sperm mingles with the protoplasm of the ovum and forms new and probably simpler combinations. These experiments were neither intended for, nor were their conditions suited to an investigation of these particular problems. The results therefore cannot be interpreted as having any bearing on them.

It may not be amiss to state, before concluding, that Vigier's[1] assumptions that unfertilized eggs of Arbacia develop into swimming larvæ in normal sea-water were invariably contradicted by my numerous experiments. Vigier says he was unable to repeat Loeb's results on artificial parthenogenesis. I have often used Loeb's methods with success in order to determine the responsive character of the eggs used in the extract series.[2] Swimming larvæ can be produced and reared to the pluteus stage with ease.

Summary of Conclusions.

The positive experimental results of Piéri should be attributed to the action of spermatozoa which had not been removed from the extracts.

Winkler's uncertain results were doubtless the effects of osmotic influences.

Extracts of the spermatozoa of Arbacia, which have been made by the ordinary methods for the preparation of enzyme solutions, and used in the proportions and under the conditions of these experiments, do not possess any power of causing proliferation of the ripe ovum.

No evidence could be furnished of the existence of a zymogen in spermatozoa.

Extracts of fertilized eggs in the earlier stages of development seem likewise to be devoid of any segmental activity.

The extracts did not produce the typical peripheral "vitelline" membrane always formed immediately in Arbacia eggs, on fusion of the male and female elements.

[1] See Loeb's criticism: This journal, 1901, iv, p. 454.

[2] See references in this connection on p. 57.

These negative results cannot be put forward as proof that there are no enzymes in spermatozoa which function during the normal process of fertilization. They do not show that enzyme action is impossible after, or at the time of union of the spermatozoön with the ovum within the latter, although the results of Series XXIX–XXXI might be interpreted as suggesting that enzymes are not thus elaborated.

In conclusion I wish to thank Professor Loeb not only for the suggestions which led me to undertake these experiments, but also for much kindness and encouragement.

CONCERNING THE POISONOUS EFFECT OF PURE SODIUM CHLORIDE SOLUTIONS UPON THE NERVE-MUSCLE PREPARATION.[1]

By HARVEY CUSHING.

[*From the Physiological Institute of Bern.*]

IT was an accidental discovery in the preparation of so-called physiological salt solution that, when sodium chloride was added to tap-water drawn from a certain source of supply, the solution was more efficacious than a corresponding one made from distilled water. Upon investigation the tap-water was found to be rich in calcium and potassium salts.

To Sidney Ringer is due the credit of recognizing this and of appreciating the significance of the fact that minute amounts of these salts would antagonize the injurious effects upon animal tissues of the pure sodium salt alone. The results given in his remarkable series of papers in the Journal of Physiology since 1880 have received confirmation on many sides, though the views as originally expressed in explanation of the process have of necessity undergone some alteration.

More recently the extraordinary series of papers by Loeb[2] and his pupils has further laid especial emphasis on the directly injurious or "poisonous" effects of the pure sodium solution when used alone. His study, originally directed toward certain low forms of marine life, has shown that the pure solution of sodium chloride, even though isotonic with the sea-water from which the animals were taken acts as a strong poison whose effects are due to the unantagonized sodium ions. The same is true of an equimolecular solution of any individual salt, as calcium chloride, etc. The presumptive explanation is that the various ions form injurious combinations with the proteids of the tissues and in order to prevent this it is essential to have a medium

[1] It is a pleasure here to express my gratitude to Professor Kronecker for giving the incentive which led primarily to the making of these observations, and as well to Dr. Asher for many helpful suggestions during their progress.

[2] Loeb: This journal, iii, iv, and v.

in which an interchange of ions between the protoplasm and the solution no longer takes place. It is the existence of such a medium which accounts for the superiority of the so-called Ringer's solution. This fact has led Loeb[1] to remark that his experience would seem to necessitate "the introduction of a new conception, namely that of *physiologically balanced salt* solutions"; meaning by this "salt solutions which contain such ions and in such proportions as to completely annihilate the poisonous effects which each constituent would have if it were alone in solution."

Howell[2] and Greene[3] in recent papers have laid stress on the fact that the calcium salt stands in an especial relation to the calling out of cardiac contraction, and some writers go so far as to state that it is the presence of this salt alone in the blood serum which gives to it its superiority as an infusion material. Howell has brought out the interesting fact that the gradual increase in the percentage of calcium chloride in solutions in which a batrachian heart has become exhausted will reawaken its spontaneous contractility. The relation of nourishment to the prolongation of cardiac activity was first pointed out in 1875 by Kronecker and Stirling. At the same time these authors laid stress rather upon the general properties of blood than upon its saline constituents. However this may actually be, experience has shown that a more perfect supporting fluid for an isolated heart or muscle is furnished by the blood serum of certain animals than by any known saline combination.

Brunton and Ringer[4] have made some comparisons between the influence of these various solutions upon cardiac muscle and the ordinary striped muscle of the extremities.

Ringer and Locke together subsequently showed that sodium chloride solutions would influence and produce variations in the contraction curve of a muscle and that certain electrical phenomena would also be called out. Locke,[5] furthermore, in a brief provisional note has published the results of some observations upon the effect of placing an isolated sartorius muscle in baths of various salt solutions. His observations seem to have been complicated by the

[1] Loeb: This journal, 1900, iii, p. 445.

[2] Howell: *Ibid.*, 1898, ii, p. 47.

[3] Greene: *Ibid.*, 1898, p. 83.

[4] Ringer: Journal of physiology, 1887, viii, p. 22. Ringer and Buxton: *Ibid.*, p. 288.

[5] Locke: Centralblatt für Physiologie, 1894, viii, p. 166.

appearance of muscular fibrillations such as are usually produced by the action of pure solutions of sodium chloride. He also directs attention to the persistence of direct irritability after stimulation from the nerve has ceased to call forth contractions, and also that the injurious effect of the pure solution of sodium chloride could largely be counteracted by the presence in the bath of the proper proportions of calcium and potassium salts. So far as I know, his detailed report has not been published.

The observations which the writer will briefly recount are in a measure confirmatory of the results which Locke has summarized. The results, however, may have been more precise, inasmuch as they have been obtained largely by infusion methods in a comparatively intact animal rather than by simply immersing an isolated nerve and muscle in a bath. The particular method of preparation offers possibilities of obtaining more prompt and pronounced reactions than does the bath method, and its employment has led to some interesting observations. The various fluids which have been used have been brought into contact with the muscle by perfusion, through the abdominal aorta and its return veins, or by direct injection into the belly of the muscle itself.

Preparation.

The following method of preparation possesses advantages not only in the ease of its accomplishment but in the fact that it is unassociated with mutilation of the animal, so that if desired, as in the second series of these observations, the normal circulation may be continued.

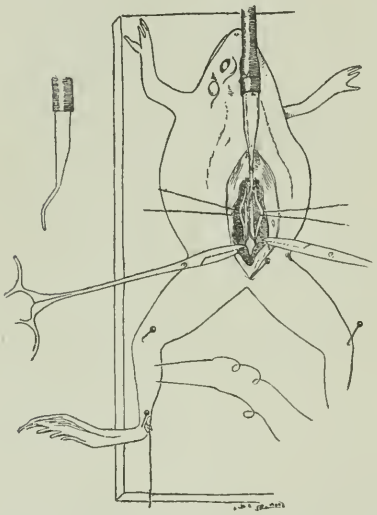
The frog is pithed and the brain and cord broken up. A vasomotor paralysis results which aids the subsequent perfusion, as the vessels remain dilated.

The gastrocnemius tendon on each side is exposed, divided, and isolated without injury to the neighboring artery and vein.

A median dorsal incision (*cf.* sketch) is then made, exposing the spine and pelvis from behind. The long *Os coccygis* is then lifted up by its unattached posterior extremity, carefully cut away from the adjoining muscles and dislocated by the insertion of a knife point at its articulation with the sacral vertebra. This entire performance should be absolutely bloodless. In the bottom of the wound thus made, the distended abdominal aorta with its bifurcation is easily

accessible. The lumbar plexus also lies exposed on each side and may be freed slightly and caught by a loose ligature in order to facilitate the subsequent introduction of the electrode under it.

A glass cannula of a particular form (*cf.* sketch) may then be readily introduced into the aorta, even in small frogs. This is a difficult matter with a collapsed vessel or by the usual method of exposure through the abdomen. The cannula is securely fastened to the animal's back and remains firmly in place without risk of tearing the vessel by any subsequent manipulations.



SKETCH illustrating method of making nerve-muscle preparation. Showing exposure of Aorta abdominalis with the Plexus cruralis on each side, after removal of Os coccygis; also form of cannula and its introduction, preparation of gastrocnemius, etc.

One of the iliac arteries may be caught with a delicate clamp, or tied, in order that the infusion shall enter one leg only while the other is preserved in normal condition for a control, or for a double preparation to be used in another irrigation.

The abdominal and iliac veins are then opened to allow the perfused fluid to pass out, and so to largely prevent any subsequent œdema.

The animal is fastened to a board by sharp pins passing through the joints and pelvis in such a way that the muscle contractions are not interfered with nor the blood-vessels compressed, although the preparation is made immovable. The irrigation should be started as soon as possible lest coagulation occur in the cannula or in the small capillaries and thus prevent a successful circulation. The circulation has usually been controlled during the perfusion by the microscopical examination of the capillaries in the web of the foot. Not only can the freedom of the circulation be thus ascertained, but from the comparative number of corpuscles remaining in the capillaries an idea can be gained of the degree of existing anæmia.

The various fluids for washing out the vessels were held in graduated burettes or Mariotte flasks which could be raised or lowered so

that the degree of pressure and rapidity of flow might be controlled and the amount of fluid passed through the extremity measured.

The muscle was stimulated by opening induction shocks, a battery of two Daniell elements being used. The irritation necessary to call forth the maximal twitch, both for direct and indirect stimuli, was first determined, and by means of a Bowditch clock and Kronecker key [1] (Spülcontact), a series of maximal opening shocks usually with a four-second interval was then employed. In all cases the muscle was unsupported and carried a weight usually of 20 grams.

The direct stimuli were given through needles inserted directly into the muscle belly (*cf.* sketch). A Pohl's commutator without cross wires was interposed between the coil and the preparation and so arranged that the effect of direct and indirect stimulation might be alternated as desired. A Kronecker [2] automatic short circuiting apparatus was inserted with the secondary coil in order to throw out the closure shocks.

Observations on the Effects of Artificial Circulation on Muscle Contractions.

Pure NaCl solutions. — If a 0.6 per cent solution, presumably isotonic with the protoplasm of the frogs' tissues, be allowed to circulate through the extremities there will be a comparatively early disappearance of irritability to *indirect stimuli*, which as a rule takes place long before there can be a complete washing out of the blood-vessels. This may be easily corroborated by the fact that the capillary circulation in the foot still contains many corpuscles and the return flow through the abdominal vein is still reddened with them. It will be found, however, that the response to *direct stimuli* persists. If the irrigation be continued for hours, however, a condition of practical bloodlessness may be reached, as shown not only by the absence of corpuscles in the circulation but by the failure to find them with the microscope on teasing the muscle substance itself. In spite of this fact *the closure contractions to direct stimuli, although usually less high than before, may persist* (*cf.* Fig. 1). I have succeeded with a frog thus washed out in obtaining them after a period of seventy-two

[1] Kronecker: Zeitschrift für Instrumentenkunde, 1889, p. 242.

[2] Kronecker: Ueber die Ermüdung und Erholung der quergestreiften Muskeln. Ludwig's Arbeiten, vi. Jahrg., p. 177. Auch K. S. Ges. Wiss. Math.-Phys. Bd. xxiii, Leipzig, 1872.

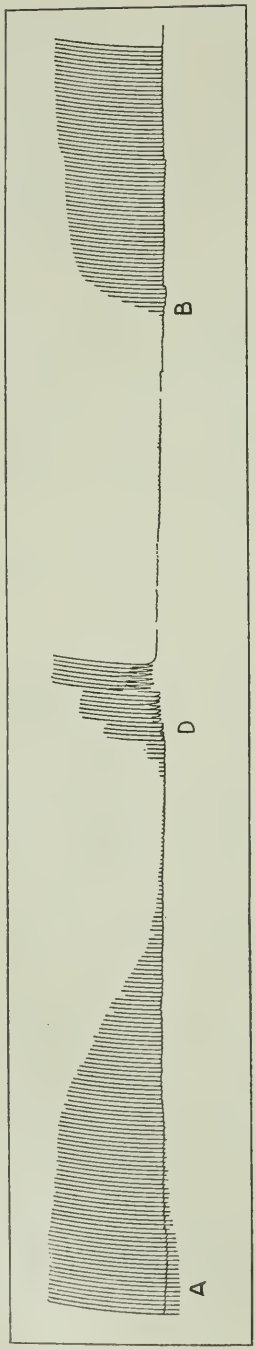


FIGURE 1. — Jan. 26, 1901. About seven-twelfths original size. *Esculenta.* Chart showing (A) rapid failure of indirect responses after perfusion of 15 c.c. of 0.8 per cent NaCl solution. (D) Persistence of direct and (B) prompt return of indirect response on perfusion with 2 c.c. of "physiologically balanced solution." In this case NaCl 0.7 : $CaCl_2$ 0.06 : KCl 0.03.

hours of constant irrigation at room temperature (with but one accidental interruption of three or four hours) and the employment of over three litres of the irrigating fluid. The presence of fibrillary twitchings in the muscle which the sodium chloride solutions often occasion have as a rule in no way complicated our "contraction curves," as may be seen in the figures.

Furthermore, this early failure of excitability from the nerve, has been shown to appear at variable times, depending upon the percentage of sodium chloride in the solution employed and the success of the perfusion. If a normal fatigue curve be made consequent upon a series of indirect maximal stimulations, the usual slow failure of response (in winter frogs) after several thousand contractions will be seen (Fig. 2). If now for comparison the other leg be washed out, with for instance a 0.6 per cent NaCl solution the failure of response will occur after a few hundred contractions (Fig. 3.) If a weaker solution be employed, in spite of its more irritating effect on the tissues, failure of response is less rapid. Even with pure water, although tetanic contractions may be produced and "contracture" be a marked feature, the muscle will continue to react when stimulated from the nerve longer than when the saline solutions are used. If we increase the percentage above 0.6 per cent the contractions as a rule fall off very rapidly after the irrigation has begun (*cf.* Fig. 1).

Inasmuch, however, as under all circumstances responses to maximal *direct stimuli* are in a great measure preserved, it is evident that the chief injurious effect from the salt occurs somewhere in the course of the nerve itself. Shifting the electrode from the exposed plexus to a section of nerve nearer the muscle, as the sciatic, gives no improvement in the response.

The effect in many ways, therefore, can be seen to be distinctly analogous to that of weak curare solutions and presumably is the result of an injury to the nerve ends themselves.

In review, it may be said that *generally speaking the number of contractions which may be called forth from a muscle by indirect stimuli after beginning an irrigation with solutions of sodium chloride diminishes with the percentage of the salt in the fluid.* Furthermore that inasmuch as direct excitability persists, the process of washing out may be considered not to lead to a more rapid exhaustion of the muscle, but rather to have an injurious effect upon the nerve-endings.

There are, however, many of my observations which would tend to show that the nerve ends themselves not only are capable of fatigue under ordinary normal circumstances, but that their susceptibility to exhaustion under

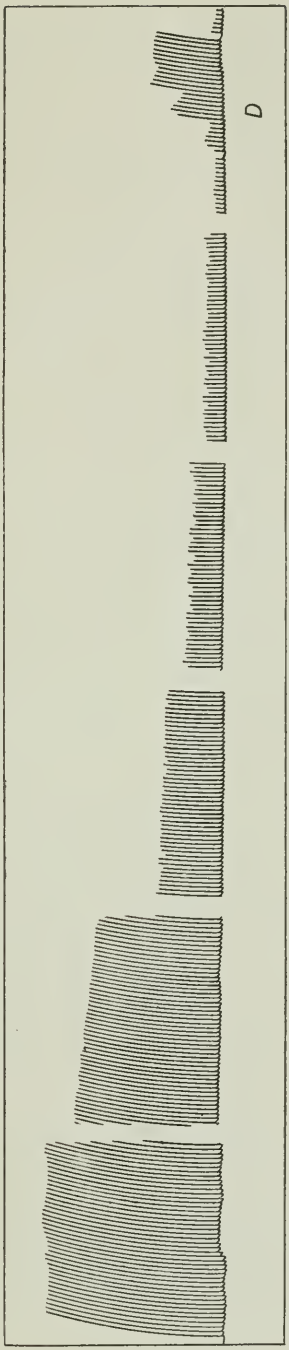


FIGURE 2.—Feb. 18, 1901. About seven-ninths original size. *Temporaria.* Fragments from a normal fatigue curve of Gastrocnemius, unsupported, carrying 20 grams and giving maximal contractions from indirect stimuli every four seconds. Portions taken from 1st, 7th, 14th, 21st, 28th, 35th hundred of contractions. Failure of response to indirect stimuli practically reached after 3600 contractions. Direct stimuli (D) still calls out contractions. For comparison with Fig. 3.

these conditions of washing out may be increased. In the first place it has been found possible by indirect stimuli to reach a period of fatigue of a non-irrigated muscle at which practically no further response from the nerve may be given even when the electrode is shifted to a new situation nearer the muscle, and yet when *directly* stimulated the muscle will contract in degree increasing with the strength of stimulus (Fig. 2). Inasmuch as nerve itself has been shown to be incapable of fatigue, this would point to a local fatigue of terminal organs apart from that of the muscle itself.

Secondly, if, during the process of washing out the muscle with a saline solution, the four-second shock be discontinued for a short period although the irrigation in the interim is kept up, it will be found on restimulating that the contractions called forth will be higher than before although lower contractions would be expected from a poisoning effect alone. It may be shown furthermore that this is not due to rest of the muscle, since, if instead of discontinuing completely the stimuli a series of direct stimuli be thrown in so that the muscle itself shall be irritated in the regular periods and yet the nerve in a measure given a rest, on returning to the indirect stimuli they will be found to have become improved.

It is thus seen that the question of increased susceptibility to fatigue, apart from the directly poisonous effects of the sodium ion, bears some relation to the problem, though presumably a relatively small one.

After a point has been reached in the irrigation at which no further indirect response is obtainable, upon continuing the perfusion, in spite of rest, no further contractions may be called out by way of the nerve. Thus with all pure sodium-chloride solutions at varying periods the so-called poisonous effect appears, though, as Loeb has shown, inasmuch as the same result follows upon the employment of such a fluid even when isotonic with the tissue fluids, the poisonous action may be considered to be a negative one and due to the *absence of other essential salts, rather than to the mere presence of the sodium itself.*

Effect of "physiologically balanced solutions." — If after a period has been reached in the process of washing out with a sodium chloride solution when no further contractions to indirect stimuli can be called forth, and then the irrigating fluid is changed for one containing the three ions in combination, contractions will reappear. Often this antidotal effect follows with astonishing rapidity, and before more

than a few drops of the fluid can have reached the muscle, the contractions will once more begin and soon may return to their maximal height (*cf.* Fig. 1).

Many factors, of course, influence this readjustment, which may not always be so prompt nor be associated with the return of the maximal contraction. This depends largely upon the percentages of salines in the solutions both of the original fluid used to exhaust the muscle response and that employed for its recovery. The exact determination of these relations would take an enormous number of observations.

It has been found, however, that a solution slightly richer in calcium elements than a "physiologically balanced fluid" is more efficacious in bringing about this recovery than one containing a smaller percentage, which is suggestive of a similarity with Howell's findings in the case of the heart. A solution, for instance, containing 0.06 per cent calcium chloride is often more efficacious than one of 0.03 per cent.

In very much the same way, and ordinarily with much more certain results, will a following irrigation of defibrinated blood lead to recovery. Often when the calcium-chloride-holding solution has succeeded in causing only a partial return of the maximal contractions, blood serum will further complete the readjustment. The blood of different animals, however, has a widely different effect, just as in the case of the various artificially "bal-

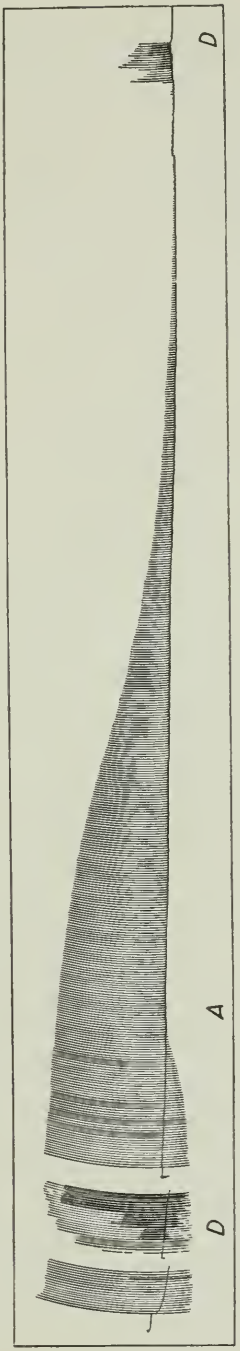


FIGURE 3. — May 2, 1901. About seven-sixteenths original size. *Esculenta*. Gastrocnemius unsupported, carrying 20 grams. Chart shows determination of indirect and direct maximal contractions; a beginning fatigue series of indirect maximal contractions; perfusion at point A with 0.6 NaCl solution; failure of indirect response after about 300 contractions and during the perfusion of 50 c.c. of the solution; the persistence of direct maximal contractions.

anced solutions." It is possible that this may bear a distinct relation to the percentages of the various ions which are contained in the serum of different species. It has been found for instance that rabbit's blood is uniformly more favorable than that of the calf or dog, and the same seems to be true for cardiac muscle as well as for that of the extremities. The exact composition of rabbit's blood and whether it be especially rich in lime salts the writer has been unable to learn and no chemical analysis has been made. In this connection it is interesting to note the view held by certain physiologists, that the toxic properties which the blood serum of one species often shows toward another are due to the saline constituents rather than to the contained alexines.

It has been further observed that, after washing out a muscle with a "balanced" solution (NaCl 0.7, KCl 0.03, $CaCl_2$ 0.03) until it ceases to respond to indirect stimuli, if the percentage of the calcium chloride be increased, to 0.06 per cent for example, contractions to the same stimuli from the nerve may reappear. On one occasion the same cycle has been repeated twice, improvement following an increase to 0.08 per cent after failure of contractions with 0.06 per cent. The stronger percentage, however, was associated with irritative signs. These observations, again, are somewhat analogous to those which Howell has demonstrated in the case of the heart.

It has been almost without exception observed that after the muscle has once failed to respond to indirect stimuli after washing out with a simple sodium-chloride solution, and recovery has been brought about by the calcium-chloride-containing fluid, a subsequent irrigation with the same sodium-chloride solution fails to produce its prompt toxic action. It is apparent, therefore, that the calcium ion enters into a more enduring combination with the tissues than do the other ions of the solution. Under these circumstances it is often a very tedious process to re-exhaust a muscle with the same solution which in the first instance had an astonishingly prompt effect. Further, it has been evident in washing out with sodium-chloride solutions that a point may be reached in the exchange of ions between tissue and perfusion fluid after which the substitution of blood or the "balanced solution" may fail to lead to a return of a functionating condition. The muscle under these circumstances remains permanently unresponsive to stimuli by way of the nerve.

Observations on the Effects of Direct Injections into the Muscle Substance.

In order to observe the effects of the various solutions on the muscle which in the mean time may receive its normal blood supply, the preparation was made in a way slightly different from that described above. In these instances the cord only was broken up, the brain and medulla being left intact so that the circulation and respiration might continue. The plexuses were exposed in the usual way from behind by the removal of the long coccyx. The exposed abdominal aorta or iliacs were clamped when it was desired to shut off the circulation at any time from one or both extremities.

Let us suppose the frog to be prepared under such conditions, and direct and indirect maximal contraction for one gastrocnemius to be determined and the series of indirect maximal contractions with the usual four-second interval to be started. If now with a hypodermic syringe we inject the fluid whose effect we wish to observe directly into the muscle belly of the contracting gastrocnemius, the following consequences will take place. The fluid (ordinarily $\frac{1}{2}$ to 1 c.c. of the solution has been used according to the size of the frog) seems to spread immediately to all parts of the muscle in an equal degree. The muscle swells evenly and symmetrically, and in consequence of the distention shortens in length. The contractions, however, which, provided the fluid be an indifferent one, continue as vigorously as before, in case NaCl solutions are employed, diminish in vigor much as in the case of the irrigated muscle. After repeating these injections two or more times the muscle fails to respond to an indirect stimulus, though still irritable to direct shocks, showing again that the chief effect concerns the inability of the nerve ends to functionate. If now a following injection be made with rabbit's serum or with a solution containing the proper ion combination the muscle immediately shows improvement, even despite its great artificial œdema, the first stimulus by way of the nerve after the injection evidencing the fact of beginning readjustment (*cf.* Fig. 4).

That the frog's blood itself does not bring about this prompt effect is shown by the following observation. Let the injections be made while the abdominal aorta is closed by a delicate clamp. When a period is reached at which the indirect response has become very slight let the artery be unclamped and the normal circula-

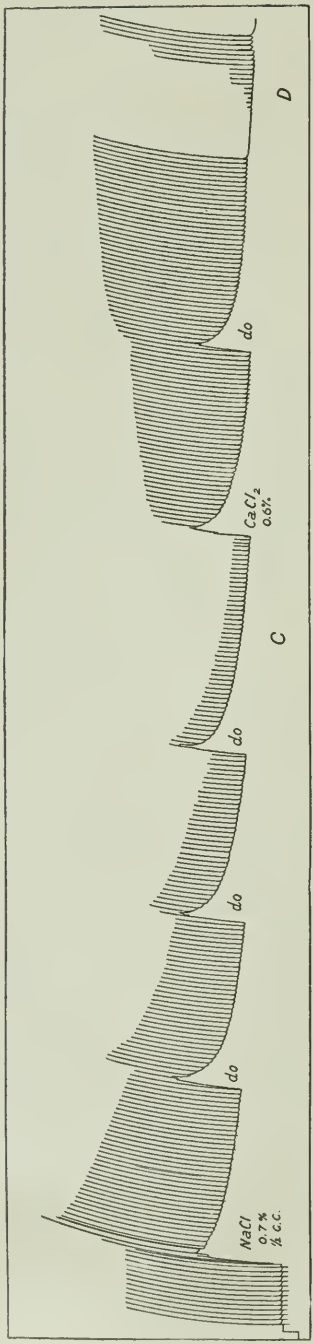


FIGURE 4. — March 2, 1901. About seven-twelfths original size. *Temporaria*, with abdominal aorta closed by a provisional ligature. Showing poisonous effect of direct injection (four times repeated) of 0.7 NaCl solution. Return of normal circulation at point C to the extremity unassociated with improvement. The injection, twice repeated, of a "balanced" solution containing $CaCl_2$ 0.06 followed by reappearance of maximal contractions. D, direct stimulation.

tion become re-established. This is followed by no improvement provided that the induction shocks by way of the nerve are continued, whereas the subsequent injection of a "balanced" fluid will immediately be followed by vigorous contractions. (Also shown in Fig. 4.) Similarly it has, I believe, been shown that the isolated frog's heart, after washing out with sodium-chloride solutions till exhausted, will respond to a few drops of the diluted serum of a rabbit more quickly than to the blood of the animal itself.

It is interesting to compare in these observations the reactions which meanwhile are taking place in the other muscles of the extremity, since all are stimulated alike from the plexus. Although by these local injections the gastrocnemius may be paralyzed, so far as response to indirect stimuli is concerned, the other muscles continue to react with their normal vigor.

The poisonous effect of the hypertonic sodium-chloride solutions, whether injected directly into the muscle or brought to it by perfusion methods is usually much more pronounced than the effect of blood saturated with carbon dioxide. An illustration of this is given in Fig. 5.

There is one further point relative to the production of

a condition simulating rigor mortis which has often been brought out by the employment of solutions containing an excessive amount of the calcium ion. On several occasions on which simple fatigue charts were made for the purpose of comparing them with the curves of various irrigation experiments, attempts have been made to resuscitate the exhausted muscle by washing out or by the direct injection of the three-ion-holding solutions. Occasionally slight beneficial effects have been seen to follow, but the usual effect was the gradual production of a state of rigor. If the entire extremity was washed out with the fluid all the muscles became permanently hardened and contracted: if the gastrocnemius alone was injected with the solution the rigor affected this muscle alone.

The relation which the presence of lime salts bears to the process of coagulation of blood and milk is well known. The passage of a muscle into a state of so-called rigor mortis is considered to be an analogous process. A fatigued muscle is known to pass more readily into a condition of rigor, post-

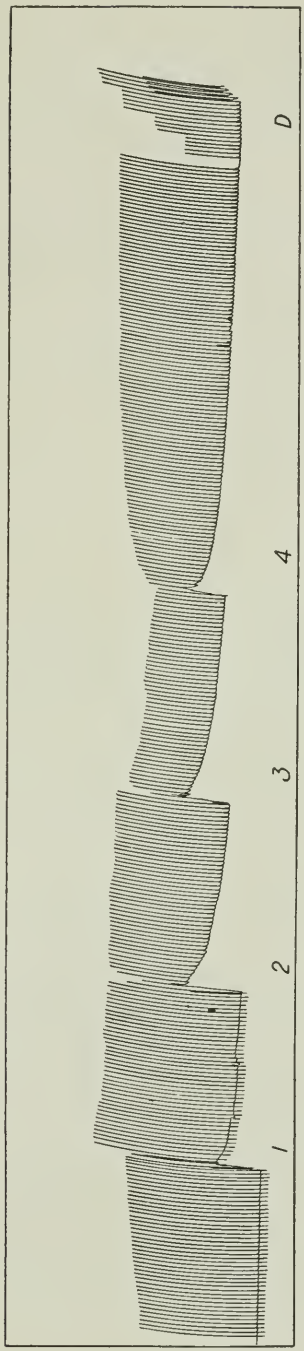


FIGURE 5. — Feb. 18, 1901. About seven-twelfths original size. *Temporaria.* Normal circulation in extremities preserved. Chart shows comparative effect of injection of various solutions directly into the gastrocnemius muscle.

1. Normal rabbit's blood. No effect on height of contractions.
2. Rabbit's blood saturated with CO_2. Slight effect.
3. 1.0 per cent NaCl solution. Marked injurious effect.
4. Balanced solution (NaCl 0.7 : $CaCl_2$ 0.06 : KCl 0.03.) Beneficial effect.

D. Direct stimulation.

mortem, than one not so fatigued: indeed the very process of active muscle contraction seemingly bears a close relation to the permanent charge associated with rigor. It is here seen that the addition of lime salts injected into a fatigued muscle may produce *intra vitam*, a condition at all events simulating rigor mortis.

It is fully realized by the writer that this series of experiments has done little more than evolve a convenient method by which future observations relative to the action of various fluids on striped muscle may be continued. Much has of necessity been left undone relative to the effects of a greater number of solutions carefully graded with respect to their content of ions. There are furthermore many irregularities which may be encountered and for the final explanation of which a more extended number of observations will be necessary. It has been noticed, for instance, after a perfusion with a sodium solution until indirect stimuli no longer call out contractions that the direct injection of a "balanced" solution into the muscle substance fails to counteract its effect, as will a perfusion of the same fluid. When the paralysis, however, has been occasioned by the injection method the same method of treatment does lead to recovery.

Observations have been made upon fifty frogs (Esculenta and Temporaria), mostly during the winter months, at which time as a rule more pronounced reactions were obtained than in the short series made in May on spring animals.

The observations permit the following conclusions: —

The pure sodium-chloride solutions are injurious to the nerve-muscle preparation.

The effect is in a measure related to the percentage of this salt in solution.

Inasmuch as the response from indirect stimulation fails, while that from direct stimulation may persist, the result primarily affects the nerve ends.

The injurious effect may be promptly counteracted by the blood or serum of certain animals or by the proper "physiologically balanced salt solution."

By varying the percentage of the calcium ion in the solution, with certain limitations, proportionately beneficial effects may be produced.

An excess of the calcium ion in certain cases of fatigued muscle may lead *intra vitam* to a permanent contraction of the muscle resembling rigor mortis.

CEREBRAL PRESSURE FOLLOWING TRAUMA.

By W. B. CANNON.

(With a Preface by W. N. Bullard.)

[*From the Physiological Laboratory of the Harvard Medical School.*[1]]

CONTENTS.

Preface.

A SOMEWHAT prolonged clinical study of traumatic cerebral lesions, and more especially those induced by blows upon the head, whether or not accompanied by fractures of the cranium, has shown that operative interference is often necessary.

It has been apparent on examination of the condition of the contents of the cranium in cases of this character, where an artificial opening of the cranium was made, that in most instances the dura was tense and the brain did not pulsate. It was also soon proved that this condition did not necessarily depend upon the increase of intracranial pressure following the introduction of an extraneous substance, such as a large hemorrhage or clot, since it occurred in the absence of hemorrhages which could thus increase the intracranial pressure. How far this marked intradural tension was abnormal was at first undecided, but later it became evident that it was frequently greater than the normal average.

It has long seemed to me that upon the experimental demonstration of the existence of this increase of intradural tension, and upon the determination of its cause, must logically depend our action in many cases of traumatic cerebral lesion. On account of the great practical

[1] The first part of this investigation was made by means of a fund established by Dr. W. N. Bullard.

importance of these questions to the neurologist and the surgeon, I induced Dr. Cannon to undertake the following research.

Dr. Cannon is entirely responsible for the method used in these experiments and for the conclusions drawn.

Introduction.

The term cerebral pressure has been applied somewhat indiscriminately by investigators to two conditions, to the pressure exerted on the brain by foreign objects in the cranial cavity, and to the pressure exerted by the brain, in response to internal forces, against the skull. It is desirable, for the sake of clearness, to separate these two conditions by means of distinctive terms. The force acting on the brain from without, for example, from depressed bone or subdural hemorrhage, results in a compression of the brain, and to this phenomenon should be applied the term cerebral compression. The term cerebral pressure may then be restricted to the force with which the brain itself pushes against the surrounding skull. Since, however, the forces within the rigid cranium are in equilibrium, the intracranial pressure must vary directly with the intensity of either of these varying factors,—the compressing foreign body, or the swelling brain. The result is manifestly the same, therefore, whichever factor increases, and consequently either of them may cause the so-called "pressure symptoms" which follow injuries of the head. This research is concerned chiefly with the second of the two forces mentioned above, the pressure exerted by the injured brain against the skull.

In the following discussion it will be well to consider, first of all, the pathological conditions found in cases of head injury, since thereby the phenomena to be explained may be presented in a schematic outline which will be serviceable throughout the subsequent treatment of the subject. The clinical findings which accompany the anatomical changes will next be regarded, and will lead to an examination of the pressure symptoms prominent in the cases. In the third division of the paper the theories put forth to explain the increase in intracranial pressure will be discussed; and finally I shall give the results of my experiments and point out what seems to me to be the significance of œdema as an active agent in the cause of death after cerebral traumatism.

I. Pathological Conditions in Traumatic Intracranial Lesions.

The careful macroscopic and microscopic examination of the pathological changes in two hundred and twenty-five cases of traumatic intracranial lesions, reported by Phelps,[1] has led to a recognition of the logic and trustworthiness of his classification and terminology of these lesions. Phelps divides the lesions primarily into two general classes: the *direct*, which include the structural changes following the injury more or less immediately, and the *indirect*, which include the secondary inflammations. The direct effects of injury are of first interest in this discussion. Of these Phelps names three groups which have clinical significance, — hemorrhages, contusions, and lacerations. It will be unnecessary to present a detailed description of the variations of these three pathological states; only such general appearances will be mentioned as have a bearing on the further development of the present argument.

Hemorrhages may be epidural, from the vessels of the diploe, the sinuses or the middle meningeal artery; pial, from rupture of vessels of the pia mater; or cortical, the direct result of injury to the brain substance. In any of these three regions the bleeding may be considerable or slight. Two effects of hemorrhage should be noted at this point, an immediate effect from profuse hemorrhage, and a more remote effect resulting from slighter effusions. Duret[2] has shown by injecting wax into the cranial cavity that a diminution of the intracranial space by five per cent produced somnolence and coma, while a diminution by eight per cent resulted in death. Epidural hemorrhages are the most liable to be profuse, and if sudden they may produce an immediate loss of consciousness, and result in death by simple mechanical compression. According to Phelps, pial and cortical hemorrhages are rarely sufficiently copious to produce marked cerebral compression; the pial hemorrhage characteristically forms merely a thin sheet over the vertex. "The general symptoms which attend these inconsiderable curtailments of the intracranial space, whatever their nature, must therefore be ascribed to other causes than a general circulatory disturbance occasioned by the contraction

[1] Phelps: Traumatic injuries of the brain and its membranes, New York, 1897.

[2] Duret: Étude expérimentale et clinique sur les traumatismes cérébraux, Paris, 1878, p. 186 *et seq.*

of cranial capacity."[1] These hemorrhages are, however, rarely uncomplicated. Almost invariably they are associated with some degree of general or local contusion, and hemorrhage and contusion together result in a vascular disturbance which leads to deficient nutrition of the brain tissues. The consequences of the deficient nutrition of the tissues will be the main subject to be considered in this paper.

The phenomena of contusion probably exist in all cases of intracranial injury, and may be general or confined to the cerebrum. The visible anatomical changes are as follows,[2] "a distention of the parenchymatous vessels, a general formation of minute thrombi, the presence of punctate extravasations, and a more or less distinct œdema. . . . The minute thrombi are the most characteristic of the several morbid conditions which have been enumerated. . . . The œdema, which is variable in amount, sometimes appreciable only after some delay and a close inspection upon section, and at other times so profuse that the fluid can be squeezed from the brain by the hand as from a sponge, is notably frequent. All these abnormal conditions, the extravasations, thrombi, and œdema, are simply measures of the general hyperemia which immediately preceded death." Contusion, therefore, like hemorrhage, manifestly presents, in the extravasations and thrombi, conditions for circulatory disturbance and a consequent deficient nutrition of the tissues; that the œdema is a secondary result of these conditions will be shown later.

Laceration of the brain is the most severe effect of external violence. It is characterized by rupture of vessels and hemorrhage so profuse that the brain substance may be broken down in all directions. The changes present in lacerations are, however, of no special interest in so far as they differ from those present in hemorrhages and contusions, and may accordingly be passed without further description.

From the foregoing review of the pathological findings in cases of traumatic injuries of the brain, it is evident that extensive lacerations and profuse hemorrhages are in themselves conditions capable of causing immediate death. With slight hemorrhages, and especially with contusions, the primary anatomical alterations are often so inconsiderable that a more general secondary process must be invoked to explain the fatal issue. What that secondary process may be remains to be discussed. As primary pathological changes, there are the slight pial and cortical hemorrhages, the minute thrombi and punctate extravasations and the vascular distention manifest in con-

[1] PHELPS: *Loc. cit.*, p. 51. [2] PHELPS: *Loc cit.*, p. 53.

tusion. The œdema, which is a constant concomitant of these primary changes, is, I believe, a secondary result from them, and is to be held accountable for the serious conditions which follow from apparently slight pathological alteration. Before considering further the manner in which œdema may act in causing death, it will be well to present the evidence offered by clinical cases.

II. Clinical Findings in Cases of Traumatic Head Injuries.

The symptoms resulting from injury to the brain vary greatly with the severity of the lesion, the nature of the pathological changes — whether hemorrhage, contusion, or laceration — and with the location of the injury in the brain. To enumerate the many variations in symptoms would not be pertinent to this discussion; it will be sufficient to state the general symptoms.

In cases of brain lesion there is usually a history of a blow or a fall on the head, or evidence of the rupture of an intracranial blood vessel. The most notable and most constant of the primary symptoms is unconsciousness. The initial unconsciousness may persist until death, or the patient may regain his senses. If consciousness returns, it is commonly lost again after gradually increasing dullness passing into stupor. The final appearances, whether consciousness is recovered or not, are deeper and deeper stupor, then coma, from which the patient cannot be aroused, and ultimately death. In this final stage certain typical signs manifest themselves. The temperature characteristically rises as the time of death approaches, the pupils do not react to light, there is stertorous breathing and slow heart-beat. Sometimes there is paralysis of the face and limbs, though there may be clonic spasms in various muscles. Passage of urine and fæces is also an occasional occurrence.

Between the symptoms preceding death from injury to the brain, and the symptoms produced in animals by increasing intracranial pressure or by otherwise causing cerebral anemia, there is a striking similarity. The pressure symptoms observed in animals have been summarized by Bergmann[1] as follows. First, there is evidence of pain due to tension of the dura mater. This is followed by stupor, sopor, and coma. When the pressure is suddenly applied, clonic spasms and sometimes roll and circus movements are noted; but

[1] Bergmann: Deutsche Chirurgie, Die Lehre von den Kopfverletzungen Stuttgart, 1880, xxx, pp. 341 *et seq.*

these phenomena are not seen when the pressure is applied slowly. A slow heart, slow, deep, snoring respiration, vomiting, and emptying of bladder and rectum are characteristic symptoms. In the experiments on animals, it was discovered that in order to produce death, the intracranial pressure must equal the carotid pressure, *i. e.*, anemia must be produced. The manifest resemblance between the clinical symptoms and the experimental phenomena has led to the application of the term "pressure symptoms" to the peculiar symptom complex following brain injuries. The evidence of intracranial pressure observed in these cases on operation confirms the validity of the term.

A further insight into clinical conditions attending intracranial lesions, which will serve to make the problem more distinct, and the nature of the explanation more clear, will be secured by an examination of a series of typical cases. These cases will illustrate three facts: 1, that there may be injury with pressure symptoms and recovery; 2, that pressure symptoms may occur with only slight gross lesion; and 3, that the œdema, which attends the cerebral lesions, is the result of a process requiring considerable time for its operation.

1. **Cases of head injury with pressure symptoms and recovery.**—Two cases reported by Walton [1] will illustrate this type.

Case I. A boy of six years was struck by a bicycle at noon one day and rendered dazed, but not unconscious. That evening he had fever and vomited, was restless at night and the next morning vomited again. During the day he became drowsy, then unconscious with unilateral paralysis including the face. Defecation and micturition were involuntary. That evening operation was considered, but, in view of slight improvement in the conditions, was postponed. On the fourth day after the accident the pressure symptoms had entirely disappeared, and on the sixth day the child was apparently well.

Case II. A child, aged three and a half years, fell from a swing and struck on her head. She was dazed, and later she vomited. The next day the left arm was paralyzed. On the third day after the injury the paralysis began to disappear, and thereafter the recovery was rapid.

In each of these cases the gradual onset of pressure symptoms some time after injury, and their gradual subsidence, should be carefully noted.

[1] Walton: American journal of medical sciences, 1898, cxvi, p. 270.

2. **Cases with pressure symptoms with only slight gross lesion.** —The phenomena presented by this class of cases are especially significant; evidence of considerable intracranial pressure is demonstrable, but on examination no hemorrhage or depression of bone sufficient to cause the symptoms is to be discovered.

Case III. Patient fell down a flight of stairs. Upon admission to hospital, semiconscious, and irritable when aroused. Dilatation of right pupil, which was irresponsive to light; no muscular symptoms. He remained in a restless, delirious, or stupid condition until his death on the seventh day. There was loss of urinary control on the fourth day, and coma, with picking at the bedclothes, and subsultus tendinum during the last twenty-four hours. The temperature, which was 98.2° on admission, rose slowly and progressively to 102° on the fifth day, to 104.2° on the sixth day, and to 105.2° one hour before death.

No fracture of the skull and no epidural hemorrhage; no superficial laceration; pia mater and cortical vessels very much congested; some opacity of arachnoid membrane, and moderate subarachnoid effusion; no pial hemorrhage; limited cortical contusion, area of one inch in diameter, at bottom of left fissure of Silvius; laceration of left optic thalamus in its central portion, one fourth inch in diameter and filled with clot. Subcortical lacerations of the left side of the pons, one third inch in diameter, in the transverse fibres; a few punctate extravasations in different parts of the brain; general hyperemia and well marked œdema.[1]

Case IV, reported by Walton and Brooks.[2] A young woman, thrown from her horse, struck on her head and was carried home unconscious. Four hours later was still unconscious, breathing quietly, with a pulse of 100. The pupils were equal, somewhat dilated, and reacted sluggishly. There was partial paralysis of the left side of the face, and complete paralysis of the left arm and leg. The patient had vomited once or twice since the accident. Restlessness supervened and consciousness partially returned. Respirations were shallow. There was incontinence of urine. The left arm and leg became rigid.

On the second day the rigidity of the left arm and leg became less marked, and limited voluntary movements appeared; but in the evening the rigidity and paralysis of the left side were very well marked, and upon efforts to arouse her no response was made.

Operation was performed at this time. A small trephine button was removed about two and a half inches above the right external auditory

[1] Phelps: *Loc. cit.*, p. 536.

[2] Walton, G. L. and W. A. Brooks, Jr.: Boston medical and surgical journal, 1897, cxxxvi, p. 301.

meatus. No fracture of either table was discoverable, but the dura was seen to be tense and non-pulsating. Upon its incision an ounce of clear fluid spurted through the opening. The brain appeared somewhat œdematous and prominent, but otherwise normal. Exploration under the dura revealed no sign of hemorrhage.

Until the fifth day after the injury there was slight improvement, but from this time on the general condition varied from moderate delirium to somnolence. The patient gradually sank. On the fifteenth day, temperature, pulse, and respiration rose rapidly, and on the sixteenth day the patient died.

At autopsy there was no sign of any fracture of the skull, and no evidence of extra- or intra- dural hemorrhage, or hemorrhage of the meninges. On section several hemorrhagic softened areas were found, — two, about the size of beans, in the left frontal lobe, and another near the outer margin of the right optic thalamus. Various minute hemorrhages were scattered over the brain.

Such cases as these have led to the opinion that the œdema itself may in some way cause the pressure symptoms. Walton states, in reference to Case IV., "that the œdema played a part in the production of the hemiplegia can hardly be doubted in view of the disappearance of rigidity and improvement in motion following the relief of pressure by operation," and he remarks, furthermore, "the importance of remembering that a fatal result may follow concussion without tangible gross lesion, unless, indeed, the two small hemorrhages in the frontal lobe, with subsequent softening, are considered adequate cause for a fatal issue."[1] Bullard[2] had previously called attention to these same phenomena. "The cause of increased intracranial tension," he declares, "is not altogether plain. It is not by any means, as is sometimes supposed, always a pressure from intracranial hemorrhage. . . . In many cases there is no evidence of any severe hemorrhage, and yet the increased pressure is apparent. Again, the increased pressure in all probability occurs in cases of so-called concussion and in other mild cases where unconsciousness exists, but where there can be no question of any profuse hemorrhage. What seems to occur is this: the brain in some way acts as a sponge and pushes so hard against the dura as to inhibit or diminish pulsation. If in these cases the dura is incised, the cerebral pulsation again becomes visible, and the relief to the patient is instantaneous and

[1] Walton and Brooks: *Loc. cit.*, p. 304.

[2] Bullard: Boston medical and surgical journal, 1895, cxxxii, p. 74.

extraordinary." In another paper Bullard[1] again declares that the pathological process resulting in increased intracranial pressure is apparently a swelling of the brain itself, due in part to a filling and dilatation of the blood vessels, in part to œdema of cerebral tissue resulting therefrom, and in part also to the excess of fluid between the pia and the dura. That the subdural fluid is not alone the cause of pressure symptoms is shown by the fact that in many cases the increase in fluid is not very great and may not be apparent. Furthermore, the tension and protrusion of the brain after cutting the dura indicates that the intradural pressure is not in any great degree due to the fluid which has escaped. The pressure is, therefore, due to a swelling of the brain itself.

The third fact to be illustrated, namely, that *the œdema attending cerebral lesions results from a process requiring appreciable time for its operation*, is made clear by a comparison of the findings in cases like those cited above, in which the time element is noteworthy, with the findings in other cases observed soon after the reception of the injury. The presence of œdema in the former condition is usually in sharp contrast with the absence of œdema when the brain is seen immediately after the accident. A single case will illustrate this negative evidence.

Case V. Female, fifty-two years old, fell from an electric car, was admitted to the City Hospital shortly after. Conscious; pupils equal; no paralysis anywhere. There was a depression in the right temporal region. The surgeon trephined in this region, the operation being done under ether. No extradural clot. The dura was tense and scarcely pulsated. On cutting the dura the brain bulged. No excess of cerebro-spinal fluid noted: recovered well and quickly.[2]

In this instance an almost immediate operation gave no time for the œdematous condition, so characteristic an accompaniment of brain injury, to develop. The bulging pulseless condition of the dura at this early stage will be explained by the experimental data to be presented.

A summary of the points thus far made in the discussion will perhaps serve to make clearer the further development of the subject. From a study of the pathological alterations after injuries to the brain it was evident that there might be hemorrhages and lacerations so

[1] Bullard: Boston medical and surgical journal, 1898, cxxxviii, p. 272.

[2] Bullard: Medical and surgical reports, Boston City Hospital, 1895, p. 64.

severe as to result in almost immediate death. It was also evident that in many instances the initial lesions — the scattered minute thrombi and punctate extravasations — were so slight as to be in themselves no adequate cause for a fatal issue. That this inference is true is indicated by the recovery from the initial loss of consciousness, — a result hardly to be expected if the primary lesions were intrinsically fatal. In cases of this nature, however, the recovery of consciousness was not infrequently followed by a slow subsidence of conscious life, with a progressive increase of the so-called pressure symptoms until death supervened. Leyden [1] long ago showed that in order to produce death intracranial pressure must equal arterial pressure, and this conclusion has been confirmed by Duret,[2] Cybulski,[3] Hill,[4] and others. Apparently what occurs in these puzzling cases is this: the intracranial pressure somehow rises higher and higher until it reaches a degree equal to the blood pressure in the arteries. At this point the circulation in the cerebral blood vessels comes to a standstill, the vital centres in the bulb no longer receive their normal nutrition, they become paralyzed and life ceases. Now the question presents itself: in what way is the intracranial pressure gradually and progressively increased until the pressure in the blood channels to the brain is overcome and death results? It is clear that the pressure symptoms which slowly manifest themselves are dependent on secondary processes following the primary lesions of the brain; it is clear also that the œdema follows the primary lesions of the brain. Is there any causal relation between these two phenomena?

III. Theories to explain the Increase of Intracranial Pressure after Trauma.

The most noteworthy theory advanced to explain the secondary increase of cerebral pressure following injuries of the brain is that of Bergmann. This same theory is held by Hill. More recently an explanation has been presented also by Courtney.

The position taken by Hill [5] and Bergmann [6] has been clearly stated

[1] Leyden: Archiv für pathologische Anatomie, 1866, xxxvii, p. 519.

[2] Duret: *Loc. cit.*, p. 183.

[3] Cybulski: Centralblatt für Physiologie, 1890, p. 835.

[4] Hill: The physiology and pathology of the cerebral circulation. London, 1896, p. 168.

[5] Hill: *Loc. cit.*, p. 188, *et seq.*

[6] Bergmann: *Loc. cit.*, p. 420.

by Hill as follows. The primal condition is an intracranial hemorrhage which acts as a localized foreign body within the cranium. Since the brain is inclosed in a rigid case, and the brain substance itself is incompressible, this localized foreign body must occupy the space of a certain vascular area, that is, it must cause an obliteration of the capillaries and veins in the region it occupies. As a consequence the local cerebral tension will be equal to that of the arteries which normally feed the affected area. In the obliterated area there will be complete stasis of blood. The transmission of the increased tension through the brain substance to the veins and capillaries of the border areas will cause a higher blood pressure and a lessened blood flow in these vessels. In more distant areas the circulation is more normal and the blood-flow may have even a compensatory increase of speed. According to Hill, the secondary increase of pressure may now be established in two ways, the first of which alone will be described, as the second does not concern the class of cases under consideration.

The high blood pressure in the border areas will lead to increased transudation of fluid, because plasma may pass more easily into the brain substance than blood through the compressed capillaries. The transudation will take place at almost arterial tension, will increase the volume of the foreign body, and so lead to compression of other capillary areas. Thus is established a vicious circle of processes, and the cerebral anemia may spread indefinitely.

The noteworthy feature of this theory is the assumption that the transudation occurs because of high blood pressure in the capillaries of the border areas. It should be observed, however, that the high pressure in these vessels is due, not to a general increase in blood pressure, but to the fact that external pressure in the brain substance about the vessels increases until it partially overcomes the internal pressure in the capillaries themselves. If "the plasma may pass more easily into the brain substance" (where tension is so high that the blood vessels are being compressed) "than blood through the compressed capillaries" (in which flow can still occur) there would be the obviously impossible condition of fluids passing from a region of less to a region of greater pressure.

But even the assumption that plasma does pass from the vessels will not remove every difficulty. For if, as the theory states, the secondary increase in intracranial pressure is due to such transudation from the capillaries, this pressure must be dependent upon the pressure of the plasma. The pressure of the plasma is, in turn, dependent on blood

pressure, and is as much *less* than blood pressure as the resistance which the tissue about the capillaries offers to the outflow from them. Hill declares that to produce death intracranial pressure must equal the blood pressure in the carotids. The difficulty arises in attempting to induce a method of compression manifestly less effective than arterial blood pressure to produce such a result; for in the end by this reasoning the direct pressure of the blood through the free ways of the vessels must be greater than the lessened pressure of the transudate, and the flow will persist.

This theory that the secondary increase in intracranial tension takes place chiefly in the border areas and is due to pressure of the plasma has, moreover, the great defect of leaving out of account the processes taking place in the portion of the brain substance in which the circulation is impaired. It will be shown that in this neglected region swelling and pressure occur wholly independently of any blood pressure whatever.

Another theory of the cause of the increase of intracranial pressure after trauma was offered by Courtney[1] in 1899. According to Courtney, blows on the head paralyze the cerebral vasomotor nerves. Paralysis of these nerves results in dilatation of the vessels which they control, and dilatation of the vessels is accompanied by *acute anemia* of the brain substance. Courtney does not make clear the manner in which dilatation of the arterioles would cause anemia. Howell[2] has shown, by perfusing the cerebral vessels of dogs with defibrinated blood, that, no matter how high the arterial pressure, the venous outflow was always proportional in amount. Evidently the higher pressures would in effect dilate the arterioles in a manner satisfactory to Courtney's theory, and yet Howell's records show no indication of even a temporary blocking of the circulation in the brain.

The sequence of events when the vascular paralysis is permanent, according to Courtney, is "arterial stasis, with enormous rise of intracranial (venous) pressure, thrombus formation, transudation." That dilatation of the arterioles does not produce arterial stasis has already been shown; it has rather the opposite effect of lessening the resistance to the flow and increasing the rate. The reason for assuming an "enormous rise of intracranial venous pressure" is not given. Howell's experiments on the dilatation of the cerebral arteries under high pressures certainly do not support any assumption of a consequent venous

[1] Courtney: Boston medical and surgical journal, 1899, cxl, p. 347.

[2] Howell: American journal of physiology, 1898, i, p. 69.

resistance. That such a result does *not* occur is explained by Howell by the application of the general rule that the total cross area of the veins in any region is greater than the total cross area of the arteries. Since this is true, the expansion of the cerebral arteries is manifestly always relatively less than the diminution in the size of the veins, and therefore the blood flow is not impeded in arterial dilatation.

The transudation which Courtney assumes to be a result of the arterial dilatation cannot be absorbed because of the exceedingly high pressure in the veins. It must therefore remain and compress the capillaries, thus still further impeding the circulation and taking the first turn in a vicious circle, similar to that noted by Bergmann. Inasmuch as the accumulation of the transudate depends on the high pressure in the veins, and the assumption of a high pressure in the veins has been shown to be unwarrantable, this part of the theory falls to the ground.

Moreover in this theory again a mechanical filtration of fluid is called upon to raise a pressure greater than its source, and this feature is, therefore, open to the same objections previously noted in discussing the statements of Hill. The theory Courtney has developed has, furthermore, the defect of failing to regard sufficiently the processes occurring in brain tissue deprived of its proper blood supply. The nature and results of these processes will be presented in the next section of this paper.

IV. Experimental Inquiry into the Cause of Brain Pressure.

It was noted in the citation of clinical cases of chief interest in this investigation — the cases with least evident cause for the pressure symptoms which they manifested — that in many of them two stages were to be observed. The first stage followed immediately after the injury and was marked by loss of consciousness or by a peculiar dazed condition. From this primary stage the patient might recover, only to pass after a time into a secondary stage characterized by a gradual increase of pressure symptoms and frequently closed by death. The question arises, can a more reasonable explanation of these phenomena be given than the theories already cited?

About two years ago Dr. W. N. Bullard pointed out to me the importance of knowing the cause of the increased pressure of the brain after trauma of its substance. The results of the study undertaken upon this suggestion may be divided into two groups, (1) those concerned with the immediate effects of injury to the brain, and (2) those concerned with the more remote effects.

Primary effects of cerebral trauma. — The method employed to determine the immediate effects of head injuries was a modification of the method used by Hill[1] in his investigation of brain pressure. The apparatus consisted essentially of an inner and an outer brass tube, as shown in Fig. 1. The outer tube (O) was bevelled at its lower end and threaded so that it could be screwed tightly into the trephine hole. The inner tube (I) had at its lower end a membrane (M) of thinnest rubber; at its upper end a rubber cork (C) securely fastened by pins passing through both the tube and the cork. The inner cylinder was held rigidly within the outer cylinder by means of a screw (S). Fitting closely around the inner, and tightly over the outer tube, was a rubber capping (R) which effectually prevented any fluid from passing out of the brain case. A curved glass tube led from the rubber cork (C) and was connected by rubber tubing to a straight glass tube (G). The whole inner apparatus was filled with water. A line was marked on the glass tube (G), at which the water level stood when the membrane (M) was even with the base of the inner cylinder. If now, after the dura has been removed, the inner cylinder is inserted with the membrane on a level with the dura and the brain exerts a pressure against the membrane, the membrane will rise in the cylinder and cause the water level in G to rise to a corresponding amount. But the glass tube (G), because of rubber connections, may be raised[2] and thus the water level within it brought to a greater height above the membrane (M) and the pressure on M thereby increased. As the pressure on M increases, the brain pressure is more and more overcome, the brain sinks toward its normal position, and as it sinks the fluid system falls until the upper level is again at the original mark on the glass tube (G), indicating that the membrane is again

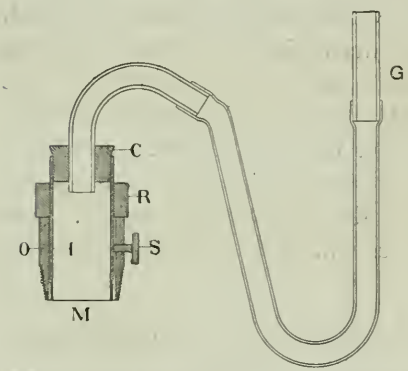


FIGURE 1. — Apparatus for measuring cerebral pressure while preventing the brain from bulging through the opening in the skull.

[1] HILL: *Loc. cit.*, p. 9.

[2] The variations in the level of the water due to raising and lowering the rubber tubing were found to be relatively insignificant.

even. By this means not only can the brain be kept in its normal relations within the cranium, but by measurement of the height of the upper level of the water above M the pressure which the brain is exerting can at the same time be ascertained. By connecting the tube (G) with a piston recorder a continuous record of the pulsations of the brain and the slight changes in its level can be obtained, and the times noted at which the tube is raised or lowered. The pressure which the brain is exerting, as indicated by the height of the water column, is written above the record, whenever the pressure changes. (See curve *a*, Fig. 2.)

Cats were used for the experiments. In every instance the animal was fully anæsthetized with ether before being operated upon. The cylinders were attached sometimes before, sometimes after the injury. Owing to the elasticity of the bones of the skull and to the force of the concussion the rigid outer cylinder would not keep its place, but would be driven out. To obviate this difficulty side projections, level with the scalp, were attached to the cylinder, and strong rubber bands passed over them to keep the apparatus in position. The concussion was caused by blows of a hammer on the skull. Since the results of injury are to be differentiated from the normal conditions, a description of the normal conditions will first be given.

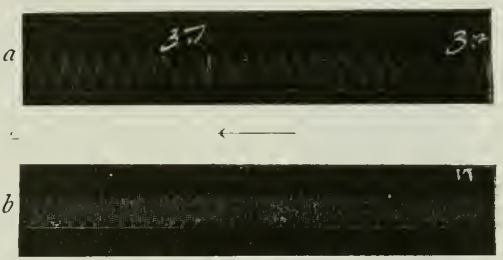


FIGURE 2.—Records of brain pulsations obtained by attaching a piston recorder to the apparatus shown in Fig. 1. The upper curve (*a*) was taken after considerable hemorrhage; the numbers indicate the water pressure required to keep the brain from bulging. The lower curve (*b*) is typical for normal pressure (17 cm. water).

The uninjured brain of an etherized cat will bulge through the trephine opening after the dura is cut, unless pressure is applied to keep the organ in place. The height of the water column necessary to prevent the bulging is a measure of intracranial pressure. In one instance this pressure during two hours of observation varied widely within the limits of 4 cm. and 20.4 cm. of water, and showed an average of 12.7 cm. of water. In another instance during two hours of observation the pressure varied between 9.4 cm. and 16.9 cm. water, and had an average of 13 cm. In still another case the average

pressure during five hours was 13 cm. of water. This observation accords with the observations of Hill on dogs, in which he found the intracranial pressure in normal conditions about 10 to 13 cm. of water.[1] The variations from the average were largely due to different degrees of etherization; renewing the ether invariably caused an increase of brain pressure, and as the effect of the ether was gradually passing away, the brain pressure would slowly fall. Every factor which increased general blood pressure, such as compression of the abdomen, movement of the limbs, stimulation of a peripheral nerve, had an instant effect in heightening intracranial tension. A hemorrhage at the time of trephining, if at all severe, caused a marked diminution of brain pressure, which ranged in one case of considerable bleeding from 0.8 cm. to 6.8 cm. of water with an average during two hours and a half of 2.8 cm. Hemorrhage caused also a characteristic change in the nature of the pulsations of the brain; the excursions through which the surface passed at inspiration and expiration were increased in amplitude, while the excursions due to the heart-beat were diminished. A comparison of curve *a* (Fig. 2) of the brain pulsations after severe hemorrhage, with curve *b*, which is typical for normal conditions, will make clear this difference.

FIGURE 3. — Curves of cerebral pressure (*a*) and blood pressure (*b*) at the time of injury (5) and immediately thereafter.

The average brain tension, then, in a normal uninjured animal is about 13 cm. of water. How does injury to the brain affect this tension? The immediate effect of a severe blow on the head is an enormous increase of intracranial pressure at the time the injury is received. If the cylinders are introduced into a trephine hole and held in place by strong rubber bands, as above described, and if the water system of the apparatus is then placed in connection with a membrane manometer, so as to avoid extensive displacement of the water by the violence of the traumatism, an indication of the effect of the traumatism on the brain may be secured. In Fig. 3 the upper curve is a record of the movements of the brain thus taken. The extensive movements

[1] HILL: *Loc. cit.*, p. 73.

of the writing lever at the point marked 5 were caused by five concussions. As the head was supported in a uniform position by means of a block, the movements could be due only in a very slight degree to the inertia of the water in the cylinders; they must be almost wholly the consequence of great variations in intracranial pressure. To understand the result of a sudden great increase in intracranial pressure, the general conditions of the skull and its contents must be recalled. The brain case, under ordinary pressures, may be regarded as a fairly rigid box, but when exposed to a violent force it manifests a considerable degree of elasticity. Within this case are the brain substance and cerebro-spinal fluid, both incompressible, and the blood, which is the single variable factor. When a severe injury is inflicted on the head, the bones yield under the blow, there is a sudden violent increase in intracranial pressure, which is distributed by the incompressible brain substance and cerebro-spinal fluid. In so far as this increase in pressure overcomes the pressure of the variable factor, the blood, it must result in a checking of the blood flow into the brain.[1] This result appears in a characteristic diminution of the amplitude of the brain pulsations immediately after the injury, as is shown in the upper curve of Fig. 3. The pulsations soon return, however, to their original extent.

Following the injury there are changes in respiration, in general arterial pressure, and in intracranial tension. Severe concussion frequently causes paralysis of the respiratory centre; respiration entirely ceases, although the heart continues beating for some time. Under these circumstances, if artificial respiration is persisted in, the respiratory centre may wholly recover its function. In this matter, which is of direct practical importance, my observations completely confirm those of Polis,[2] Horsley,[3] and Kramer.[4] The typical changes in general arterial pressure were recorded from the femoral artery in order to avoid, so far as possible, any interference with the cerebral circulation. At the time of the injury and a moment thereafter, a slight rise in the blood pressure was usually to be observed. The rise was followed by a considerably more pronounced fall of pressure, after which there was a gradual return to normal. When the injury caused a temporary cessation of respiration, the fall was always

[1] *Cf.* Kramer: Annals of surgery, 1896, xxiii, p. 163.
[2] Polis: Revue de Chirurgie, 1894, xiv, p. 730.
[3] Horsley: Quarterly medical journal, 1894, ii, p. 309.
[4] Kramer: *Loc. cit.*, p. 167.

greater than when the breathing continued without interruption. (Fig. 3).

The phenomenon of interest, however, is the change in intracranial tension following the injury. The upper curve in Fig. 3 indicates a higher cerebral pressure after the traumatism than before. The objection may be justly raised that in this instance the skull was opened, and the only opposition to the projection of the brain through the opening was in the elastic tension of the manometer. The concussion may, therefore, have pushed the brain outward, and thereby produced the appearance of greater intracranial pressure. That there is a real increase of brain tension after injury is proved by causing the concussion before the skull is opened, and afterward applying the apparatus for measuring the tension (Fig. 1). A record of the variations in the height of the water column needed to keep the brain even with the dura can thus be made precisely as in normal states. After injury, the average height of the water column was always greater than with the uninjured brain. In one case the cat was etherized at 11 A. M., and fifteen minutes later was struck ten times in a region afterward trephined. So soon as the trephine button was removed, the dura was observed to be bulging and pulseless with a little blood beneath. At this time the animal ceased breathing; artificial respiration was repeated several times, and then the dura was quickly opened, when regular breathing began and continued throughout the period of observation. The apparatus was applied to the edges of the trephine hole, and the variations in pressure were observed during four hours. In that time the pressure varied between 10.1 cm. and 47 cm. of water, with an average pressure of 25 cm. There was no noticeable increase of pressure in the last half over that in the first half of the experiment. Other similar operations gave similar results; in the few hours following the injury, the pressure never rose higher than 50 cm. of water, although in some instances there could be seen at the autopsy pial hemorrhages and contusion of the brain substance. Simultaneous records of the brain and the femoral artery show that there is no increase in general arterial pressure to account for the rise in brain pressure after injury. Whether this rise is to be ascribed to increased resistance in the smaller vessels of the brain, or to a relaxation of the vascular walls, has not yet been demonstrated. Mosso[1] noted a consid-

[1] Mosso: Ueber den Kreislauf des Blutes im menschlichen Gehirn, Leipzig, 1881, p. 200.

erable increase in the brain pulsations after releasing the carotids from compression, and he maintained that the effect was merely local, and that it was caused by a relaxation of the vessel walls in consequence of the interruption of the circulation within them,— a phenomenon which can be demonstrated in the arm. It is possible that the concussion and the initial checking of the blood stream through the cerebral vessels cause a similar relaxation of their walls, and a consequent greater pressure of the brain against the skull.

A consideration of the phenomena attending the reception of an injury to the head, and following soon thereafter, indicates that in cases of simple concussion (*i.e.*, without severe intracranial hemorrhage or laceration of the brain) the only pressure which might account for the primary pressure symptoms is that observed at the moment of injury. Men remain conscious through the spasms of strychnine poisoning, although, according to Hill,[1] the pressure within the skull must, under these circumstances, rise to 50 mm. of mercury, an equivalent of 68 cm. of water.

Evidently the highest pressure after injury secured by the method I have employed, 47 cm. of water, is not sufficient to account for either the primary or the secondary unconsciousness, and the other symptoms of intracranial tension following head injuries. It is clear that the force of a severe blow on the head will overwhelm any blood pressure in the cerebral vessels, and result in circulatory disturbances likely to take a considerable time for their readjustment. The paralysis of the respiratory centre for some moments after the injury is an indication of the lasting primary effects. The readjustment of the circulation in the brain may be further hindered by the general fall in arterial pressure immediately after trauma. In one instance the arterial blood pressure fell from 135 mm. Hg to 95 mm. Hg almost directly following the trauma. Since there is in the brain at this same time a process of recovery from a checked blood flow, the factors effective in producing ordinary syncope are present. The primary unconsciousness after concussion seems thus to be accounted for by circulatory disturbances alone, although in so sensitive a structure as nervous tissue the possibility of molecular changes should not be overlooked.

Secondary effects of cerebral trauma. — In the preliminary study of pressure symptoms it was pointed out that in order to cause them the intracranial pressure must equal the general arterial pressure.

[1] Hill: *Loc. cit.*, p. 73.

It was also made clear that these symptoms frequently appeared after the primary effects of injury had passed away, or as a continuation and intensification of the primary effects. In these accidental experiments what may be sought as an explanation of the results? Before entering into a discussion of these results three antecedent conditions must be regarded: the anatomical arrangement of the cerebral blood vessels, the anatomical changes caused by injury, the effects of these changes on the circulation in the brain.

The central nervous system is peculiar in having over its surface a richly anastomosing network of blood vessels, from which branches pass into the substance of the organs. On the surface of the brain the vessels form a vast canaliculate reservoir with very free communications from one part to another. An injection into an afferent artery first fills the network and then goes more easily into other afferent vessels than into the nourishing arteries of the tissue.[1] The unity of the surface system is manifest. From the common reservoir there pass into the brain substance the nourishing arteries, some to the cortex, some to the deeper fibre tracts. Each of these vessels is isolated, independent, terminal, not anastomosing with other vessels from the surface or with branches from the central supply of the brain. The physiological conception of a terminal artery does not signify, however, that there is no communication with other sources of blood supply;[2] it means simply that the communication, *i. e.*, by capillaries, does not permit the easy establishment of a collateral circulation after the interruption of the arterial supply to a part. The brain substance is, then, nourished by terminal arteries preceded by an anastomotic network.

The importance of the arrangement of the cerebral blood vessels in understanding the effect of injury is seen when the pathological changes caused by trauma are recalled. Contusion of the brain exists in all cases of intracranial injury and, as has been noted, it is characterized by a diffuse formation of thrombi, the presence of punctate extravasations, and often also by thin patches of hemorrhage in the meninges. Associated with these changes is œdema, which is, however, of secondary development.

The effect of the pathological changes on the circulation is apparent from the nature of the anatomical arrangement of the cerebral

[1] CHARPY: Système nerveux, Part iii of Traité d'anatomie humaine, edited by Poirier, p. 700.

[2] BAUMGARTEN: American journal of physiology, 1899, ii, p. 245.

vessels. Because of the free anastomosis in the surface network a compressing hemorrhage in any part must force the blood to take the easier paths and thus partially or completely shut off the blood from the nourishing arteries plunging inward from that part of the net. Moreover, owing to the terminal nature of the arteries the general formation of thrombi and extravasations must result in a general diminution of the blood supply of the injured tissue. Proximal to the interruption there would be stasis, distal to the interruption, anemia, and both conditions must result in impaired nutrition of the brain. In this relation the interference with the normal food supply is not so important as the deprivation of oxygen. As will be made clear, the problem of a secondary increase of intracranial pressure is essentially the determination of the action of brain tissue deprived of its normal nutrition and especially of its supply of oxygen.

The effect on protoplasm of cutting off its oxygen supply may be seen in such widely different structures as unicellular organisms, the muscular tissue of the frog, and in nerve cells. Budgett [1] noted that when paramœcia were deprived of oxygen they began to absorb water, the contractile vacuole increased in size and new vacuoles appeared, then from the surface there protruded vesicles from which some of the vacuoles usually escaped. Finally the vesicles burst and the cell contents were extruded. Poisons, such as potassium cyanide, caused similar changes to take place more rapidly. When potassium cyanide was applied to a sympathetic nerve cell of the frog the cell soon became larger than normal and continued swelling until there was a marked increase in size. Budgett believed that the swelling was probably due to an extensive splitting of the molecules within the cell by means of the poison, and a consequent rise of intracellular osmotic pressure and absorption of water. Loeb [2] has noted in the frog's gastrocnemius similar changes from lack of oxygen. In a large number of frogs he ligatured the leg on one side and after a time removed and weighed the two gastrocnemii. Normally the two muscles are of the same weight; but under the circumstances of the experiment, the muscle deprived of its oxygen supply took up water so that in eighteen hours its weight was from one to three per cent greater than that of the undamaged muscle. After forty-eight hours there was a difference of fifteen per cent and at the end of seven days

[1] Budgett: American journal of physiology, 1898, i, p. 211.

[2] Loeb: Archiv für die gesammte Physiologie, 1898, lxxi, p. 470.

muscles showed a difference of weight from twenty-five to forty per cent. Loeb concludes that his experiments leave no doubt that the assumption of water by a muscle deprived of its blood supply is due to chemical changes in the muscle increasing the internal osmotic pressure and that these chemical changes are probably due to lack of oxygen.

These examples of the effect on various tissues of cutting off the oxygen supply show that the active agent in the production of swelling is osmotic pressure. Loeb found that a frog's gastrocnemius, placed in a 4.9 per cent salt solution, at first loses water, but later begins to take it up from this strong solution. The osmotic pressure of this solution is more than thirty atmospheres, the osmotic pressure of the normal tissue is that of normal salt solution or about five atmospheres. The original loss of water in this instance must therefore have caused chemical changes in the muscle which raised its osmotic pressure more than twenty-five atmospheres. Evidently in osmotic pressure there is a force acting which is very much greater than any blood pressure and which is entirely independent of blood pressure in producing a swelling of the tissues.

These results may now be applied to the conditions in the brain after injury. The initial changes are hemorrhages and contusion with thrombi and extravasations. The result of the changes is an impaired blood supply to the injured region and a consequent lack of oxygen. Both experiment and clinical observation prove that œdema follows. Dean[1] placed a glass disc between the brain and the dura and sewed the dura in place again. The disc produced anemia of the compressed area. After from three to six days Dean found that the parts about the foreign body were œdematous; a piece of brain taken from the compressed area contained 3 per cent more water than a similar piece from the opposite hemisphere. In clinical cases Bergmann says:[2] "One finds about the region of injury merely a small zone, which is scattered with specks of blood and colored yellowish red; farther on, as far as the swelling reaches, the brain substance appears moist, glistening, soft, — that is, highly œdematous." Now the question arises, is this œdema, this result of the primary pathological changes, this concomitant of the secondary pressure symptoms, a passive transudate due to blood pressure, as has been held hitherto; or is it the effect of chemical changes in the brain sub-

[1] Dean: Journal of pathology, 1893, i, p. 39. See also Duret, *Loc. cit.*, p. 194.
[2] Bergmann: *Loc. cit.*, p. 420.

stance, resulting from diminished blood supply and causing the taking of water into the tissues by increased osmotic pressure? The former process, when required to produce the equivalent of arterial pressure, encounters the difficulties already mentioned in criticising previous theories; the latter process provides a force amply sufficient to overcome any possible blood pressure.

Normal salt solution has the same osmotic pressure as the blood and therefore the same osmotic pressure as the tissues. Cohnheim [1] found, after introducing normal salt solution in animals even to 40 per cent of the body weight, not the slightest trace of œdema in the central nervous system. Magnus [2] has gone still farther and infused animals with normal salt solution to 110 per cent of the body weight within one hundred and forty-three minutes and produced no general œdema. Since normal salt solution is isotonic with the tissues, so that in its presence water does not pass into them when they are nourished, the passage of water from the solution into the tissues when they are not nourished indicates an increase of osmotic pressure within them. Upon this statement was based the following series of experiments with brain tissue.

The first question to be settled was with regard to the action of the brain when normal salt solution is passed through the vessels at ordinary blood pressure. To this end a cannula was tied into each common carotid artery of the cat and a solution of 0.8 per cent sodium chloride allowed to pass into the cerebral vessels. The solution ran from an elevated source and the pressure of the fluid was recorded by a mercury manometer. The height of the reservoir was such that the manometric pressure was approximately blood pressure, — 120 mm. of mercury. One or two minutes after the solution began passing into the arteries the head of the animal was severed from the body, the vertebraterial canals and the spinal canal plugged with cotton soaked in glycerine, and the skull trephined for the measurement of cerebral tension. For this purpose the two cylinders shown in Fig. 1 were used, but the inner cylinder was attached by rigid glass connections with a mercury manometer, with mercury conduction between the two. Before the inner cylinder was applied the level of the exposed surface of the mercury was made even with the level of the membrane, and over the mercury surface

[1] Cohnheim: Lectures on general pathology, London, 1889, p. 459.

[2] Magnus: Archiv für experimentelle Pathologie und Pharmacologie, 1899, xlii, p. 261.

was placed a thin paraffine plug fitting closely into the tubing of the manometer. The head was now placed at such a height that the trephine hole was even with the mercury level and the membrane, and the cylinder (I) was then slipped into its holder (O). In case the brain was bulging beyond its normal level the mercury in the manometer would rise and carry up the paraffine plug. Thereupon mercury was dropped into the manometer until the plug was forced downward to its former position, which indicated that the brain was returned to its normal level and at the same time showed the force which the brain was exerting against the membrane.

The following figures from an experiment in which was employed the procedure above described will show how the brain pressure changed as time passed. The flow of the salt solution began at 10.45 A.M.

Time.	Pressure of salt sol.	Brain pressure.
11.00 A. M.	11.1 cm. Hg.	1.6 cm. Hg.
11.20 [1]	11.2	2.9
11.30	12.5	3.5
12.15 P. M.	12.5	7.1
1.15	12.5	9.2
3.15 [2]	12.5	12.1
5.00	12.5	12.1

[1] At this time the flow from the veins had changed from a running stream to a rapid dropping.

[2] At this time there was only a very slow dropping from the veins.

In this experiment the brain pressure remained at the height of 12.1 cm. of mercury until 1 P. M. the following day, when the apparatus was removed. The pressure from the salt solution was removed first, but this did not result in any fall of the mercury level in the manometer; the brain had apparently taken in water until it completely filled the cranial cavity. Care was taken that the turgid tissues in the neck did not prevent the solution from passing into the brain, but it was impossible to keep the brain itself from swelling and shutting off its own supply. This probably accounts for the fact that the brain pressure did not rise above the pressure of the solution;

for in other cases in which the tissue was allowed in the early stages of the swelling to press outward as a hernia from the trephine hole, a pressure of more than 28 cm. of mercury was supported for hours without diminution of the swelling and with a solution pressure of only 0.7 cm. of mercury. Evidently under circumstances of non-nutrition the brain will take up water from a solution isotonic with the blood and will thereby exert a pressure sufficient to exclude the blood from the cerebral vessels.

The question raised in criticising the previous theories of increase of brain pressure now arises again, — is the increase of pressure due to the formation of a transudate from blood pressure, or is it the result of an active process in the tissues themselves? That brain tissue deprived of its proper blood supply will take up water from a solution isotonic with the blood, entirely independently of any mechanical pressure of the solution, can readily be demonstrated by removing a brain and placing it in 0.8 per cent sodium chloride solution. Under these circumstances it will almost immediately begin to increase in weight; as will be seen upon examining the curve of weight (A, Fig. 4) of such a brain, the increase during the first four hours is rapid, and thenceforth is slow and persistent for days. In one instance after five days the brain had increased 33.2 per cent; since the specific gravity of the brain and the solution is approximately the same this increase of weight means an increase in size of about one-third. As has been previously stated, a diminution of the intracranial space by one twelfth results in death. It is evident that impairment of the nutrition of the brain as a whole may cause it to take up water to a degree far greater than that necessary to produce death. Now what is true of the brain as a whole may be assumed to be true also of any parts deprived of their blood supply, and localized regions thus affected would take up the water of the plasma from

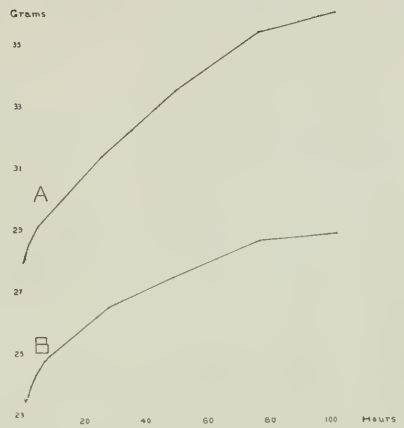


FIGURE 4. — Curve A showing the increase in weight when the brain is placed in normal salt solution, 0.8 per cent; B when the brain is placed in 2.0 per cent salt solution.

neighboring regions and swell in the same manner in which the brain as a whole will swell.

That nerve cells increase in size, as does the brain, when deprived of nutrition, has been shown by Scagliosi.[1] After repeated slight concussion in rabbits he found definite changes in the cells of the brain, varying according to the duration of life after the injury. The microscopic lesions consisted in varicosities of the dendrites, degeneration and swelling of the cell body with formation of vacuoles within it, and a more and more homogeneous appearance of the nucleus till the cell almost completely disappeared. The similarity of these changes with those recorded for infusoria deprived of oxygen is striking, and indeed Scagliosi attributes the alterations to disturbances of the circulation preventing normal metabolism.

The swelling of nerve cells when deprived of normal nutrition may be observed directly by placing in salt solution under a sealed cover, one of the small sympathetic ganglia of the frog. It will be seen from drawings of such a cell, made with a camera lucida, that the dimensions become greater and greater as the time passes, and that finally the cell begins to disintegrate and lose its definite outline. The last of such a cell is a formless granular mass. It is very possible that this is the series of changes through which the nerve cells pass in the production of areas of softening in the brain.[2] A brain which had been in salt solution for five days was declared by Dr. E. W. Taylor, instructor in neuropathology, Harvard Medical School, to show internally the typical appearance of softening.

The observations on brains and nerve cells prove that blood pressure is not a necessary factor in causing cerebral swelling and œdema. It may be, as Hill and Bergmann maintain, that there is transudation from the cerebral blood vessels when intracranial pressure is locally increased, but they have not shown that the transudation is a mechanical result of the increased pressure, nor have they shown that, even with a mechanically produced transudate, the pressure resulting therefrom is sufficient to produce death. They have, moreover, not regarded the factors increasing osmotic pressure within the tissues. Hill[3] states, "under normal pressures, the secretion and absorption of cerebro-spinal fluid does no doubt follow osmotic laws;" he overlooks the fact that deprivation of nutrition

[1] Scagliosi: Archiv für pathologische Anatomie, 1898, clii, p. 522.

[2] See Case IV, p. 98.

[3] Hill: *Loc. cit.*, p. 23.

causes within tissues chemical changes which affect a force much more powerful than mechanical blood pressure.

A conception of the force involved in the increase of weight, when the brain is placed in normal salt solution, may be obtained by means of a method used by Loeb. Curve B of Fig. 4 shows the changes in the weight of a brain placed in a 2 per cent solution of sodium chloride. For four hours there was scarcely any increase in weight. At first there was even a diminution of weight, because the osmotic pressure outside was greater than that within the tissues. Soon there was a sharp rise, however, and after about ten hours the slow, persistent increase in weight began. The osmotic pressure of a 2 per cent solution of sodium chloride is about 14.5 atmospheres. The pressure within the tissues, therefore, must have developed to this great height in order that the water should pass into them. Inasmuch as the osmotic pressure of an 0.8 per cent salt solution is only 5 atmospheres, there is evidence of a pressure increase of 9 atmospheres, which is about fifty-seven times ordinary blood pressure. Naturally such a pressure never becomes fully operative in the tissues; disruption must take place long before that result could occur. The internal osmotic pressure is almost balanced by the external, but because of the greater internal pressure, fluids pass inward till the mechanical pushing of the tissues overcomes the push of the blood, thereby shutting out the source of fluid supply. It is clear, therefore, that in the chemical changes taking place in dying brain tissues, there is a force present abundantly able to overwhelm the blood pressure and cause death.

It may be objected that the method used is too unnatural, too remote from actual occurrences, to prove the contention that the increased brain pressure after trauma is due to osmotic changes from malnutrition of the tissues. In this relation, the phenomena attending the formation of a hernia cerebri are instructive. The average pressure required to prevent the brain of an etherized cat from projecting through a trephine hole is about 13 cm. of water. In case a hole over the frontal region is left uncovered, however, the brain begins to rise through it, and as time passes the level becomes higher and higher, until the outer surface of the bulging brain is even with the scalp. At this point a considerable compressing force is needed to drive the bulging brain back into the skull. The surface of the hernia shows no pulsations, the vessels may contain blood, but the circulation is much impaired. How may this phenomenon be

explained? The brain presses against the edge of the trephine hole. As it does so, a circular area of blood vessels in the pial network may be regarded as separated by pressure from the remainder of the superficial reservoir. Since the anastomoses in the pial vessels are very free, the blood takes a course of less resistance than that offered at the edge of the trephine hole. The nourishing vessels pass into the substance of the brain at right angles to the surface; the part of the brain within the hole, therefore, would not be normally nourished. Under these circumstances the osmotic pressure in the tissue rises; the plasma from neighboring areas has the osmotic pressure of the blood; it is consequently taken into the disturbed tissue, and the process of swelling begins, and continues persistently. In this instance nothing is introduced into the cranial cavity to increase blood pressure there; the pressure in neighboring brain areas cannot be much greater than 13 cm. of water, and yet a hernia is produced which drives outward the pial coverings of the brain, and in the end offers a marked resistance to reduction. Examination of the hernia shows an evident œdema.

A review of the evidence thus far presented shows that in cases of brain injury, pathological changes are brought about which result in an impairment of the nutrition of the injured region. In conditions of impaired or interrupted nutrition, tissues undergo internal changes leading to increased osmotic pressure, and thereby to increase of water-content and greater size. The swelling takes place, therefore, by means of a force much greater than blood pressure. The conditions of the brain are peculiar in that the organ lies in a rigid case. Swelling of a part consequently compresses the only compressible portion of the contents of the cranial case, — the blood vessels. Thereby new areas are shut out from normal blood supply, and changes now take place in these tissues as well, with the result that water passes into them; thus the swelling spreads until the blood-flow is so greatly excluded from the brain that life is no longer possible.

It is by this process that the cases of head injury resulting in death from secondary increase of brain pressure [1] may be explained. Cases in which death results soon after the reception of severe injury may also be supposed to be due in part to this same process. The remaining [2] class of cases, those in which the pressure symptoms develop after injury but do not result in death, remain to be consid-

[1] See p. 97. [2] See p. 96.

ered. In attempting to explain these cases, the fact should be remembered that in osmotic action not only does the water pass inward through the membrane to the stronger solution, but also the dissolved particles pass outward and tend to equalize the osmotic pressure. It is conceivable that, if the immediate result of the blow is slight, the passage of water into the tissues will be only slight, before the diffusion of decomposition products from the tissues has taken place to so great an extent as to bring about an equilibrium. Or the progress of the developing necrosis may be slow and diffusion occur gradually, and œdema may not, therefore, result. In this connection it would be interesting to know the careful quantitative urine analysis of the body salts in the cases of secondary pressure symptoms after head injury.

The occurrence of accumulations of fluid under the dura or in the ventricles in some of the recorded cases[1] seems to offer evidence against the theory of pressure here propounded; for why does not the fluid pass into the tissues rather than accumulate if the tissues have the great osmotic pressure attributed to them? Before this question can be definitely settled, the osmotic pressure of these fluids must be determined. It is probable that the gatherings of fluid are encapsulated. The diffusion of salts from the injured tissues into even a slight amount of fluid in an encapsulated space would render that fluid of higher osmotic pressure than the blood plasma. The plasma would thereupon pass into the encapsulated space in obedience to osmotic laws, and thus increase the volume of the fluid and its compressing effect. Further change in the injured tissues would lead to greater swelling in them, and to diffusion of more of the dissolved products of decomposition. The diffusion into the encapsulated fluid would still further increase its osmotic pressure, and result in still more plasma coming to increase its volume. Thus there would be a passage of salts from tissues to blood in a series of decreasing concentrations, and a passage of fluids to tissues in a series of increasing osmotic pressures. And since water will pass more rapidly than salts through the membranes, the result is usually a greater and greater pressure till death supervenes.

These theoretical considerations merely point the way to further experimental work and more exact clinical observation. The facts of the ground here covered remain; that injuries to the brain interfere with its proper blood supply, that such interference causes an in-

[1] See Case IV, p. 97.

creased osmotic pressure within the tissues, and a consequent taking up of water from the surrounding plasma. The swelling and œdema of the brain after head injuries, therefore, are not wholly due to passive transudation, as Hill and Bergmann have maintained, but are mainly the result of an active process in the tissues themselves, a force many times greater than blood pressure, and amply sufficient to produce all the pressure symptoms, and account for all the signs of intracranial tension which the clinical cases of cerebral trauma often manifest.

Summary.

At the moment of injury the intracranial pressure rises to a height sufficient to check the blood-flow into the brain.

Immediately after the injury the general blood pressure usually rises for a moment, then falls. Thereafter a gradual recovery of normal blood pressure occurs, with a simultaneous increase in the extent of the pulsations of the brain.

The paralysis of the respiratory centre following head injury may be recovered from if artificial respiration is persisted in, and the heart action remains strong. (Horsley, Polis, Kramer.)

The primary loss of consciousness after a blow on the head is apparently due solely to circulatory disturbances, though minute changes in the nerve cells must also be considered.

The normal cerebral pressure is about 13 cm. of water; after injury the brain pressure may rise to an average of 25 cm. of water. Since this increase is not sufficient to account for the symptoms present in clinical cases, there must be other secondary processes causing increased pressure. The secondary increase in pressure is due mainly to three factors: deprivation of normal nutrition in injured parts, passage of water into these parts with consequent swelling, and the rigid inclosure of the brain, causing the swelling in one region to affect markedly neighboring regions.

The thromboses, extravasations, and hemorrhages, which accompany contusion, impair the blood supply of the injured region, especially since the nutrient arteries of the brain are terminal.

Brain tissue deprived of blood undergoes chemical changes resulting in greater internal osmotic pressure and the passage of water into the tissue. Brains placed in normal salt solution increase continuously in weight, even to 33 per cent. The swelling which the tissue

undergoes must cause it to compress neighboring regions, and thus further impair the circulation so that new regions are involved in the process. Thus a vicious circle is established.

The main force effective in causing swelling is probably osmotic pressure, which, in brain tissue, may attain a degree so much greater than blood pressure, that the blood would really be prevented from entering the cranium.

Recovery from head injuries after pressure symptoms, and the accumulation of fluid under the dura and in the ventricles, are possibly to be accounted for by diffusion of the products of destruction from the tissues.

ON THE ANALOGY BETWEEN THE EFFECTS OF LOSS OF WATER AND LOWERING OF TEMPERATURE.

By ARTHUR W. GREELEY.

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IN his experiments on heliotropism, Loeb[1] observed that the larvæ of Polygordius and certain Copepods can be made positively heliotropic either by raising the concentration of the sea-water, or by lowering the temperature, thus indicating an analogy in the effects of a loss of water and a reduction of temperature. Other instances may be cited. Thus among plant lice that exist in two forms, one winged and the other wingless, the growth of wings in the wingless forms can be produced either by lowering the temperature or allowing the animals to dry. Loeb[2] has also shown that division of the protoplasm in the fertilized Echinoderm egg can be prevented by raising the concentration of the sea-water or lowering the temperature. From these facts Loeb[3] concluded that: — "raising the concentration of the salt solution, in which an animal or a tissue lives, has qualitatively and quantitatively the same effect as lowering the temperature." It is well known that raising the concentration of the salt solution causes the organism or tissue to give off water, but why a reduction of the temperature has an analogous effect has never been explained. It is hoped that the present experiments will throw some light upon this problem.

At first the effect of changing the temperature was tried on the common blue-green Stentor cœruleus. The Stentor were kept in small covered dishes, and the water was renewed each day from the aquarium in which the Stentor had been grown. This contained an abundance of food material. The animals were divided into three lots: —

Lot 1 was kept at the room temperature (20° C.) as control material.
Lot 2 was surrounded by a mixture of ice and salt.
Lot 3 was placed in a thermostat at a temperature of from 25° to 28° C.

[1] Loeb: Physiology of the brain, 1900, p. 198.
[2] Loeb: Journal of morphology, 1892, vii, p. 253. [3] *Ibid.*

When the temperature was suddenly lowered to the freezing point, and ice was allowed to form in the dish containing the Stentor, the animals immediately became quiet. Death took place when the freezing point was reached. The cell outlines became extremely irregular and shrunken, and the blue-green pigment, stentorin, was rapidly diffused through the water. These irregular cells remained intact for a short time, but soon disintegrated.

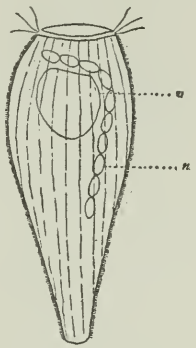

FIGURE 1. — Camera lucida drawing of a Stentor exposed to a temperature gradually lowered to 2° C. and maintained for thirty minutes.

A very different result was obtained when the temperature was lowered gradually. The dish containing the Stentor was at first loosely surrounded with ice, so that the temperature sank to about 10° C. More ice and salt were then added, and the temperature allowed to fall for an hour, until zero was reached, or until a film of ice began to form around the edges of the dish. Under these conditions the Stentor soon became quiet, except for a lively contraction of the buccal and peristomal cilia, and remained so for from one to three hours. Large vacuoles (*v*, Fig. 1) began to appear in the protoplasm, but otherwise the organism was unchanged. Finally the cell slowly assumed a spherical form, the vacuoles (*v*, Fig. 2) increased in size, and the nuclear nodes (*n*, Fig. 2) separated. The peristome then closed over, the mouth and œsophagus with their cilia disappeared, and the only evidence of continued life was a slow contraction of the small body cilia (*b c*, Fig. 3) found over the entire cell wall. While these changes were taking place, the Stentor still remained irritable and frequently changed their shape, but soon the peristome and body cilia disappeared, the striations of the ectosarc slowly faded out, the protoplasm became densely granular in appearance, and the cell ultimately assumed a more or less regular spherical form. This cell shrunk in size because of the absorption of

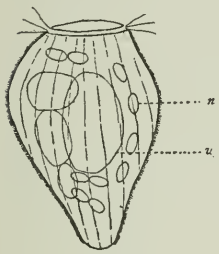

FIGURE 2. — Stentor exposed to a temperature of 2° C. during one hour and twenty-five minutes.

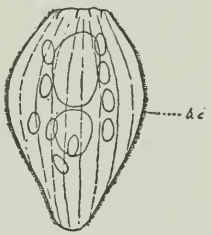

FIGURE 3. — Stentor exposed to a temperature of 2° C. during two hours.

the vacuoles, the ectosarc became separated from the rest of the cell, and some of the nuclear nodes came to the surface, and were given off with the ectosarc. The remaining nodes of the nucleus were embedded in the endosarc, and later were found fused together, although the protoplasm became so opaque that it was difficult to follow the process. Finally there was formed a simple resting cell, consisting only of the endosarc, from which all the complex structures of the living animal had disappeared, and which resembled a cyst in every respect, except that it lacked the distinctive tough cell wall of the latter. Such a resting cell is shown in Fig. 4. The ectosarc (*ec*) is seen adhering to the cell wall. Three of the nuclear nodes (*n*) are visible.

It is generally thought that a reduction of temperature produces only a cessation of the vital activities of an organism, and that these activities may be resumed when the normal temperature is again reached. But this experiment shows that in the case of Stentor a lowering of the temperature brings about certain well defined structural changes, that are not necessarily incidental to a permanent suspension of the vital functions of the cell. The low temperature induces a definite transformation of the organism which does not involve its death.

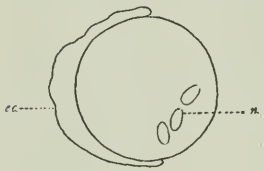

FIGURE 4. — Typical resting cell of Stentor, after an exposure to a temperature of 2° C. during two hours and a half.

That this transformation is a vital and not a lethal phenomenon is shown by the fact that when the temperature again becomes normal these resting cells undergo a reverse process, and become active Stentor. This retransformation of the artificially produced resting cells into the normal animals was observed to be complete in three or four cases. In several cases, although the first stages of the process were seen, the retransformation was never completed. In all instances, the resting cells lived in the small dishes for from two to three weeks before disintegrating, showing that the Stentor had not been immediately killed by the low temperature. In the few instances in which the complete retransformation was observed, the spherical resting cells were removed from the cold water by means of a pipette, and isolated in small covered dishes at the room temperature. In from three to six hours after removal from the cold water the retransformation was begun by the cell gradually lengthening until it assumed the form of an ordinary Stentor. The nucleus appeared as

a solid band (*n*, Fig. 5) formed by the fusion of the nuclear nodes, but it slowly divided until the common chain-like form was reached. The first indication of the peristomal cilia was a longitudinal ciliated band (*p c*, Fig. 5) which extended over the anterior end of the cell. The lower end formed a slight spiral depression which grew deeper, and finally became the mouth (*m*, Fig. 5). The upper end of this ciliated band curved slowly about the anterior portion of the animal and at the same time the lower end with the mouth depression was drawn up, until the band formed a circle around the anterior end of the cell with the mouth at one side (*m*, Fig. 6). This circle became the peristomal circle of cilia (*p c*, Fig. 6), inclosing the disk or peristome (*p*, Fig. 6). The cilia extended spirally down the mouth, forming the cilia of the gullet. While these changes were taking place the ectosarc and its striations appeared, and a typical Stentor resulted.

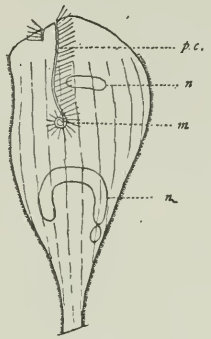

FIGURE 5. — First stage in the formation of an active Stentor from the resting cell, twelve hours after the temperature has become normal.

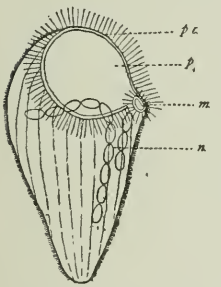

FIGURE 6. — Second stage in the formation of an active Stentor from the resting cell, sixteen hours after the temperature has become normal.

These stages in the formation of the peristome, and the division of the nucleus correspond closely with a periodical change which Balbiani[1] observed in Stentor. He saw the disappearance of the old mouth and peristome, and their regeneration, with the attending modification of the nucleus as described above. This experiment recalls also Loeb's[2] experiments on the transformation of organs among Hydroids, in which the material of the polyps was modified into stolons by a contact stimulus, and the process then reversed.

None of these changes was observed in the Stentor that were exposed to a higher temperature (25°–28° C.), but another process was noted. As described by Bütschli[3] and others an increase of temperature greatly stimulated cell division among the Infusoria. The con-

[1] BALBIANI: Zoologischer Anzeiger, iv, pp. 312 and 323.
[2] LOEB: This journal, 1900, iv, p. 178.
[3] BRONN'S Thierreich, i, 3, p. 1243.

trol specimens, kept at the room temperature, showed very few cases of cell division, but in almost every instance, the Stentor began to divide immediately upon being put into the thermostat. The divisions followed in quick succession for from five to seven bi-partitions, without a marked diminution in the size of the Stentor. After three or four hours, division ceased, and the Stentor soon died. This result indicates an increase of irritability, corresponding with an increase of water in the cell, and brings out very clearly the antagonism in the effect upon these organisms of raising and lowering the temperature, the one stimulating cell division and rapid growth, and the other producing a contraction of the protoplasm and the formation of a spherical resting cell. This antagonism is still further emphasized by the observation that lowering the temperature will not only inhibit cell division, but will bring about a reverse process, causing a fusion of the partially divided halves. This experiment was performed on Stentor in the process of division, and the fusion of the dividing portions was shown very clearly.

The shrunken condition and general behavior of the Stentor which had been exposed to a lowering of the temperature suggested the possibility that the process had been accompanied by a loss of water. To test this hypothesis, the concentration of the water was raised, that it might be ascertained whether the same result could be obtained by this means. The results were as follows: when the Stentor were placed in a $\frac{m}{5}$ [1] or $\frac{m}{10}$ solution of cane-sugar, the effect was the same as that produced by suddenly lowering the temperature to the freezing point. The cells became much shrunken from excessive plasmolysis, and soon disintegrated. If, however, a $\frac{m}{0}$ solution of cane-sugar was used, the Stentor became quiet in a short time, and formed spherical resting cells, which could hardly be distinguished from those formed by a gradual reduction of the temperature. The same stages were passed through, and the two processes seemed to be identical in every respect, although I was unable to bring about the reverse process in this case. This experiment makes it probable that a lowering of the temperature causes the cell to lose water, as is the case when the concentration of the surrounding medium is raised. To test further this hypothesis, some experiments were tried upon Spirogyra in which the plasmolysis can be easily studied.

[1] m indicates *mol.*, or one gram molecular weight in a litre.

Several years ago, Klebs[1] succeeded in producing parthenogenetic spores of Spirogyra by placing the filaments in a $\frac{m}{5}$ solution of cane-sugar. The cell contents shrunk through plasmolysis, and formed oval spores in the centre of the ordinary cells of the filament. I repeated this experiment and at the same time exposed another lot of Spirogyra filaments to a lowering of the temperature (from 20° C. to 1° or 2°). The filaments were kept at this temperature for about three hours, and then removed to that of the room. Examination showed that plasmolysis in these filaments had been as perfect as in the others. Regular oval spore-like bodies were formed in the centre of each cell (see Fig. 7). Upon removal to the room temperature, the cells gradually took up water, the chromatophores expanded, and the cells resumed their normal appearance. Another experiment, well known to plant physiologists, which was brought to my notice by Mr. B. H. Livingston, shows very strikingly that the Spirogyra cell loses water when the temperature is lowered. If the filaments be carefully dried and placed in olive oil, and then exposed to a low temperature, the water may be seen to escape from the filaments, and collect in small drops in the oil. If the temperature be still further lowered, these drops will freeze, and the ice crystals will be readily seen.

FIGURE 7. — A cell of Spirogyra showing plasmolysis produced by an exposure to a temperature of 2° C. during three hours.

The author desires to express his obligation to Dr. Loeb, under whose direction this work was done.

SUMMARY.

1. In Stentor a lowering of the temperature does not produce simply a suspension of the vital activities of the cell, but it brings about certain well defined morphological changes. The same changes can be produced by increasing the osmotic pressure of the surrounding solution.

2. These changes consist in the absorption of the cilia and the gullet and the throwing off of the ectosarc; and finally there is formed from the endosarc alone a spherical, cyst-like cell, which may be called a resting stage of the Stentor.

[1] KLEBS: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen, Jena, 1896.

3. If the temperature be again raised, a reverse process will take place, and the cyst-like cell will develop into an active organism.

4. In Spirogyra, a typical plasmolysis can be produced by a reduction of the temperature.

5. This fact makes it probable that a reduction of the temperature and a loss of water have similar effects, because the cell loses water when the temperature is lowered, as well as when the concentration of the surrounding medium is raised.

NOTES ON REGENERATION AND REGULATION IN PLANARIANS[1] (*continued*).

By FRANK R. LILLIE.

THE experiments herewith recorded have been performed as occasion offered and have consequently extended over a considerable period of time. They have been selected from a large body of notes, because the facts are themselves new, and, the experiments being made on genera not hitherto studied from this point of view, the results may serve to control the theoretical deductions made from the study of *Planaria*, the genus of aquatic triclad Turbellaria hitherto exclusively studied.

II. Regeneration of the Head in Dendrocœlum Lacteum.

In Dendrocœlum, which is closely related to Planaria and similar in structure, the capacity for regeneration is very much less, and as concerns the head is limited to the anterior third of the body, approximately, though a new tail may regenerate at any transverse level.

My first experiment was to cut a single specimen in two through the pharynx, July 23, 1899. The cut surface healed, and the farther fate of the parts was as follows. The posterior part formed no new tissue, although it lived for twelve days. From the anterior portion, on the other hand, there grew out a pointed piece, which formed a tail. I afterwards repeated the experiment several times with a similar result. Thinking that the failure to regenerate a head might be due to the presence of the pharyngeal pouch, I then cut fourteen specimens transversely immediately behind the pouch. Two days after the operation I noticed an interesting difference in the reactions of the two kinds of parts. While the anterior pieces reacted in all respects like intact individuals, migrating to shaded parts of the dish, the posterior parts showed no definite reaction of this sort, but re-

[1] Number 1 of these notes, "The Source of Material of New Parts and Limits of Size," appeared in volume xxxiv of the American Naturalist, March, 1900, pp. 173–177.

mained scattered irregularly over the bottom, some with the ventral surface up. In four days five of these pieces died, in five days two more were dead, and in six days all of the posterior pieces were dead without having shown any signs of regeneration; while all of the anterior pieces, kept in the same dish, were living and regenerating new tails. This experiment was repeated with similar result, and I soon became convinced that, in this form, while a tail might regenerate at any transverse level from the pharynx back, a new head could not be formed from tissue behind the pharynx.

The question now was, could a new head be formed in front of the pharynx at any level? Specimens were cut transversely immediately in front of the pharynx. The result of these experiments was that, while a very narrow border of new tissue might be formed at the cut end of the posterior pieces, there was never regeneration of even the semblance of a head.

In the next experiment the heads of five individuals were removed by a transverse cut just behind the eyes. The heads, thus removed, did not regenerate, but in five days it was apparent that new heads were forming on the decapitated pieces, and in one of them the eyes could already be seen. In six days new eyes could be seen in all. The capacity for regeneration of a head was thus demonstrated.

Two questions now remained: first, is the development of a new head due to the position of the cut, or to the size of the piece? and second, how far back does the capacity for regeneration of a head extend? The first question received an answer in a very simple way. The head was first cut off just back of the eyes, and then from the anterior end of the major piece a small transverse part was cut. In six days a rudimentary head with eyes developed on one of three such small transverse parts. (The two parts that did not form heads were probably cut too small.)

How far back does the capacity for regeneration of a head extend? We have seen that it cannot be formed from tissue just in front of the pharynx, but that it can be formed just back of the eyes. Twelve specimens were cut transversely about half-way between the anterior end of the pharynx and the tip of the head. The reactions of both kinds of parts were quite normal, though the headless parts reacted much less rapidly than the head-bearing ones. In seven days both parts were rapidly regenerating, and eyes had appeared in the posterior parts; in each head-bearing part a new pharynx and tail were forming. Both kinds of parts then completed

the regeneration rapidly, though in nineteen days the normal proportions were far from being restored.[1]

Thus in Dendrocœlum, while tissue may grow out at any transverse level, behind the region immediately back of the eyes, in the form of a tail, the capacity for regeneration of a head is limited to the anterior third or fourth of the body. I do not mean, of course, to state that the formation of a head back of this region is completely impossible. Some one may, at any time, demonstrate by operating on a sufficiently large number of individuals, that a head may exceptionally regenerate back of this level. But these experiments demonstrate very clearly that the power to regenerate a new head is limited in Dendrocœlum, by other conditions than size of the piece or presence of certain parts of the intestine.

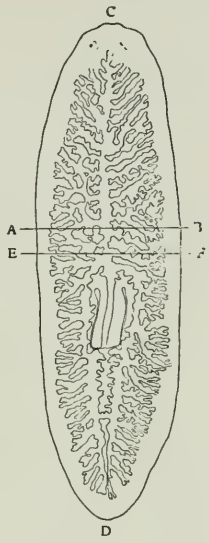

FIGURE 1. — Exact camera drawing of a stained specimen of Dendrocœlum. Note extensive anastomosis of gut-diverticula especially on left side. In other specimens more than one transverse anastomosis between posterior gut rami may occur.

Dendrocœlum differs in this respect from both Planaria and Phagocata, but strangely enough resembles the earthworm *Allolobophora fœtida*. In this form, according to Morgan's[2] observations, regeneration of a head does not ordinarily occur back of the fifteenth segment and never behind the middle of the body, although a new tail may regenerate at any level back of the tenth segment.

Why is it that embryonic tissue will continue to grow and differentiate on the posterior end of ABC (Fig. 1), but not on the anterior end of ABD? It is not because the cells of the ectoderm or of the mesenchyme or of the gut are incapable of growth

[1] This point deserves emphasis. In Planaria and also in Phagocata regenerating parts rapidly assume the normal proportions, after the differentiation of the new tissue ; this involves great changes in the form and positions of the organs. The completely regenerated individual thus comes to possess not only all the parts of a normal worm, but also the same proportions. Morgan [Biological Lectures from the Marine Biological Laboratory, Woods Holl, 1899, Boston: Ginn & Co., 1900] has proposed the term "morphallaxis," for this phenomenon of translocation of tissues by which the normal proportions are reassumed. In Dendrocœlum, morphallaxis is very slight, or in some parts entirely lacking.

[2] MORGAN, T. H.: Archiv für Entwicklungsmechanik der Organismen, 1897, v, pp. 570–586.

and differentiation at the level AB, because the same tissues, ABEF, that grow out into a tail, if forming part of CEF, will not grow at all if forming part of ABD.

The explanation of this peculiar fact must lie in some conditions of the piece ABC not found in ABD. I believe that the determining condition is the presence of the brain and anterior part of the central nervous system in the anterior piece.

How may the presence of this part of the central nervous system be thought to influence regeneration? The possibilities would seem to be:—1. That it may exercise a "trophic effect." 2. That it may act by coördinating the activities of the piece, and in consequence establishing normal stimulation in the regenerating part. 3. For regeneration of a brain we might suppose that certain cells not found in sufficient number back of a given region are necessary.

All of these possibilities presuppose differences, which actually occur, between the central system of Dendrocœlum on the one hand, and of Planaria and Phagocata on the other, cephalization being more advanced in Dendrocœlum than in the other genera.

I am unable to determine whether the brain does or does not exercise a "trophic effect," or whether the statement that it does would have any different meaning from what follows. Certain facts concerning the reaction of parts of Dendrocœlum incapable of regenerating a head lead me to place particular emphasis on the second possibility. Some years ago Loeb made the interesting observation, often since repeated (see Parker),[1] that decapitated specimens of Planaria react to the usual stimulus of light like normal individuals, but more slowly. Any symmetrical piece of Dendrocœlum capable of regeneration tends to come to rest in the shaded parts of the dish precisely like a normal individual. No doubt the coördination of movements upon which so definite reactions depend, is regulated by the portion of the nervous system within the piece. However in Dendrocœlum, all parts incapable of regenerating a head also become incapable, after a day or two, of performing the usual reaction to light. Thus my notes concerning twelve specimens of Dendrocœlum cut in two immediately behind the pharynx read: "Two days after the operation all of the anterior pieces go to the side of the dish and remain in the angle between the bottom and side; but none of the posterior pieces

[1] G. H. Parker and T. L. Burnett: This journal, 1900, iv, p. 373.

shows such reaction; these remain scattered over the bottom, about half of them with the ventral surface up." I have other similar records in my notes; but also some in which the reaction seems to have continued normal.

However, there seems to me to be a connection between the lack of correlation of movement and the inability to regenerate a head.

It is therefore quite possible that the fate of the undifferentiated mass may be determined by the stimulation of the normal movements; if at the anterior end, the forward extensions and contacts may furnish such stimuli; if at the posterior end the stimuli would be of a different nature. In fact, the stimuli would differ for all variants in position of the differentiating mass.

But, though I am led by the conditions found in Dendrocœlum to attach particular importance to these normal stimuli, I certainly agree with Bardeen[1] that the internal conditions prior to regeneration are of great importance. It seems to me that earlier observers have erred chiefly in not paying attention to the conditions of the internal parts. It is especially the physiological actions of these parts that must be taken into account. Bardeen has insisted on this in the case of the intestine principally. But similar relations obtain also in the muscular system and in the nervous system. The contraction of the muscular coats of the body wall is of the nature of a peristaltic wave. In any piece therefore different conditions, brought about by the direction of the contraction-waves, would obtain at anterior and posterior ends. The direction of the nervous impulses within the portion of the nervous system contained in the regenerating part would be typical. Thus in the gut, in the musculature, and in the nervous system the anatomical and physiological relations clearly distinguish anterior and posterior ends. Possibly similar anatomical and physiological conditions prevail in other parts. Before we have recourse to such dimly conceived hypotheses as "polarity," the possible differential effect of known anatomical and physiological conditions should be considered.

It seems to me, therefore, that the factors determining the fate of embryonic tissue at the anterior and posterior ends respectively are probably of this nature: — in the first place, the actual anatomical and physiological relations of parts, in all of which in any transverse part there is some antero-posterior differentiation; in the second

[1] Bardeen, C. R.: This journal, 1901, v, p. 1.

place, the coördination of all organs comprising a separated piece, depending probably on the nervous system, and leading to normal stimulation of any exposed embryonic tissue.

III. Regeneration of the Pharynges of Phagocata.

The specimens of *Phagocata gracilis* with which I experimented were found in the same pond with *Planaria maculata* and *Dendrocœlum*. The manner of life is very similar to that of the other two genera. Apparently it does not reproduce by fission. The power of regeneration of this genus is equal to that of Planaria; for instance, a single specimen may be cut into sixteen parts capable of complete regeneration. Phagocata, as is well known, possesses a very long pharyngeal pouch (Fig. 2), containing a large number of pharynges; one of these occupies the usual position at the anterior end of the pouch, while the others are attached laterally and communicate with short branches of the posterior gut rami.

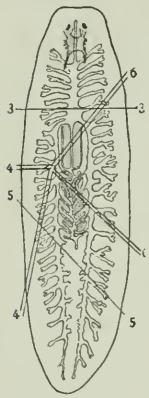

Figure 2. — Phagocata gracilis (slightly diagrammatic). The lines 3–3, 4–4, 5–5, 6–6, indicate approximately the source of the parts shown regenerated in Figs. 3, 4, 5, and 6.

a. In most parts, after three or four days, several pharynges are found regenerating simultaneously, though the more anterior ones are more advanced, and posteriorly the earliest rudiments are found (Fig. 3).

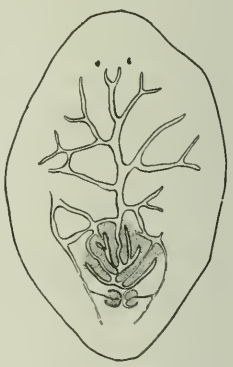

Figure 3. — Phagocata gracilis. Regeneration of the part in front of the line 3–3 (Fig. 2).

The formation of the pharynx and pharyngeal pouch from the mesenchyme has already been noticed by Woodworth[1] for Phagocata and Bardeen for Planaria. The former author gives a detailed account. The first rudiment is an accumulation of cells of embryonic type; near the periphery of this a semi-lunar cavity appears and forms the rudiment of the pouch; the tissue projecting into the pouch differentiates into the pharynx. The mouth is a secondary perforation of the floor of the pouch. It may be worth

[1] Woodworth: Bulletin of the Museum of Comparative Zoölogy. Harvard, 1891, xxi, p. 1.

while to notice in passing that this differs from the ontogenetic method of origin, both pharynx and pouch arising in the latter case from an ectodermal invagination.

b. The origin of the pharyngeal pouch as a cavity in the mesenchyma surrounding the free end of the pharyngeal rudiment has the following curious effect: the definitive common pouch arises as a series of separated cavities, which secondarily fuse together. The original partitions are recognizable for a long time as pointed projections of tissue into the pouch between the pharynges, Figs. 3 and 4. But in some cases of regeneration the fusion is incomplete or entirely absent, so that two or more pouches may be present, each containing several pharynges, Fig. 4. A curious abnormality is represented in Fig. 5: two pharynges have a common termination; evidently two buds arose very near together and partly fused.

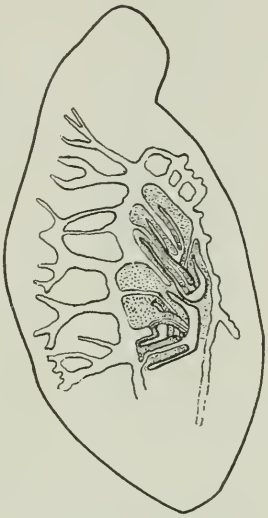

FIGURE 4. — Phagocata gracilis. Regeneration of the part bounded by the lines 4–4 (Fig. 2).

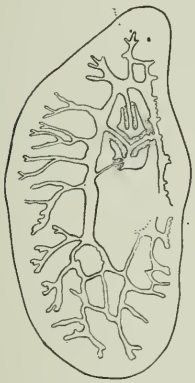

FIGURE 5. — Phagocata gracilis. Regeneration of the part behind the line 5–5 (Fig. 2).

c. As indicating that there is a special relation between the regeneration of the gut and the pharynges, the regeneration of lateral pieces offers some features of interest. The pharynges form near to the cut edge, as does the single pharynx in Planaria. Fig. 4 shows a case of this sort; there is a long pouch near the cut edge, in this case divided in two parts by a partition, and all of the pharynges are on the side towards the old tissue, with a single exception. Connecting with each pharynx, however rudimentary, is a branch of the intestinal system. The pharynges never begin to develop in such pieces on the outer side of the pouch until a branch from the intestine supplies the neighborhood. It will be seen that a new branch, destined to form the posterior ramus of the regenerating side, has grown along outside the pouch. This branch is very slender, but in connection with it a new pharynx has already differ-

entiated; other pharynges form along this side later. But in many observations made on the regeneration of lateral pieces, I never found the regeneration of pharynges along the cut side begun, until the posterior ramus of the intestine of the same side was formed. (See also Fig. 6.)

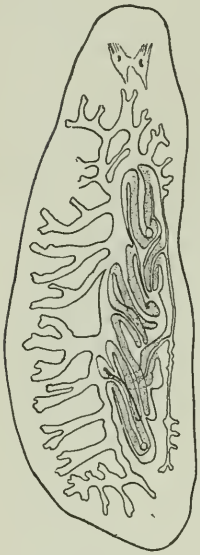

FIGURE 6. — Phagocata gracilis. Regeneration of the part bounded by the lines 6–6 (Fig. 2).

d. Usually the regeneration of the various parts takes place in a coördinated fashion, the pharynges developing in relation to the intestine, etc., and all parts in such a manner that normal, *i. e.*, adaptive, relations are established. Fig. 7 shows an exception to this rule. The part figured was as nearly as possible a $\frac{1}{32}$ part of a normal individual; the individual in question was divided into sixteen equal transverse pieces, and each of these was cut through its centre. None of these parts regenerated completely. Their history was as follows. Operation, Aug. 1st, 1899, thirty-two pieces; Aug. 2nd, twenty-one pieces living, the smaller pieces very thick dorso-ventrally; Aug. 4th, two pieces living. These were killed and stained, and a drawing of one of these is shown in the figure.

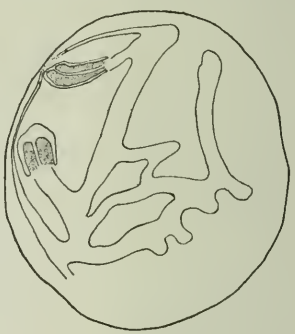

FIGURE 7. — Phagocata gracilis. Regeneration of a $\frac{1}{32}$. The gut branches were very broad, irregular, and branching in more than one plane; the superimposed branches are not represented.

The points of interest are that, while the piece has not regenerated a whole, the intestine has grown out in all directions through the piece, and at each of two places a pharynx has regenerated. These pharynges are widely separated and turned in opposite directions, a species of heteromorphosis. The side toward which they lay was evidently the injured side. Certain regenerative processes may go on in such a piece, but there is not full coördination, and the result is non-adaptive.

IV. Theoretical and Critical.

The phenomena of regeneration offer many problems, some of which not only appear insoluble in the present state of our knowledge, but actually offer no point of attack. For instance, if one were to ask why the regenerating rudiment of the head develops into such different forms in Planaria, Dendrocœlum and Phagocata, it could only be said that we do not know; nor are we able to say why Phagocata has many pharynges and the other genera of planarians only one. No physiological explanation of these phenomena can at present be offered. But if we leave out of account such problems, there still remain certain problems of regeneration that may be attacked with good expectation of success. The localization of regenerating organs is one of these. In the case of the regeneration of Hydroids, Loeb[1] has treated this question with great success, by showing that the regeneration of hydranths or rhizoids depends on certain external stimuli, such as, in different cases, light, gravitation, or contact.

A recent paper by Bardeen[2] seems to me to make for the planarians a decided advance in this direction inasmuch as he pays particular attention to the relations of the regenerating parts to the organs originally present in the piece. I propose, therefore, to devote some attention to Bardeen's theory in the light of experiments described in the second and third of these notes, which deal with different genera of planarians from the one (Planaria) used by Bardeen. I shall therefore first state his conclusions at some length.

1. "Embryonic tissue is formed in the specimens here studied at two places only, (1) at or near a cut surface, and (2) in the region of the piece just posterior to the point of least intestinal pressure." The author offers the following suggestions as to why the embryonic tissue should be formed at these places. The formation of embryonic tissue at the cut surfaces may be due to one or other of these causes: (1) slight change in the osmotic pressure; (2) exposure of the internal tissues to the water; (3) enzymes set free by the injury.[3] "The cause of the formation of embryonic tissue just pos-

[1] Loeb, Jacques: Biological Lectures Delivered at the Marine Biological Laboratory of Woods Holl, 1893, p. 37, Ginn & Co., Boston, 1894.

[2] *Loc. cit.*

[3] Loeb: This journal, 1900, iv, p. 60.

terior to the point of least intestinal pressure is equally dark. The process is much slower and is preceded by a retrograde metamorphosis in the pre-existing adult tissue. We might assume that here certain intestinal fluids are set free by pressure."

2. "The differentiation of this embryonic tissue depends on its relations to the intestinal apparatus of the animal. (1) If it lies anterior to the main axial gut, it becomes converted symmetrically into a head region." (2) "If the embryonic tissue lies lateral to the axial gut or to a line extending directly posterior to this, it becomes converted into a new lateral region." (3) "If the embryonic tissue lies at the posterior end of the axial gut, *i. e.*, behind the point of least intestinal pressure, it becomes converted into a new pharynx and pharyngeal pocket." (4) "If the embryonic tissue lies posterior to the pharyngeal region, it becomes converted into a new tail."

The author concludes from these facts that the intestinal system has a specific action in determining the nature of the parts developed from the embryonic tissue, rejecting the idea that the development of the intestinal system may be merely a coincident phenomenon. He also develops the outline of a theory of the way in which the intestinal system may be supposed to exert such specific effect. There will be no need, however, to consider this, if it can be shown that the rejected alternative is the correct one.

The whole theory is based on the relation of the "axial gut." Now what is the axial gut? It is what zoölogists call the anterior ramus of the intestine, and it extends from the base of the pharynx to the head. (See Fig. 1). That is, it is something that can be defined only by its relation to the pharynx and the head. It cannot, therefore, be otherwise than as stated, if regeneration is to take place at all. The head must form in front of it, the pharynx behind it, and to its sides the sides of the body. If it happened otherwise there would be no regeneration, but the development of a monstrosity.

But the author meant more than a mere truism of this sort; he meant to assert that, however much embryonic tissue might form, it would remain undifferentiated until the establishment of an axial gut, which then exercises its all-compelling influence. How far this conclusion is justified by the mere coincidence of the phenomena dealt with we may now pass on to inquire.

In Bardeen's opinion, then, the localization of regenerating organs depends entirely on the prior regeneration of the axial gut. I think that it can be shown that this conclusion is incorrect in some respects. We

have seen what is the criterion of the axial gut in normal individuals; what is the criterion in regenerated parts? Curiously enough, Bardeen does not dwell on this essential point; but I think that one who reads his paper with sufficient care will see that the criterion actually employed is the same here, *i. e.*, relation to the head and pharynx. If this be so, Bardeen has simply argued in a circle. The consideration of one class of cases discussed by Bardeen will serve to show that this has actually been done. "In the case of a very oblique crosspiece (in front of the pharynx) *a lateral branch of the old axial gut may be transformed into an extension of the axial gut.* The head then develops symmetrically around the tip of this, and hence somewhat lateral to the axis of the parent worm."[1] Why in this case does Bardeen regard a certain lateral branch of the original axial gut as transformed into an extension of the latter? For no other reason than that the head appears lateral to the cut end of the original axial gut. There is, in fact, in such cases, no other criterion of the axial gut. The figures illustrating this very case are conclusive in this respect. Regeneration of head and axial gut are really coincident phenomena. I have moreover a large number of observations that show the form of the regenerating gut to be dependent on the form of the new external parts, and *not vice versa*.

The observations recorded in the second of these notes show that the presence of a certain amount of embryonic tissue in front of an unquestionable axial gut does not furnish all the conditions necessary for the regeneration of a head. It was shown, that in Dendrocœlum there is a region extending a certain distance in front of the pharynx, in which regeneration of a head will not take place. A piece including this region after about two days has a certain amount of embryonic tissue on its cut end; it possesses also a certain portion of the original axial gut; yet a head does not form. Other conditions are necessary.

Concerning the localization of the pharynx in regenerating parts, it is held by Bardeen that wherever gut pressure is least, *i. e.*, at the place in the system where waste matters tend to accumulate, there the new pharynx forms. The criterion is not so indefinite here, because in all parts containing any of the three original main rami of the gut, by "place of least pressure" is meant simply the most

[1] *Loc. cit.*, p. 35. The development of the new head lateral to the axis of the parent worm in the case of oblique cuts was noticed first by Morgan and received extensive treatment at his hands.

proximal part in relation to the old pharynx. But I do not see how it is possible to determine the place of least pressure in the regenerating system when no part of the original three main rami are present, until they are again established. But by this time the regeneration of the pharynx is usually begun, so that it is often difficult to say what is the determining factor in phenomena that are so nearly coincident.

But here again comparison of other forms in which the conditions are only slightly different is very instructive. In Phagocata it is very evident that intestinal pressure has nothing at all to do with the regeneration of the lateral pharynges. Reference to Figs. 3, 4, 5, 6, will show that the lateral pharynges develop at the ends of short intestinal branches that are extremely delicate, and in which the intestinal pressure must be higher than anywhere else.

It is at least probable that there is an intimate correlation between regeneration of the intestine and of the pharynx or pharynges. I think that it is also probable that the stimuli inducing pharynx formation proceed from intestinal cells of new formation. But all new portions of the intestine do not induce the formation of new pharynges. There is another determining factor. What is this? It seems to me improbable that Bardeen's answer is in any way correct, because it presupposes a typical form of regeneration of the gut that does not exist. As a matter of fact, the regenerating gut is at first in most cases extremely irregular, and the restoration of its typical form is a matter of secondary regulation, due, I believe, to the form of the body, position of the pharynx and the tendency of the intestinal fluids to flow in the paths of least resistance.

In studies 2, 3, and 4 I have attempted to show the following facts: —

1. The differentiation of exposed embryonic tissue may be dependent on the external stimuli to which such tissue is exposed. The functional correlations of all the parts of a piece capable of regeneration are the internal factors, and the various stimuli from without thus induced in normal sequence are the external factors which determine the location of organs. The case of Dendrocœlum appears to indicate that functional correlation is dependent on the nervous system. The regeneration of a head lateral to the axis of the parent worm in the case of parts cut off obliquely is thus explained, because stimuli which normally would fall upon the head are received by the most advanced part, which is lateral to the original axis in

the case of such an anterior cut surface. The regeneration of a tail is similarly explained.

2. The intestinal system regenerates in relation to the new external parts, and not *vice versa*, as maintained by Bardeen; from which it follows that the location of new parts cannot be due primarily to the form of the gut.

Woods Holl, August, 1901.

ARTIFICIAL PARTHENOGENESIS PRODUCED BY MECHANICAL AGITATION.

By A. P. MATHEWS.

[*From the Marine Biological Laboratory, Woods Holl, Mass.*]

THE observations of Loeb[1] and Morgan[2] have established the fact that the unfertilized eggs of many different kinds of animals can be started in development either by increasing the osmotic pressure of the sea-water and thus depriving the eggs of water, or by the action of certain specific ions. The observations in the present paper show that mechanical agitation of eggs of the star-fish (Asterias Forbesii?) without any change whatever either in chemical constitution or osmotic pressure of the sea-water will produce the same result. The mechanical disturbance necessary to start the process is in certain conditions of the eggs so slight as to render this a serious source of error in all experiments in artificial parthenogenesis.

Some years ago I discovered that vigorous shaking of the unripe eggs of the star-fish for a few moments would cause the unripe eggs to mature, to extrude their polar globules and to be ready for fertilization. Morgan[3] confirmed and extended these observations. He showed that shaking ruptured the membrane of the germinal vesicle, that this increased above the normal the speed of development of such eggs after fertilization and that the unripe eggs of the sea-urchin, Arbacia, acted similarly. At the time and subsequently I attempted to induce the eggs to continue their division and to develop parthenogenetically but always with negative results owing, as I have since found, to my having shaken the eggs too early. Morgan, however, observed that in some of his shaken cultures certain eggs developed to gastrulæ even without the addition of sperm, but he attributed these to the accidental introduction of spermatozoa through the sea-water in the pipes of the laboratory.

The process which finally led to a successful result is, in brief, as

[1] LOEB: This journal, 1901, iv, p. 423.

[2] MORGAN: Archiv für Entwickelungsmechanik der Organismen, 1899, viii, p. 448.

[3] MORGAN: Anatomischer Anzeiger, 1893, ix, p. 141.

follows. The eggs are shed into sterile sea-water and allowed to remain there from two to four hours until both polar globules have been extruded and the female pronucleus has re-formed and reached a considerable size. The eggs are then removed to a test-tube with some sea-water and shaken vigorously five or six times back and forth and poured into a large dish with plenty of sea-water. The eggs then undergo the changes described later and a certain number, varying from less than one to more than fifty per cent, depending on the state of ripeness of the eggs, develop into actively swimming blastulæ and gastrulæ which live for from twenty-four to forty-eight hours and, under favorable conditions, occasionally reach the bipinnaria state.

The amount of agitation necessary to start the process of development varies in different individuals and depends in part upon the length of time the eggs have been lying in sea-water. After from four to six hours in the water the mere transference of the eggs from one dish to another by a pipette, or the jar caused by setting the dish containing the eggs down sharply on the table is sufficient to start development in a small portion of the eggs, with the result that swimming blastulæ appear by morning. Even the most careful transference of the eggs with a large mouth pipette from one dish to another into which they are gently introduced with the mouth of the pipette below the surface of the water will sometimes suffice to yield one or two embryos among several thousand eggs, while the untransferred eggs are wholly undeveloped.

In the experiments here cited all dishes and pipettes had been sterilized by washing in fresh water containing hydrochloric acid, followed by thorough washing in fresh water to remove the last traces of the acid. My hands and instruments were similarly sterilized and all sea-water had been heated to 70° C. before using. The individual star-fish were thoroughly washed with a brush under running tap-water to remove any adherent sperm before opening them. With such precautions I have never seen one fertilized egg out of thousands examined. Only in a single instance did the non-transferred controls show any advanced embryos, though here and there the first segmentation stages might rarely be met with. The single exception mentioned was the discovery of one irregular parthenogenetic blastula among many thousand untransferred eggs of a very ripe female. It must not be inferred from the precautions taken that the sea-water in the pipes of the laboratory contains sperm, as has been often stated. For I placed a large number of ripe eggs in a

thin layer in the bottom of a large dish and allowed the water from the pipes to flow over them for twelve hours and at the end of that time a careful examination of twenty thousand eggs taken from different parts of the dish did not reveal a single fertilized egg. The precautions described were taken to remove any possible doubt of fertilization. Following are protocols of some of the experiments.

Experiment 52. August 5. — Unfertilized Asterias eggs in water four hours. 2.45 P. M., shaken slightly in a test-tube. 3 P. M., the eggs have put out fertilization membranes and look like fertilized eggs. 5 P. M., the eggs begin to divide, some into 2–4 cells very regularly, others irregularly. 9 P. M., clear normal looking morulæ are common ; a clear area (nucleus ?) visible in each blastomere. 9 A. M., many eggs are dead and opaque ; many ill-shapen swimming blastulæ observed. Some dwarfs, some as large as normal. Several embryos are swimming in the fertilization membranes surrounded by the debris of dead cells.

Experiment 54. — 3.30 P. M. Same star-fish shaken hard. 8.45 P. M., many 16–32 cell stages observed. More than half the eggs are dividing irregularly with the fertilization membranes out. Next morning almost all dead.

Experiment 58. August 7. — Non-mature eggs shed at 10.30 A. M. Shaken at 11.15 A. M. 3.40 P. M., all are mature and a large number have fertilization membranes. 8.30 A. M., next day : several normal looking non-ciliated blastulæ found, but most eggs are dead and disintegrated.

Experiment 89. August 12. — Female Asterias. Eggs shaken after thirty-five minutes in sea-water. Aug. 13, 9 A. M., a few fairly regular segmentation stages. No swimming embryos. Another lot shaken harder was also negative.

Experiment 107. — Shaken when one polar body had been put out. 2 P. M., next day : almost all eggs dead. A few blastulæ found after long search.

Experiments 126, 127, 128, 129, 130, and 131. — 2.30 P. M. Shaken in test-tube with progressive severity after three hours' ripening in sea-water. 5 P. M., all ripe eggs have fertilization membranes and the unripe eggs are maturing. About one tenth of all the eggs are in the 2–8 cell stage. Many appear very regular and look exactly like fertilized eggs. 8.30 P. M., one tenth of the eggs are developing from two cells to normal blastulæ. 9 A. M., next morning : every pipette full of eggs has many swimming blastulæ except those from eggs shaken hardest. The blastulæ are many of them abnormal in appearance, being small and irregular in outline. Most eggs have died after a partial development.

Experiment 132. — 2.30 P. M. Same eggs drawn up in pipette and shot into another dish of water. 9 A. M., next day : vast majority have no development ; but here and there a swimming blastula is found.

Experiments 133 and 134. — 3 P. M. Same eggs as preceding drawn up in a narrow pipette and shot forcibly into a dry dish and then covered with water. 8 P. M., 2–16 cell stages are common. The immature eggs have not matured. 9 A. M., next day: two or three swimming blastulæ form in each lot of eggs examined (2000 eggs).

Experiment 136. — Same eggs transferred from the control dish to fresh sea-water with great care using a large mouth pipette introduced beneath the surface of the water. 9 A. M., next morning: after long search among many thousand eggs, one embryo was found.

Experiment 137. — Control of the preceding experiment. The eggs were left in the dish in which they matured. Long search the next morning failed to reveal a single embyro.

The effect of different degrees of mechanical violence is shown in the following experiments:

Experiment 147. August 17. — Female Asterias. Eggs left in water from 12 M. to 4.30 P. M. Not all ripened, as too many eggs were in the vessel. Transfers were made from this lot of eggs before the female pronucleus had re-formed.

Experiment 147 (a). — A large pipette filled and transferred with great care. 9 A. M., next morning: in 6000 eggs only one undergoing an irregular segmentation (into three cells). All other ripe eggs are dead without development. No fertilization membranes to be seen.

Experiment 147 (b). — A few eggs from the same star-fish shed into a large amount of sea-water and left undisturbed. Not an egg shows any sign of development.

Experiment 147 (c). — Same eggs as 147 (a) squirted into a dry dish and sea-water added. 9 A. M., no embryos.

Experiment 147 (d). — Squirted and drawn up and squirted once more. No segmentation. No embryos.

Experiment 147 (e). — Squirted four times into a dry dish. 9 A. M., three irregular non-swimming blastulæ in 1500 eggs. Nine other eggs irregularly segmenting.

Experiment 147 (f). — Squirted eight times. 9 A. M., some swimming blastulæ. Many non-swimming. 46 embryos in 2000 eggs.

Experiment 147 (g). — Squirted sixteen times. 9 A. M., one perfect swimming blastula, many imperfect non-swimming. 43 embryos ranging from early segmentations to blastulæ. Many eggs disintegrated.

Experiment 147 (h). — Same eggs. Shaken very lightly in a test-tube. No development.

Experiment 147 (i). — Shaken harder. No development.

Experiment 147 (j). — Shaken still harder in a test-tube. 9 A. M., many entirely normal looking segmenting eggs from 2–4 cells onward. Out of

2000 eggs, 107 observed in various stages of development. No swimming embryos seen. Few eggs of this star-fish developed when fertilized.

Experiment 170. **August 18.** — Another star-fish. Eggs in water three hours before transference. Female pronucleus re-formed. 4.30 P. M., carefully transferred under water. 9 A.M., next morning: out of 2500 eggs only one early development of two cells found. Only one third have matured.

Experiment 170 (a). — Same as Experiment 170. No embryos.

Experiment 170 (b). — Squirted twice. In 2500 eggs a few 3, 8, and 16 cell embryos found.

Experiment 170 (c). — Squirted four times. 9 A.M., 15 embryos in 3500 eggs. Irregular blastulæ and segmentation stages.

Experiment 170 (d). — Squirted eight times. 9 A.M., 24 embryos in 2200 eggs. Only one or two well developed and normal in appearance.

Experiment 170 (e). — Squirted sixteen times. 9 A.M., six embryos mostly 2–4 cells. Almost all dead.

Experiment 170 (f). — Shaken greatly in a test-tube. 9 A.M., two or three have begun to develop in 2000 eggs. In this star-fish also very few of the eggs could be fertilized and the development of these was mostly abnormal.

The following experiment shows that the eggs must be shaken after maturation is complete in order to insure a successful result.

Experiment 189. **August 19.** — Very ripe female. Nearly all eggs mature. 9.30 A.M., eggs of set A were transferred as soon as shed into sea-water without sperm. 1.30 P. M., the second set (B) transferred after the formation of the female pronucleus.

Experiment 189 a (1). — Transferred carefully. No development of any kind.

Experiment 189 a (2). — Eggs not transferred. No development at all.

Experiment 189 a (3). — Eggs squirted into water. No development.

Experiment 189 a (4). — Eggs squirted into dry dish. No development.

Experiment 189 a (5). — Eggs squirted into dry dish four times. No development.

Experiment 189 a (6). — Eggs squirted into dry dish eight times. A few fertilization membranes out. No development.

Experiment 189 a (7). — Squirted twelve times. No development.

Experiment 189 a (8). — Squirted sixteen times. No development.

Experiment 189 a (9). — Shaken in test-tube hard. The female pronucleus re-forms before that of the unshaken eggs and is abnormally large. No development. These eggs, though not developing, are so sensitive that transferring them with a pipette to a small watch glass causes all to put out fertilization membranes and develop irregularly.

Experiment 189 b (1). — 1.30 P.M., transferred carefully as maturation is complete. 3.50 P. M., about one in eighty have fertilization membranes and nuclei have disappeared. 8 A.M., next day: one normal swimming early gastrula found in 2000 eggs.

Experiment 189 b (2). — Squirted into sea-water. 4 P.M., one half have fertilization membranes and nuclei gone. Several have divided into 2–3 cells. 7.50 P.M., about 80 per cent have fertilization membranes and are budding off irregular clear protoplasmic masses. 9.30 A.M., about 90 per cent of the eggs are dead and disintegrated. No swimming blastulæ found in 700 eggs, but many non-swimming.

Experiment 189 b (3). — Squirted into dry dish. 4.10 P.M., about one tenth have fertilization membranes out. 7.30 P.M., many 2–4 and 8 cell stages. 9 A.M., most are dead. Two swimming blastulæ found in 700 eggs.

Experiment 189 b (4). — Squirted twice. 4 P.M., one in seven has fertilization membranes out. 9 A.M., two swimming blastulæ found in 1200 eggs.

Experiment 189 b (5). — Squirted four times. 9 A.M., four ciliated embryos in 1500 eggs.

Experiment 189 b (6). — Shaken in test tube. 9 A.M., many have started, but only four in 2000 eggs have reached the blastula stage. A similar negative result was obtained in star-fish 217, transferred with and without shaking at 9.30 A.M., before maturation. The same eggs transferred at 1.30 P.M., after maturation was complete, gave some swimming embryos in every culture and in that squirted eight times thirty swimming gastrulæ in 4000 eggs were found next morning.

Experiment 274. — 11 A.M. Eggs shed into sea-water. No eggs develop in the untransferred control. Lot A was transferred at 1.45 P.M.; lot B at 3 P.M.; lot C at 6 P.M.; and lot D at 8 P.M.

Experiment 274 a (1). — 1.45 P.M. Eggs picked up in a pipette and shot into water. 10 A.M., next day: no embryos in 3000 eggs examined.

Experiment 274 a (2). — Squirted twice into a dry dish. 10 A.M., no embryos swimming.

Experiment 274 a (3). — Squirted four times. Seventy-five ciliated blastulæ and gastrulæ in 2500 eggs found next morning. Many eggs had disintegrated.

Experiment 274 a (4). – 1.45 P.M. Shaken very lightly in a test-tube. 11 A.M., no swimming blastulæ. Many 2, 4, and 8 cell stages up to blastulæ with a normal fertilized appearance. All eggs alive. Fertilization membranes out.

Experiment 274 a (5). — 1.45 P.M. Shaken harder. 11 A.M., many eggs undergoing perfectly regular segmentation. No swimming larvæ, but farther along than 274 a (4). About one third are developing. None dead.

Experiment 274 a (6). — Shaken still harder. 11 A.M., fully one third are developing and many blastulæ are just beginning to swim.

Experiment 274 b (1). — 3 P.M. Transferred carefully to another dish and then the dish set sharply down on the table and the eggs drawn up in a pipette and shot out. 6 P.M., about one seventh of the eggs segmenting directly into four, eight, or sixteen cells. Three to four clear areas visible in other eggs. 8 P.M., 64 and 128 cell stages are numerous. At 8 A.M. 65 ciliated blastulæ found in 500 eggs.

Experiment 274 b (2). — 3 P.M. Squirted into water. 2 P.M., next day: many irregular groups of cells and blastulæ.

Experiment 274 b (3). — 3 P.M. Shaken in a test-tube. 2 P.M., next day: the dishes are full of swimming gastrulæ and blastulæ and swimming pieces of embryos. About one third of all the eggs are swimming. Some disintegrate after partial development. Of the control eggs almost one half not matured.

Experiment 274 b (4). — 3 P.M. Shaken harder. 2 P.M., next day: fully one half the eggs are swimming blastulæ and gastrulæ.

Experiment 274 c (1). — 6 P.M. Squirted into water. 8.30 A.M., 65 embryos mostly segmental stages of 100 cells and upward, but some swimming blastulæ found in 2000 eggs.

Experiment 274 c (2). — 6 P.M. Squirted into water. 11 A.M., next day: 157 larvæ, many just beginning to swim, were found in 3000 eggs.

Experiment 273 d (1). — 8 P.M. Squirted into water. 11 A.M., no swimming blastulæ, but about one seventh of the eggs are going as large morulæ and irregular heaps of clear cells. Many non-dividing eggs are full of clear areas (asters?).

Experiment 274 d (2). — 8 P.M. Squirted into water. About one fifth develop chiefly into blastulæ. A few ciliated and swimming.

Experiment 274 d (3). — 8 P.M. Shaken hard. 9 A.M., next day: almost all eggs have put out fertilization membranes, swollen and disintegrated. No swimming embryos.

The foregoing experiments show clearly that the eggs of the star-fish can often be made to develop with great regularity into blastulæ and gastrulæ. As a rule the embryos die in from twenty-four to thirty-six hours and are abnormal in shape and appearance, being generally smaller, more opaque and thicker walled than the normal. The riper the eggs, however, the more normal are the embryos, and in many instances the embryos could not be distinguished from the fertilized gastrulæ of the same star-fish. I have not as yet succeeded in getting them well into the bipinnaria stage, but it happened that the star-fish at this season of the year were not entirely ripe and only a relatively small number of the eggs even when fertilized would, without the aid of pilocarpine, go beyond the gastrula stage.

I do not think my results can possibly be attributed to any accidental infection with sperm nor to any self-impregnation by any possibly hermaphrodite individuals, which Cuenot[1] claims exist. For in every instance but one the eggs not transferred, or transferred before maturation was complete, showed no development whatever. I have repeated these controls at least fifty times with a constant result except in the one instance mentioned.

The great sensitiveness of the eggs after maturation to mechanical shock was very surprising. The majority of all ripe eggs will, if shaken, begin to develop, though, as already stated, only a few of them reach the blastula stage. Merely drawing the eggs into a pipette to transfer them to another dish may bring about development. An inspection of the experiments shows also that the eggs differ in sensitiveness at different periods in their maturation. Immediately after shedding into sea-water shaking causes no development. After two hours larvæ begin to appear on shaking. At four hours, hard shaking produces a very large proportion of larvæ, while merely transferring gives but one or two. A few hours later, transferring the eggs will cause a large number to begin to develop, though as a rule the development does not go beyond late segmentation stages. At this time shaking causes all the eggs to begin to develop, but none reach the blastula stage.

The question arises whether the star-fish is normally parthenogenetic, as McBride[2] says Asterina gibbosa is. Greef[3] in one instance observed the eggs of Asterias rubens develop without sperm, and Hertwig[4] has recorded similar observations upon Asterias glacialis and Astropecten. It can hardly be possible, however, that the general parthenogenesis of Asterias could be overlooked. Certainly the eggs of the animals I observed were not naturally parthenogenetic, since, if left undisturbed, they never developed. Hence, I am inclined to believe that this star-fish while not normally parthenogenetic is nevertheless on the verge and that it may be started in several ways. I feel somewhat doubtful about the extent of parthenogenesis which may occur naturally because in almost all my experiments a certain number of the eggs, and in some cases a large proportion, failed to put out the polar globules when shed, and were hence presumably not

[1] Cuenot: Zoologischer Anzeiger, 1898, p. 273.
[2] McBride: Quarterly journal of microscopical Science, xxxviii, p. 339.
[3] Greef: Cited from Viguier: Annales des sciences naturelles, 1901, p. 117.
[4] Hertwig: Jenäische Zeitschrift für Naturwissenschaft, 1890, xxiv, p. 304.

fully mature. It is possible that the examination of the fully mature individuals in June, when, according to Mead, they shed their eggs naturally, may show a different condition. It may be said, however, that in my experience as the eggs approach a condition in which they fertilize readily and develop normally their parthenogenetic development becomes easier to bring to pass. It may be said positively, however, that the individuals observed during the present work were certainly not parthenogenetic unless the eggs had been disturbed. It may be that Greef's and Hertwig's observations were due to disturbing the eggs.

I strongly suspect that a large number of eggs of other animals will be found to be in a similar state of unsteadiness, making it necessary to handle them with extreme care in all experiments involving the production of parthenogenesis in other than mechanical ways. Loeb and Fischer permit me to announce that they have already confirmed my results for Amphitrite, Chætopterus and Nereis, which are also easily started in this same manner. The possible inference that the parthenogenesis already observed by Loeb in these and other forms may have been due to his transfers of the eggs from one medium to another instead of to the chemical action of the reagents he employs, is, I feel sure, not justified. So far as Arbacia goes, I have repeatedly tried to secure development by agitation without success, and attempts by Loeb and Lewis at Woods Holl this summer have also been negative. In the other forms Loeb employed, with the possible exception of the star-fish, the number of larvæ obtained by the methods employed by Loeb, *i. e.*, raising the osmotic pressure, or the action of H, K, or Ca ions, give far more larvæ than the controls which are transferred in the same manner. It is, of course, not impossible that in some instances at least the loss of water by the protoplasm, or the action of certain chemicals, may so raise its instability that a very small mechanical shock will suffice to start parthenogenesis. Many of Loeb's results, however, are not open even to this objection, to which, at the most, I attribute no great weight, since the eggs were not retransferred from the altered sea-water to the normal, but left in the former subject to the same conditions as the control. It may be well in passing, however, to mention the fact that if ripe star-fish eggs are transferred, after maturation, with a sterile pipette to a small, clean watch-glass for microscopic examination, and are then retransferred to the original dish, a few embryos are almost certain to appear the next morning in the dish. I have observed this several times. It

may be that the occasional parthenogenesis of various sea-urchins described by Viguier[1] may have been produced in this manner. But the difficulty he appears to have experienced in repeating his experiments successfully, when strict precautions were taken against sperm infection, lead me to suspect that he was dealing rather with some accidental fertilization than, to use his own phrase, an "accidental parthenogenesis."

The microscopical changes in the egg caused by shaking or agitation are remarkable. It is surprising that so slight a shock can produce so profound a structural effect. These changes have not been studied in sections, so that I can describe only what may be seen in the living egg, which, however, is large and fairly transparent. The first and most striking change is the development of the fertilization membrane, which appears a few minutes after shaking. At the same time the shape of the egg changes from spherical form to a flattened ellipsoid, the flattening generally appearing in the neighborhood of the polar bodies of the fertilized egg. The nucleus which before shaking is present as a large single nucleus, or as a collection of from three to seven separate spheres, undergoes a change. The nuclear membrane fades away either within a few moments or after several hours and the nucleus quite disappears from view. As a rule, its loss is followed after a time by the appearance of from two to thirty clear areas in the egg, or by a peculiar budding off of clear portions of the protoplasm about the periphery, although this budding may at times take place without the disappearance of the nucleus. As a result of this budding there is formed a morula-like mass of cells with a larger or smaller undivided mass in the centre. These budded off portions which look like small cells probably do not contain nuclei since they soon go to pieces. It not infrequently happens that the large undivided piece of the cell subsequently develops into an embryo which acquires cilia and rotates rapidly in the débris of the dying buds. A large number of clear areas may develop in a single egg, and the egg segment at once into a very large number of cells. Blastulæ so formed do not, however, so far as observed, develop farther, but die in the course of two hours; but I have not made a careful study of this point.

The manner in which shaking brings about development is uncertain. In the case of unripe eggs the maturation is ushered in norm-

[1] VIGUIER: Annales des sciences naturelles, 1901, p. 88; Comptes rendus, 1901, June 10 and July 15.

ally by the disappearance or dissolution of the nuclear membrane at one spot where the centrosome appears. If eggs do not naturally mature the nucleus persists unchanged. If now the eggs be shaken, the nuclear membrane either breaks mechanically or is dissolved. This has been observed by Morgan and myself. The ripening of the egg appears to be connected in some way with the disappearance of the nuclear membrane and the presumable discharge of nuclear matter into the cytoplasm. Similarly, in the eggs after maturation, shaking causes a change which manifests itself by a dissolution or disappearance of the nucleus. This change of the nucleus, however, is not, in the majority of instances, due to mechanical rupture, for it is frequently inaugurated from many minutes to from two to four hours after shaking has stopped. These observations, together with the well known fact that the centrosome originates as a rule close to the nucleus suggests that the dissolution of the nuclear membrane is a determining factor in karyokinesis. It is possible that if the agitation cause dissolution in one place only, such as normally occurs, the normal division into two cells results, but that if dissolution occur in the periphery, generally many asters are found leading to division all at once into a large number of cells. In this way the appearance of polyspermy might easily be brought about.

On the other hand there is also the possibility that mechanical agitation causes the cell to lose water, as happens in some plant-cells when they are stimulated, for example, in the leaves of a sensitive plant. A condition might result similar to that produced by raising the osmotic pressure of the surrounding liquid. Such a loss of water could most probably be produced by a lowering of the osmotic pressure of the cell and this only by a reduction of the number of molecules in the egg. This would mean a combination between possibly the organic and inorganic constituents of the protoplasm, which in turn might lead to dissolution of some of the cell elements. The whole subject is at present in such a state that no definite conclusions may be drawn, but I believe many facts of cell physiology might be explained by such a rhythmical or unstable combination between the organic and inorganic constituents of protoplasm. This is a matter which I hope to consider more carefully later.

Too strong shaking of the eggs causes a dissolution of the whole egg. After shaking has ceased and after the process of nuclear disappearance already described has taken place the egg begins to swell and ultimately either dissolves altogether or remains a swollen mass

of débris. In such case an increase in osmotic pressure would appear to be the result.

The fact brought out by these observations that karyokinesis may be started mechanically is, I think, possibly of general application to protoplasm. Loeb and Fischer's observations of similar processes in the Annelids there indicate this. May it not help us to understand how irritation of the skin may cause the development of callous areas, or irritation of bone the production of bony growths?

The change, whatever be its nature, which is set up by mechanical agitation lasts for several generations of cells and causes a hastened development. This Morgan had already seen in fertilized eggs. It is visible also in the unfertilized eggs. In Experiment 274a (4, 5, 6) it is seen that those eggs shaken very gently were the next day in the early stages of segmentation up to one hundred and twenty-eight cells or more; those shaken harder were blastulæ just ready to swim; those shaken still harder were swimming blastulæ and those shaken hardest of all were already beginning to gastrulate. Perhaps this change may be correlated with the apparent diminution of surface tension which exhibits itself in the change of shape of the egg from a sphere to an ellipsoid and in the budding off of portions and irregularity of the outline of the periphery of the egg. That the cohesion is reduced is shown also by the ease with which such eggs may be shaken to pieces, being strikingly different in this respect from the unripe eggs. This, however, ushers us into an at present unknown field.

Summary.

1. The ripe eggs of Asterias Forbesii may be made to develop to the bipinnarian or late gastrula stages by mechanical agitation or shock.

2. The amount of agitation necessary varies in different individuals from a hard shaking in a test-tube to transferring the eggs from one dish to another.

3. The speed of development is in narrow limits roughly proportional to the amount of shaking the eggs have received.

4. The parthenogenetic developing eggs have, most of them at least, fertilization membranes and many look exactly like fertilized eggs.

5. The eggs become more sensitive the longer they lie in sea-water up to seven hours. The most favorable time to obtain the largest number of swimming embryos appeared to be about three hours after shedding and with relatively hard shaking.

6. The ease with which development may be started in this way makes this a serious source of error in any study of artificial parthenogenesis by other means.

7. The microscopical changes observed in the eggs consist in the development of fertilization membranes, the dissolution of the nuclear wall, the frequent appearance of many clear areas (asters) in the substance of the egg, and the segmentation of the egg, often directly, into many cells.

THE COMPOSITION OF TENDON MUCOID.[1]

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CONTENTS.

IN their paper on the glucoproteid of white fibrous connective tissue Chittenden and Gies[2] stated that the average amount of sulphur in three analyzed preparations of tendon mucoid[3] was 2.33 per cent. Loebisch,[4] who previously had been the only one to analyze this substance completely, found in it an average of but 0.81 per cent of sulphur, and ascribed to it the formula $C_{160}H_{253}N_{32}S_1O_{80}$ with a molecular weight of 3936. Referring to the unexpectedly high results of their sulphur determinations, as compared with those obtained by Loebisch, Chittenden and Gies wrote: "We present these figures

[1] Some of the results of this work were reported before the American Physiological Society. See the Proceedings, CUTTER and GIES: This journal, 1900, iii, p. vi.

[2] CHITTENDEN and GIES: Journal of experimental medicine, 1896, i, p. 186.

[3] Following COHNHEIM'S suggestion (Chemie der Eiweisskörper, 1900. p. 259) we use the term "mucoid," instead of the previously accepted "mucin," to designate this substance. We agree with Cohnheim that, for the sake of definiteness, the term "mucin" may be best applied to the glucoproteids elaborated by true secretory cells, and the term "mucoid" to similar substances in the tissues. In the present unsettled state of our chemical knowledge regarding these bodies, such a distinction is at best of only temporary convenience. The original differences have little importance in the light of the results of recent researches.

[4] LOEBISCH: Zeitschrift für physiologische Chemie, 1886, x, p. 40.

with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the mucin molecule."[1]

The divergent results of these two investigations naturally throw some doubt on the question of the elementary composition of tendon mucoid. We have attempted not only to ascertain definitely the amount of sulphur in tendon mucoid, but also to explain the previous discrepancy in experimental data relating to sulphur content. In addition to the results in this particular connection, certain others of significance obtained by us may be appropriately given with them.

I. Content of Sulphur and Nitrogen.

Historical. — Rollett[2] was the first to show that tendon contains mucin-like material. He described some of the qualities of the substance, but made no elementary analyses of it. Eichwald[3] merely verified Rollett's qualitative results, in this connection.

Loebisch used Rollett's method to prepare sufficient quantities of tendon mucoid for analysis. Only three preparations were analyzed by Loebisch. But one sulphur determination was made on each, with the following results: (a) 0.82 per cent; (b) 0.80 per cent; (c) 0.82 per cent. Chittenden and Gies, who were the next to analyze this particular glucoproteid material, used improved methods of preparation and purification and, in sulphur analysis, obtained seven concordant results on three purified products, with the following averages: (a) 2.34 per cent; (b) 2.35 per cent; (c) 2.31 per cent. The difference is very striking.

With respect to the amount of nitrogen in tendon mucoid, a similar though not so decided analytic difference was established in these two investigations. Loebisch made only four determinations of nitrogen in his three purified preparations. The average of two closely agreeing results for his first preparation was 11.80 per cent; for the second the single result was 11.84 per cent and for the third it was 11.59 per cent. Chittenden and Gies made ten determinations in three preparations with the following averages of results in close

[1] Chittenden and Gies: *Loc. cit.*, p. 197.

[2] Rollett: Untersuchungen zur Naturlehre des Menschen und der Thiere (Moleschott), 1859, vi, p. 1. Also, *Ibid.*, 1860, vii, p. 190.

[3] Eichwald: Annalen der Chemie und Pharmacie, 1865. cxxxiv. p. 177.

agreement: (a) 11.94 per cent; (b) 11.80 per cent; (c) 11.51 per cent. They found, further, that the nitrogen content of a series of very carefully prepared fractional products varied between 11.51 per cent and 12.26 per cent, data which seem to suggest, though they do not establish, the existence of several related mucoids as components of ordinary tendinous tissue.

Preparation of Fractional Products. — At the outset of these experiments we assumed that tendon contains more than one glucoproteid. This seemed probable for several reasons. Among the latter is the fact that the larger tendons show considerable variation in texture throughout their length. Thus the tendo Achillis of the ox, from which the previously analyzed tendon mucoids were extracted, is comparatively soft and very tough in the main shaft, but toward its connections with the bones becomes more compact, and outwardly somewhat resembles cartilage. The superficial qualities of the thick sheaths enveloping the two large branches of the Achilles tendon in this animal also resemble those of cartilage to a certain extent.

These physical modifications within the tendinous tissue naturally suggest chemical differentiation of the constituents. Previous analytic variations respecting tendon mucoid may have been dependent on extraction of different mixtures of distinct though closely related bodies. Loebisch does not state which portions of the tendons were employed in his work. Chittenden and Gies used sections of the main shaft, together with portions of the two branches and the sheaths of the latter. In our own experiments these parts were extracted separately.

General Method. — In the preparation of mucoid for use in these experiments the Achilles tendon of the ox was employed. Following the usual method, the tissue, immediately after removal from the animals, was thoroughly freed of extraneous matter and cut into very thin cross sections. These pieces were washed in water and then extracted in half-saturated calcium hydroxide. The mixtures were shaken at regular intervals. The mucoid was precipitated from the filtered extract with dilute hydrochloric acid.[1] The precipitated substance was repeatedly washed; first in dilute hydrochloric acid, to

[1] We have always found that mucoids may be precipitated from lime-water or sodium carbonate solution much more satisfactorily with dilute HCl than with any other acid. The substance seems to separate much more quickly and completely in the presence of slight excess of this acid. Chlorides have comparatively slight solvent action on mucoids in the presence of free HCl, unless admixed in excess.

remove all traces of adherent proteid impurity, then in water until it was free of acid. It was next redissolved in dilute alkali and reprecipitated once with dilute hydrochloric acid. The washing process was repeated. Finally the acid-free substance was dehydrated and purified by long-continued treatment with large quantities of boiling alcohol-ether; then dried *in vacuo* and weighed.

First Experiment. Series A and B. — In this experiment two parallel series of fractional extractions were made and the mucoid in each separated and analyzed. 4600 gms. of the main shaft of the tendon about five inches in length, with from two to three inches of its bifurcations, were employed in Series A. In Series B only the sheaths of the branches, weighing 1900 gms., were used. Both lots of finely divided tissue were given identical treatment at each stage of the experiment. All extractions were made with 2 c.c. of half-saturated lime-water per gm. of moist tissue. After the extracts had been strained through cloth, the tendon pieces were thoroughly washed in water to prevent adherent dissolved mucoid from becoming part of the succeeding extract. The first extracts in each series were readily precipitated and brought to the flocculent condition with very slight excess of 0.2 per cent hydrochloric acid. Subsequent extracts, however, became only turbid with large excess of 0.2 per cent HCl — even with an equal volume. It was necessary, therefore, to add stronger acid (1.5% HCl) to separate the mucoid in flocks.[1] In purifying, the substance was redissolved in half-saturated lime-water. Powdered thymol, used in the second experiment also, entirely prevented bacterial action.

The summary, Table I, on page 159, gives additional significant facts relating to these fractional preparations.

A striking feature of these preparations was the fact that precipitation became more and more difficult with each extraction. More acid was required in each successive extract (except the fourth of Series B) to bring the mucoid to the flocculent condition. It will be seen from the data in Tables I and II that this was entirely independent of the proportion of contained mucoid. The alkali could not have effected decomposition, and thereby possible variations, because it was too

[1] In each instance the acid was added slowly in small quantities. The mixtures were thoroughly stirred and allowed to stand for flocks to form. After waiting a sufficient time, more acid was added if separation had not taken place. At first 0.2 per cent HCl was used. If after an equal volume of the acid had been stirred in, flocks failed to separate, 1.5 per cent HCl was added little by little. Separation took place instantly upon reaching the proper amount of acid. On reprecipitating, the same procedure was followed. The proportion of acid required was not recorded in the latter case, but great variations were observed. This method was employed in the second experiment also.

weak. Further, the volumes of fluid in each series were kept constant and the temperature was always about the same, so that the salts formed on acidification of the alkali of the extracts had essen-

TABLE I.

Extract.		Time of extraction:	Amount of HCl present to completely precipitate.[1]	Weight of purified product.[2]
Number.	Volume c.c.	Hours.	Per cent.	Grams.
Series A.				
First	9200	24	0.04	6.52
Second	9200	24	0.18	9.79
Third	9200	24	0.26	3.55
Fourth	9200	48	0.32	3.13
Series B.				
First	3800	24	0.03	4.23
Second	3800	24	0.17	1.65
Third	3800	24	0.46	0.93
Fourth	3800	48	0.37	

[1] The figures for per cent of HCl necessarily present to precipitate in flocks express approximate values. The precise amount of acid neutralized by the $Ca(OH)_2$ was not directly determined. It was the same of course throughout each series. Greater exactness would have emphasized the facts made significant by the above data.

[2] These weights are for substance dried *in vacuo*. The amount of each preparation could not be exactly quantitative, of course, because of slight losses during their purification. The mucoids are very difficult substances to handle and their preparation is decidedly laborious. Every effort was made to reduce inevitable loss to a minimum, however, and, as the loss was relatively the same in each preparation, the weights are entirely reliable for the intended comparisons.

tially the same influence throughout. The extracts were strained quickly at practically the same time and were promptly treated with acid, so that no changes could have occurred by reason of delay in final treatment.

The figures for weights of substance in each extract suggest variable resistance, on the part of the mucoid, to the solvent action of the dilute alkali. None of the extracts were ever saturated and all were distinctly alkaline. The peculiar behavior of these preparations harmonizes with the view that the tissue contains two or more glucoproteids, and that the products separated by the usual method of mucoid extraction are mixtures of different bodies.

(*c*) *Second Experiment. Series C and D.* — A second set of preparations was made in essentially the same way as in the first experiment. 6600 gms. of the main shaft of the tendon and its branches, of the same size as heretofore, were extracted in Series C; 4200 gms. of sheath in Series D. The periods of extraction were shorter at the beginning and longer at the close of this experiment than previously. In purifying, the substance was redissolved in 0.5 per cent sodium carbonate.

The summary of results given in Table II, page 161, connected with preparation, is directly comparable with Table I.

In this experiment, also, successive increase in proportion of acid was necessary for precipitation, the results harmonizing in detail with those of the first experiment. Variations in the quantities of separated mucoid again pointed to variable resistance to the action of the extractive. Fractions of a single substance would hardly act so differently at successive intervals under essentially the same conditions.

Analytic results. — Although the differences in the action of our several products indicated the existence of two or more mucoids in tendinous tissue, more direct evidence than qualitative variation was necessary to justify such a conclusion. We very carefully analyzed these products, with results that confirm the original deduction.

The amounts of nitrogen and sulphur in mucoids furnish excellent data for general comparisons of composition. Table III, on page 162, summarizes our results for percentage content of nitrogen and sulphur in the ash-free substance dried at 105–110° C. to constant weight.[1] The analyses were made by the customary methods — Kjeldahl for the nitrogen; fusion with NaOH over alcohol flame, and precipitation with $BaCl_2$, for sulphur.

[1] The proportion of ash in these preparations was usually much less than 1 per cent. In only four was it more than that, and in none of these did it exceed 1.78 per cent. It consisted mostly of phosphate and chloride; only a trace of sulphate was present.

These results seem to prove that more than one substance has been extracted — that mixtures have been obtained. The results for every member of each series differ decidedly in one respect or

TABLE II.

Extract.		Time of extraction.	Amount of HCl present to completely precipitate.[1]	Weight of purified product.[1]
Number.	Volume cc.	Hours.	Per cent.	Grams.
Series C.				
First	13200	17	0.03	14.56
Second	13200	20	0.15	24.88
Third	13200	26	0.17	17.26
Fourth	13200	30	0.38	2.04
Fifth[2]	13200	65	0.45	4.09
Series D.				
First	8400	17	0.02	11.85
Second	8400	20	0.15	13.41
Third	8400	26	0.45	3.19
Fourth	8400	30	0.39	0.29
Fifth	8400	65	0.35	0.59

[1] See notes under Table I.

[2] A sixth extraction lasting 124 hours was made in Series C. A trifle more than a gram of unpurified substance was obtained. The presence of nearly 1 per cent of HCl was necessary in order to bring it to the flocculent condition. This substance was true mucoid — on decomposition it yielded a reducing substance. It is evident from these results that it is very difficult to completely extract glucoproteid from tendinous tissue.

another from the rest in the group, and this, too, in spite of the fact that the analyses of all were conducted by uniform methods and under conditions as nearly the same as it is possible to attain. The extremes in percentage content are too far apart to be due to unavoidable analytic errors.

TABLE III.

CONTENT OF NITROGEN AND SULPHUR.

Extract.	Experiment I.				Experiment II.			
	Series A.		Series B.		Series C.		Series D.	
Number.	Nitrogen.	Sulphur.	Nitrogen.	Sulphur.	Nitrogen.	Sulphur.	Nitrogen.	Sulphur.
First	13.17 13.33 13.25	2.25 2.36 2.25 2.36 2.31	13.14 12.96 13.05	2.11 2.34 2.22	12.49 12.55 12.70 12.58	2.20 2.20 2.20	12.64 12.64 12.64	2.70 2.91 2.81
Second	12.85 12.94 12.71 12.83	1.81 1.66 1.74	12.41 12.46 12.43	2.67 2.72 2.70	11.77 11.79 11.78	1.75 1.86 1.81	12.68 12.70 12.69	2.28 2.39 2.34
Third	13.29[1] 13.25 13.27	1.49	13.47[1] 13.59 13.70	2.23	12.74 12.70 12.54 12.66	1.84 1.87 1.85	13.89[1] 13.92 13.91	2.47 2.28 2.38
Fourth[2]	13.84[1] 13.88 13.86	1.23 1.41 1.32			13.98[1] 14.06 14.02	1.55	14.06[1]	
Fifth					15.02 14.95 14.98	1.49 1.54 1.52	14.56[1]	
General averages.	13.25	1.87	13.02	2.41	13.11	1.81	13.39	2.51

[1] Not ash-free. There was not sufficient substance left for additional determinations. The average amount of ash in all the other preparations was 1.03 per cent. See note, page 160.

[2] The substance of the third and fourth extracts of Series B was combined for analysis; the amounts were too small to be dealt with separately.

It will be noticed that the nitrogen of the mucoids of the first extracts is greater in amount than in the second — just as was found in the single similar experiment by Chittenden and Gies. With one exception the nitrogen of the mucoid in the second extract is much less in each series than in any of the others of the group but becomes greater with each succeeding extraction. The sulphur, on the other hand, shows gradual decrease in Series A and C, but remains much the same in the other two. The average content of sulphur in the mucoids of Series B and D (prepared from the sheaths) is appreciably higher than in the others. The nitrogen average is practically the same in all.[1]

II. Complete Elementary Composition.

We made complete analysis of several of our preparations in order to obtain additional evidence in the connections just discussed, and to add if possible to our knowledge of general composition.

Closely related members of Series C and D of our preparations were selected for this purpose. The methods of analysis were those commonly in use. We followed those outlined in detail in a recent paper on a similar subject from this laboratory,[2] so that their description may be omitted here. Special care was taken to keep as nearly uniform as possible all conditions known to affect analysis, so that the results would be directly comparable.

No. 1. Mucoid of first extract of Series C.

Carbon and Hydrogen. 0.3550 gm. substance gave 0.6120 gm. CO_2 and 0.2100 gm. H_2O = 47.02 per cent C and 6.57 per cent H; 0.4120 gm. substance gave 0.7140 gm. CO_2 and 0.2480 gm. H_2O = 47.26 per cent C and 6.69 per cent H.

Nitrogen. 0.2275 gm. substance gave 0.0282 gm. N = 12.40 per cent N; 0.1484 gm. substance gave 0.0187 gm. N = 12.61 per cent N; 0.1894 gm. substance gave 0.0236 gm. N = 12.46 per cent N.

Total Sulphur. 0.5665 gm. substance gave 0.0905 gm. $BaSO_4$ = 2.19 per cent S; 0.6547 gm. substance gave 0.1045 gm. $BaSO_4$ = 2.19 per cent S.

Sulphur combined as SO_3. 0.4210 gm. substance, after boiling in HCl, gave 0.0413 gm. $BaSO_4$ = 1.33 per cent S; 0.2880 gm. substance, after boiling in HCl, gave 0.0286 gm. $BaSO_4$ = 1.35 per cent S.

Ash. 0.1727 gm. substance gave 0.0012 gm. Ash = 0.69 per cent Ash.

[1] Compare with results for carbon and oxygen, also, in Table IV, page 168.

[2] Hawk and Gies: This journal, 1901, v, p. 403.

Percentage Composition of the Ash-free Substance.[1]

								Average.
C	47.34	47.59	....	....	....	....	....	47.47
H	6.63	6.74	....	....	....	....	....	6.68
N	....	....	12.49	12.70	12.55	....	....	12.58
S	....	....	....	....	....	2.20	2.20	2.20
O	....	....	....	....	....	....	....	31.07

No. 2. Mucoid of second extract of Series C.

Carbon and Hydrogen. 0.1252 gm. substance gave 0.7320 gm. $H_2O = 6.50$ per cent H; 0.1903 gm. substance gave 0.3292 gm. CO_2 and 0.1122 gm. $H_2O = 47.18$ per cent C and 6.55 per cent H; 0.1303 gm. substance gave 0.2245 gm. CO_2 and 0.0760 gm. $H_2O = 46.99$ per cent C and 6.48 per cent H.

Nitrogen. 0.2523 gm. substance gave 0.0295 gm. N = 11.70 per cent N; 0.3037 gm. substance gave 0.0355 gm. N = 11.68 per cent N.

Total Sulphur. 0.6541 gm. substance gave 0.0830 gm. $BaSO_4 = 1.74$ per cent S; 0.7209 gm. substance gave 0.0974 gm. $BaSO_4 = 1.85$ per cent S.

Sulphur combined as SO_3. 0.4798 gm. substance, after boiling in HCl, gave 0.0567 gm. $BaSO_4 = 1.62$ per cent S; 0.3760 gm. substance, after boiling in HCl, gave 0.0437 gm. $BaSO_4 = 1.59$ per cent S.

Ash. 0.1989 gm. substance gave 0.0017 gm. Ash = 0.85 per cent Ash; 0.1200 gm. substance gave 0.0009 gm. Ash = 0.75 per cent Ash.

Percentage Composition of the Ash-free Substance.

								Average.
C	....	47.56	47.36	....	....	....	....	47.46
H	6.56	6.60	6.53	....	....	....	....	6.56
N	....	....	....	11.79	11.77	....	....	11.78
S	....	....	....	....	....	1.75	1.86	1.81
O	....	....	....	....	....	....	....	32.39

No. 3. Mucoid of third extract of Series C.

Carbon and Hydrogen. 0.1194 gm. substance gave 0.2063 gm. CO_2 and 0.0709 gm. $H_2O = 47.12$ per cent C and 6.60 per cent H; 0.0973 gm. substance gave 0.1694 gm. CO_2 and 0.0566 gm. $H_2O = 47.48$ per cent C and 6.46 per cent H.

[1] Only traces of phosphorus were present, equal in amount to the phosphorus in the ash. This was ascertained for each preparation. The quantity was greatest in this particular product — 0.26 per cent and 0.24 per cent in two determinations.

Nitrogen. 0.2181 gm. substance gave 0.0275 gm. N = 12.61 per cent N; 0.3675 gm. substance gave 0.0462 gm. N = 12.57 per cent N; 0.2831 gm. substance gave 0.0351 gm. N = 12.41 per cent N.

Total Sulphur. 0.7412 gm. substance gave 0.0982 gm. $BaSO_4$ = 1.82 per cent S; 0.6574 gm. substance gave 0.0887 gm. $BaSO_4$ = 1.85 per cent S.

Sulphur combined as SO_3. 0.6686 gm. substance, after boiling in HCl, gave 0.0653 gm. $BaSO_4$ = 1.34 per cent S.

Ash. 0.1720 gm. substance gave 0.0018 gm. Ash = 1.04 per cent Ash.

Percentage Composition of the Ash-free Substance.

								Average.
C	47.62	47.98	....	....	....	....	....	47.80
H	6.66	6.53	....	....	....	....	....	6.60
N	....	....	12.74	12.70	12.54	....	....	12.66
S	....	....	....	....	....	1.84	1.87	1.85
O	....	....	....	....	....	....	....	31.09

No. 4. Mucoid of first extract of Series D.

Carbon and Hydrogen. 0.0770 gm. substance gave 0.1372 gm. CO_2 and 0.0480 gm. H_2O = 48.60 per cent C and 6.93 per cent H; 0.0968 gm. substance gave 0.1721 gm. CO_2 and 0.0578 gm. H_2O = 48.48 per cent C and 6.63 per cent H.

Nitrogen. 0.3946 gm. substance gave 0.0495 gm. N = 12.55 per cent N; 0.3154 gm. substance gave 0.0396 gm. N = 12.55 per cent N.

Sulphur. 0.5967 gm. substance gave 0.1159 gm. $BaSO_4$ = 2.68 per cent S; 0.7591 gm. substance gave 0.1603 gm. $BaSO_4$ = 2.89 per cent S.

Sulphur combined as SO_3. 0.8904 gm. substance, after boiling in HCl, gave 0.0886 gm. $BaSO_4$ = 1.36 per cent S.

Ash. 0.1983 gm. substance gave 0.0015 gm. Ash = 0.75 per cent Ash.

Percentage Composition of the Ash-free Substance.

							Average.
C	48.97	48.87	....	....	....	....	48.92
H	6.98	6.68	....	....	....	....	6.83
N	....	....	12.64	12.64	....	....	12.64
S	....	....	....	....	2.70	2.91	2.80
O	....	....	....	....	....	....	28.81

No. 5. Mucoid of second extract of Series D.

Carbon and Hydrogen. 0.1779 gm. substance gave 0.3101 gm. CO_2 and 0.1028 gm. H_2O = 47.54 per cent C and 6.42 per cent H; 0.0608 gm. substance gave 0.1066 gm. CO_2 and 0.0365 gm. H_2O = 47.82 per cent C and 6.69 per cent H.

Nitrogen. 0.3046 gm. substance gave 0.0380 gm. N = 12.48 per cent N; 0.2545 gm. substance gave 0.0316 gm. N = 12.45 per cent N.

Sulphur. 0.7143 gm. substance gave 0.1226 gm. $BaSO_4$ = 2.35 per cent S; 0.9841 gm. substance gave 0.1608 gm. $BaSO_4$ = 2.24 per cent S.

Sulphur combined as SO_3. 0.7130 gm. substance, after boiling in HCl, gave 0.0805 gm. $BaSO_4$ = 1.55 per cent S.

Ash. 0.3477 gm. substance gave 0.0059 gm. Ash = 1.69 per cent Ash; 0.1665 gm. substance gave 0.0031 gm. Ash = 1.86 per cent Ash.

Percentage Composition of the Ash-free Substance.

							Average.
C	48.40	48.67	....	....	....	....	48.54
H	6.54	6.81	....	....	....	....	6.68
N	....	....	12.70	12.68	....	....	12.69
S	....	....	....	....	2.39	2.28	2.34
O	....	....	....	....	....	....	29.75

Discussion of results. — The general summary of our results for complete elementary composition, Table IV, may be compared with similar data obtained in the previous investigations. It will be observed that although there is some variation within each series — very slight in Loebisch's, quite marked in our own — the three group averages are very nearly the same. This is particularly significant in this connection. It suggests that mixtures of generally uniform composition resulted in each of the previous studies. Leobisch varied his method very little and obtained practically uniform products; Chittenden and Gies varied theirs more decidedly, and the result was distinct variation in composition of substance extracted. By the fractional method in our own experiments, still greater differentiation was effected.

We do not mean to suggest that our own products are chemical individuals. They are mixtures, just as all the previously described tendon mucoids have doubtless been. Further research, with more elaborate methods, and particularly with reference to inner groupings of the elements, will be necessary for definite differentiation, if such is possible while we remain in our present profound ignorance of the structure and peculiarities of proteid molecules.[1]

The amounts of nitrogen in our preparations appear to be slightly greater than those previously determined, although the nitrogen con-

[1] Hawk and Gies: *Loc. cit.*, p. 414 *et seq.*

tent of preparation No. 2 (Second extract, Series C), which was the largest in quantity of all our products,[1] conforms closely with the generally accepted figures for content of this element.

The only particularly discordant results in the general averages are those for content of sulphur and oxygen (by difference) obtained by Loebisch. We had hoped that this low figure would be explained by our results, but none of our products contained so little sulphur. Our figures in this connection accord very well with those given by Chittenden and Gies. As has already been stated, Loebisch made only a few analyses — only one determination of sulphur in each of his three preparations. He duplicated results in only half of the analyses he reported.

In referring to the differences in composition observed among their products, Chittenden and Gies stated: "Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. . . . There is at the present time no standard of purity with regard to this body, and it is quite as probable that fibrous connective tissue contains two or more mucins as that there is only one mucin in the tissue, and that any deviation from the figures obtained by Loebisch or by us in preparation No. 3 is due to the presence of a larger or smaller amount of proteid impurity."[2]

We can no longer believe that proteid impurity is responsible for the observed variations. In the first place the quantity of soluble proteid in tendon, other than mucoid, is very slight. Experiments in progress in this laboratory indicate that it is less than 0.3 per cent. If, however, it were possible for all of this small quantity to combine permanently with the precipitated mucoids, it could not account for the regular rise and fall of nitrogen content observed in each series of our experiments.[3] Although it is conceivable that the mucoid of the first extract could be so affected, such an assumption would not explain the rise of nitrogen in the third and subsequent extracts, particularly in view of the marked fall of the same in the second. Then, too, each product was so thoroughly washed in excess

[1] See table on page 161.

[2] CHITTENDEN and GIES: *Loc. cit.*, p. 194.

[3] See the table on page 162.

TABLE IV.

AVERAGE PERCENTAGE COMPOSITION OF TENDON MUCOIDS.

Investigators.	LOEBISCH.				CHITTENDEN AND GIES.				CUTTER AND GIES.					
Number of preparation.	1	2	3	Average.	1	2	3	Average.	1	2	3	4	5	Average.
C	48.24	48.34	48.32	48.30	49.29	48.74	48.26	48.76	47.47	47.46	47.80	48.92	48.54	48.04
H	6.44	6.43	6.53	6.47	6.63	6.46	6.49	6.53	6.68	6.56	6.60	6.83	6.68	6.67
N	11.79	11.84	11.59	11.74	11.94	11.80	11.51	11.75	12.58	11.78	12.66	12.64	12.69	12.47
S	0.82	0.80	0.82	0.81	2.34	2.35	2.31	2.33	2.20	1.81	1.85	2.80	2.34	2.20
O	32.71	32.59	32.74	32.68	29.80	30.65	31.43	30.63	31.07	32.39	31.09	28.81	29.75	30.62

All of the above preparations were made by similar methods. Loebisch extracted for 48 hours in half-saturated lime-water in the proportion of 2 c.c. of the latter per gram of moist tissue. His first preparation was precipitated with 1–5 per cent acetic acid, the second with 0.1–0.2 per cent hydrochloric, the third with 1–5 per cent acetic acid. Only the third was purified by reprecipitation from alkaline solution. It was redissolved in 0.5 per cent sodium carbonate and again thrown down with acetic acid.

Chittenden and Gies used the above proportions of tissue and half-saturated lime-water in their first preparation and continued the extraction 48 hours. The tissue was re-extracted for the same time and the precipitates from the extracts united and purified together. They precipitated with 0.2 per cent hydrochloric acid, re-dissolved in half-saturated lime-water and reprecipitated again with dil. HCl. Their second product was separated from tissue which had previously been extracted in 10 per cent sodium chloride solution for 36 hours. From this point exactly the same process was employed as that used for preparation No. 1, except that the substance was redissolved in 0.5 per cent Na_2CO_3. In their third preparation the tissue was first extracted in 10 per cent salt solution for 24 hours, then in the usual proportion of half-saturated lime-water for 60 hours. The mucoid in this extract was treated separately in a special experiment. (By fractional precipitation two products were obtained from it, the first with 12.26 per cent of nitrogen, the second with 11.91 per cent. Compare with the nitrogen of their Preparation No. 3 and our own results.) The tissue was again extracted in the usual amount of dilute lime-water and the mucoid in this second extract purified as Preparation No. 3 (with 11.51 per cent N).

Of our own preparations, Nos. 1, 2, and 3 were the first three fractional products of Series C (made from the main shaft of the tendon and its bifurcations). Nos. 4 and 5 were the first two fractional products of Series D (made from the sheaths of the tendons used in Series C). See page 160.

of 0.2 per cent hydrochloric acid, that unless very intimate and unusual chemical union resulted, lymph proteids must have been quickly and completely dissolved from the precipitates. We know of no other substance in tendon which would resist the washing treatment and, by mechanical admixture or chemical combination, account for the orderly variations observed in the analytic series.[1]

It is much more probable, we think, that an answer to these considerations will be found in the fact that the mucoids are labile bodies of great variety in the tissues and with more than one function to perform. Their acid radicles doubtless make them prone to enter into numerous ion combinations. The very complexity of these substances makes it natural to assume that exactly the same proportions of the constituent radicles would in metabolic changes be the exception rather than the rule.

All of the products separated in these experiments were true glucoproteids, responding to each of the well known reactions and yielding reducing substance in abundance.

We have repeated the experiments of Chittenden and Gies on the osazone substance obtainable with the reducing body and, working with a larger quantity of mixed mucoid products by the same and also improved methods, obtained a crystalline product melting at 182° C.[2] In microscopic appearance the crystals are identical with those of glucosazone. We have not yet been able to free the substance entirely from the brownish globules that occur with it and which persist

[1] Since this paper went to the printer we have seen NERKING'S recent note on fat proteid compounds, in the Archiv für die gesammte Physiologie, 1901, lxxxv, p. 330. His results indicate that various proteid products, which have been purified by the usual methods, contain fat or fatty acid in close combination ; further, that this fatty radicle may be broken off, and extracted, by DORMEYER'S method. No such combination with ovomucoid was shown, but about three per cent of extractive matter was found to be combined with submaxillary mucin. NERKING does not state, however, that the mucin was thoroughly extracted in hot alcohol ether during the preliminary process of purification, in the customary manner. No results are presented for tendon mucoid; but LOEBISCH, and CHITTENDEN and GIES have already called attention to the fact that tendon mucoid when freshly precipitated is admixed with extractive matter that is removable only after long continued extraction. All our preparations were given careful and extended treatment in boiling alcohol-ether, and we do not believe that the variations in composition noted are due to such fat combination. We hope that studies which have lately been in progress in this laboratory, will soon furnish direct evidence concerning this and related questions.

[2] The product obtained by CHITTENDEN and GIES melted at 160° C.

in spite of all our attempts to purify the crystals. It seems certain that glycuronic acid and glucosamin, or very closely related bodies, are formed together in the decomposition of tendon mucoid with hot dilute mineral acid.

III. Relation to other Connective Tissue Glucoproteids.

Composition. — It appears to be definitely established by the numerous results of these and the preceding experiments that the amount of sulphur in tendon mucoid is relatively high — almost the same as in chondromucoid and osseomucoid — and that Loebisch's data in this particular connection can no longer be accepted as correct. We have never been able to prepare a tendon mucoid having less than 1.3 per cent of sulphur.[1]

The sulphur is present in at least two combinations, as in the case of chondromucoid and osseomucoid. After boiling with alkali, lead sulphide may be obtained on addition of lead acetate. The amount combined in the form of SO_3 is relatively large, varying as the analytic data for each preparation show, between 1.33 and 1.62 per cent of the whole molecule. The average amount of SO_3 sulphur in chondromucoid is 1.76 per cent. In osseomucoid it equals 1.40 per cent. Levene[2] has lately separated from tendon mucoid a substance very similar to chondroitin sulphuric acid. The quantity of this substance separable from the mucoid has not been estimated.

Two years ago, in our preliminary report, we made the following statement:[3] "Before these experiments were started, the similarity in the percentage composition of Mörner's chondromucoid and the tendon mucin analyzed by Chittenden and Gies suggested to us that the two substances are perhaps closely related. This was further emphasized by the fact that the osazone crystals they obtained had the same general appearance as the crystals of glucosazone, and, therefore, might have arisen from glucosamin, one of the decomposition products of chondromucoid." Levene's results and our own increase the probability that the two substances are very much the same.

The following summary of average elementary composition shows the general relationship of very nearly identical products:

[1] See table, page 162.

[2] Levene: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 395.

[3] Cutter and Gies: *Loc. cit.*

	C	H	N	S	O
Chondromucoid MÖRNER	47.30	6.42	12.58	2.42	31.28
Tendomucoid (*a*) CHITTENDEN and GIES	48.76	6.53	11.75	2.33	30.63
(*b*) CUTTER and GIES (1) .	47.47	6.68	12.58	2.20	31.07
Osseomucoid HAWK and GIES . .	47.07	6.69	11.98	2.41	31.85
Average . .	47.65	6.58	12.22	2.34	31.21

Heat of Combustion. — Heat of combustion furnishes important means of estimating chemical relationships, though its indications are not, perhaps, so delicate as those of elementary analysis. The determinations in these experiments were made by the method described by Hawk and Gies. In Table V we give the heat of combustion of our five completely analyzed preparations, together with comparative

TABLE V.

COMBUSTION EQUIVALENTS.

Preparation.	Direct determinations. Heat of combustion. Small calories. Per gram of substance.			Averages for ash-free substance. Percentage content.		Heat of combustion. Small calories.	
	I	II	Average.	Carbon.	Oxygen.	Per gm. of substance.	For substance containing 1 gm. of carbon.
I. Tendomucoid. No. 1	4925	4940	4933	47.47	31.07	4967	10463
No. 2	4963	4930	4947	47.46	32.39	4986	10506
No. 3	4921	4934	4928	47.80	31.09	4979	10416
No. 4	4908	4920	4914	48.92	28.81	4951	10121
No. 5	5044	5036	5040	48.54	29.75	5131	10571
Average.	4952	4952	4952	48.04	30.62	5003	10415
II. Osseomucoid. Average of two preparations.	4972	4985	4979	47.16	31.79	4992	10589
III. Chondromucoid. Average of two preparations.	4865	4869	4867	45.87	32.90	4883	10647

data from the summary in a recent paper from this laboratory.[1] The figures show only imperfectly the differences among the tendon mucoids. They are valuable chiefly for the indication they furnish that the various glucoproteid products referred to are essentially the same compounds.

We still believe "continued investigation will show that the differences among the mucins, mucoids, and chondroproteids are not as great as their varying physical properties and behavior have suggested, but that each is a combination of proteid with a glucosulphonic acid, the qualities of each compound, just as in the case of the nucleoproteids, being dependent largely on the proportions and character of the proteid and compound acid radicles." [2]

IV. Summary of Conclusions.

The more important conclusions to be drawn from the results of this research are:

1. Tendon contains more than one glucoproteid. The average percentage composition of five preparations of mixed mucoid was as follows:

C	H	N	S	O
48.04	6.67	12.47	2.20	30.62

These figures agree very closely with those published by Chittenden and Gies.

2. The composition of mucoid from the shaft and from the sheath:

	C	H	N	S	O
Shaft (3)	47.56	6.61	12.34	1.95	31.52
Sheath (2)	48.73	6.75	12.66	2.57	29.28

3. Tendon mucoids contain an average amount of sulphur equal to that found by Chittenden and Gies — approximately 2.30 per cent. Not a single product had the very low content of sulphur ascribed to this substance by Loebisch.

4. The average composition of mucoid separated from white fibrous connective tissue by the customary methods is very nearly the same as that of chondromucoid and osseomucoid.

5. Thermochemical studies of the mucoids in tendon, cartilage, and bone emphasize the probability that these bodies are very intimately related.

[1] Hawk and Gies: *Loc. cit.*, p. 422.

[2] Cutter and Gies: *Loc. cit.*

PHLORHIZIN DIABETES IN CATS.

By JULIUS F. ARTEAGA.

[*From the Physiological Laboratory of the University and Bellevue Hospital Medical College.*]

FORMER investigations in Professor Lusk's laboratory have established the fact that when phlorhizin is frequently given subcutaneously to fasting rabbits there is a preliminary sweeping out of the body's sugar through the urine, followed by a continuous elimination of dextrose and nitrogen in a constant ratio of 2.8 grams of dextrose to 1 gram of nitrogen.[1] This ratio was that found by Minkowski[2] in fasting dogs diabetic from the removal of the pancreas. Later investigations showed that if dogs were treated with phlorhizin according to the method employed above with rabbits, the sugar excretion became much larger in relation to nitrogen eliminated, constantly averaging 3.75 grams of dextrose to 1 gram of nitrogen.[3] This represented a higher production of sugar from proteid metabolism than had ever before been obtained. Still later work done by Professor Lusk and the author[4] showed that after treating goats with phlorhizin the urinary ratio became dextrose : nitrogen = 2.8 : 1. Since the carnivorous dog showed a ratio of 3.75 : 1, and the herbivorous rabbit and goat one of 2.8 : 1, it seemed to me important to carry on similar experiments with another variety of carnivora, and to this end the cat was selected.

The disadvantage of using the cat lies in the fact that the catheter cannot be passed through the urethra because of its small size. On account of this the urine of one day was always more or less mixed with that of the preceding days. The results show that the collection of the urine must be continued several days before the ratio becomes fixed and constant. The urine was caught in a tray beneath the cage occupied by the cat.

[1] GRAHAM LUSK: Zeitschrift für Biologie, 1898, xxvii, p. 82.

[2] MINKOWSKI: Archiv für experimentelle Pathologie und Pharmakologie, 1893, xxxi, p. 85.

[3] REILLY, NOLAN, and LUSK: This journal, 1898, i. p. 395.

[4] LUSK: Festschrift zu Voit, Zeitschrift für Biologie, 1901. (Not yet published.)

In the first experiment 0.1 gram of phlorhizin was dissolved in a few cubic centimetres of a warmed 1.2 per cent Na_2CO_3 solution and injected subcutaneously every eight hours. The phlorhizin was given on March 7, after the cat had fasted four days. The result of the urinary analyses may be tabulated as follows:

Cat I. 0.1 gm. phlorhizin every eight hours after March 7.

Date, 1900.	Amount of urine in c.c.	Weight in kg.	Dextrose.	Nitrogen.	D. : N.
March 3	....	3.16			
7	80	....	?	?	
8	192	....	5.68	2.89	1.96
9	231	....	8.53	3.28	2.60
10	....	....	10.58	2.46	4.30

It is apparent that the small dose of phlorhizin (0.1 gram) became progressively more and more effective in its ability to eliminate sugar, since the highest ratio was found on the fourth day. As I wished to have the body's excess of sugar removed as quickly as possible I returned to the dose of one gram every eight hours, which had been successfully employed in the case of the rabbit. The first experiment was with Cat No. I, used above. In Cats II and III the diabetic urine was not analyzed during the first two days. The results obtained are expressed in the tables on page 175.

All these cases show in the later days a pronounced tendency to approach the urinary ratio D. : N = 2.8 : 1, and this is conclusively demonstrated in the last experiment, where, on the third, fourth, and fifth days the ratios were 2.93 : 1, 2.80 : 1, and 2.93 : 1. The slightly higher ratios of 3.19 : 1 and 3.07 : 1 on the last days of experiment with the first two animals is to be explained by the admixture of the urine of previous days when the body's sugar was being removed; hence appears the disadvantage of not being able to separate the urine by catheterization and by washing the bladder. About six hours after the last urine of Cat III was passed the animal died, apparently in diabetic coma. This last urine was observed to yield a sediment upon standing. These crystals were examined by Professor John A. Mandel, and to him I am indebted for the report that they

yielded a reducing substance on cleavage with acids, and showed the same crystalline form and melting point as phlorhizin. This discovery of phlorhizin in cats' urine seems especially remarkable, since

Date, 1900.	Amount of urine in c.c.	Weight in kg.	Dextrose.	Nitrogen.	D. : N.
Cat I. Fasting since April 7.					
April 11	59	....	4.01	1.36	2.95
12	144	....	17.04	4.03	4.23
13	163	....	12.19	3.74	3.26
14	104	2.37	9.22	2.89	3.19
Cat II. Fasting since April 15.					
April 18	?	4.05	?	?	
19	?	....	?	?	
20	178	....	16.10	5.03	3.20
21	144	3.49	13.26	4.32	3.07
Cat III. Fasting since Oct. 2.					
Oct. 3	?	2.34	?	?	
4	?	....	?	?	
5	48	....	3.39	1.16	2.93 : 1
6	130	....	4.06	1.45	2.80 : 1
7	186	....	1.94[1]	0.66	2.93 : 1

[1] 12 hours' urine.

neither Cremer[1] nor Lusk[2] were ever able to detect phlorhizin in rabbits' urine.

This inquiry into the nature of phlorhizin diabetes in cats assumes

[1] Cremer: Sitzungsberichte der morphologisch-physiologischen Gesellschaft zu München, 1895, p. 75.

[2] Lusk: Zeitschrift für Biologie, 1898, xxvii, p. 98.

added interest when considered in connection with the work of Lee and Harrold [1] which has shown the muscular fatigue in cats' muscle after the readily combustible sugars have been removed by phlorhizin.

The principal result of my research is that in the fasting cat, just as in the rabbit and the goat, the urinary ratio between dextrose and nitrogen in phlorhizin diabetes is 2.8 : 1. This is a striking example of biological uniformity.

[1] LEE and HARROLD: Proceedings of the American Physiological Society, This journal, 1900, iv, p. ix.

ON THE PRODUCTION OF ARTIFICIAL PARTHENOGENESIS IN ARBACIA BY THE USE OF SEA-WATER CONCENTRATED BY EVAPORATION.

By S. J. HUNTER.

[*From the Laboratory of Comparative Zoölogy and Entomology, University of Kansas.*]

IN the parthenogenetic development of the eggs of *Arbacia* by the Loeb method, the nature of the actions of the solutions used merits consideration. Loeb states that artificial parthenogenesis in Echinoderms is caused by an increase in the osmotic pressure of the solution surrounding the unfertilized eggs.[1]

Since density can be expressed in terms of specific gravity it is evident that we can ascertain the relative osmotic pressures of the solutions used as well as the degree of pressure required to produce artificial parthenogenesis.

The specific gravity[2] of sea-water at the Marine Biological Laboratory, Wood's Holl,[3] where this work was carried on was 1.0211.

Specific Gravities of Normal Sea-Water and of Sodium Chloride Solutions.

Sea-water	1.0211
$2\frac{1}{2}$ *n* sodium chloride solution	1.0902
Solution No. 1. — A 10 per cent solution of $2\frac{1}{2}$ *n* sodium chloride, composed of 25 c.c. $2\frac{1}{2}$ *n* sodium chloride and 225 c.c. sterilized sea-water	1.0265
Solution No. 2. — A 15 per cent solution of $2\frac{1}{2}$ *n* sodium chloride, composed of $37\frac{1}{2}$ c.c. $2\frac{1}{2}$ *n* sodium chloride and $212\frac{1}{2}$ c.c. sea-water	1.0283

All sea-water that came in contact in any way with unfertilized eggs was first sterilized by heating slowly to 65° C., then cooled to

[1] Loeb, J.: This journal, 1900, iv, p. 184.

[2] The specific gravity figures given throughout are calculated by Pemberton's tables from readings on Baume's Hydrometer. The temperature of all solutions at the time of reading was 33° C. This temperature was used since it was not convenient to reduce to 15.5° C., required by the hydrometer, and since relative densities only were desired.

[3] I am indebted to Dr. F. R. Lillie, for the ample facilities afforded and valuable suggestions given during the progress of this work.

22° C., the temperature of sea-water at Wood's Holl, and to replace oxygen which might have been driven off by the heat, the solution was passed through a glass syphon with the longer arm of very small aperture, and allowed to fall in a fine stream through the air for a distance of about four feet into a wide Stender dish. This was done twice with each flask of sterilized sea-water and three times in the case of sea-water concentrated by boiling.

It has been shown by Loeb, Wilson, and others that the 10 to 15 per cent $2\frac{1}{2}$ *n* sodium chloride solutions are the best sodium chloride solutions for the production of artificial parthenogenesis in Arbacia.

Now it seemed reasonable to suppose that if the density of normal sea-water were increased by evaporation until it stood between the figures given for Solutions 1 and 2, artificial parthenogenesis should likewise be produced. Accordingly 500 c.c. of normal sea-water were reduced by boiling slowly to 375 c.c., a reduction of 25 per cent in volume. In the same manner another quantity of 500 c.c. of normal sea-water was reduced to 250 c.c., a reduction of 50 per cent. in volume. The specific gravity readings of these two solutions were as follows: —

Solution No. 3. — Specific gravity of 500 c.c. sea-water reduced by evaporation to 375 c.c. 1.0260

Solution No. 4. — Specific gravity of 500 c.c. sea-water reduced by evaporation to 250 c.c. 1.0431

The specific gravity of Solution No. 3 was 0.0005 below that of Solution No. 1. This, however, can be accounted for by observational error. Before their use for experimentation, these solutions were cooled to 22° C. and ærated three times.

Unfertilized eggs of a single female *Arbacia* were divided, placed in Solutions 3 and 4, allowed to remain there for two hours, then removed to sea-water. The eggs from Solution 3 segmented. Some of the eggs developed into gastrulæ capable of locomotion. No segmentation or perceptible processes of development were observed in eggs taken from Solution 4. These experiments were repeated with two other females and similar results secured. The use of Solution 4 was discontinued. Sea-water that had been reduced 10 per cent in volume, *i. e.*, 100 c.c. reduced by boiling to 90 c.c., was tried with negative results. The next experiment was with sea-water which had been reduced 30 per cent in volume, *i. e.*, 100 c.c. had

been boiled down to 70 c.c. In this concentration segmentation and development took place, but not more favorably nor in larger numbers than in Solution 3. No further experiments with various concentrations by evaporation were performed. Observations were confined to sea-water reduced in volume 25 per cent. The optimum duration of time for eggs in this solution was found to be two hours and twenty minutes. To ascertain this, eggs were removed to sea-water every thirty minutes until two hours had elapsed, and then every ten minutes for the next thirty minutes. Eggs taken out and placed in sea-water, after being in Solution 3 for one hour, would begin to segment, but would not develop so far as those left in it for two hours and some minutes. The eggs of two females, however, do not always act alike under similar conditions. Hence the optimum length of time may vary slightly with the eggs of different females.

In order that there may be no question concerning the contamination of eggs with spermatozoa the method of operation may be briefly given. All dishes were thoroughly washed with hydrant water, inverted and allowed to dry. All instruments were heated in the flame of an alcohol lamp, then placed in a dish of hydrant water, just before using. Through the kindness of the Curator, Mr. Gray, the females were selected and no males were brought into the laboratory. The female from which the eggs were to be taken was washed for about three minutes under a stream of hydrant water, laid on a sterilized vessel until the hands of the operator had been thoroughly washed with soap and hydrant water, then the sea urchin was washed under a stream of hydrant water for about three minutes longer. If the female had just been brought in from the ocean,[1] and was ready to spawn, oviposition was easily brought about by pouring tepid sea-water over the ventral surface. Sometimes oviposition was incited by the hydrant water, which was somewhat warmer than the sea-water. Some of the eggs were kept in sterilized sea-water to serve as a proof of absence of spermatozoa.

Ten experiments with eggs of as many females were performed to observe the relative effects of Solutions 2 and 3. In each experiment the eggs of one female were separated into three lots; one in sea-water for control, one in Solution 2, and the third in Solution 3. With one exception the eggs in Solution 3 developed more rapidly

[1] The majority of those selected for experimentation were brought from the ocean the same day their eggs were used.

and showed a larger percentage of larvæ (locomotive forms) than appeared among the eggs developed in Solution 2.

Since my work was concerned primarily with the early cleavage stages of these eggs, I paid little attention to the length of life in those taken from Solution 3. In one case, however, I observed plutei six days old that had been developed in Solution 3.

In all, the eggs of fourteen females were placed in Solution 3 and in eleven cases satisfactory results were secured. In some of the experiments as many as 90 per cent, approximately, of the eggs began to segment. Comparatively few, however, reached the freely moving gastrula stage. In one instance I estimated that the number of moving gastrula approached 40 per cent of the eggs in the solution. Were I to prepare cultures to continue the life of the individuals, I should use fewer eggs and greater amounts of sea-water. As it was, a large number of eggs in early cleavage stages was desired.

This same solution of sea-water reduced in volume 25 per cent by evaporation, was used this season by the class in Embryology in the Laboratory and a good percentage of larvæ obtained.

These experiments with sea-water concentrated by evaporation, then, tend to strengthen Loeb's osmotic theory of artificial parthenogenesis in Arbacia. Sea-water that is condensed until it is isotonic with Loeb's 10 per cent to 15 per cent $2\frac{1}{2}$ *n* sodium chloride solutions will cause artificial parthenogenesis.[1] Sea-water with osmotic pressure perceptibly less or greater than the 10 to 15 per cent solution of $2\frac{1}{2}$ *n* sodium chloride will not produce artificial parthenogenesis. Furthermore, it is evident that a certain osmotic index or degree of pressure is essential for artificial parthenogenesis.

[1] Solution 2, the 15 per cent solution of $2\frac{1}{2}$ *n* sodium chloride, gives more satisfactory results than Solution 1, the 10 per cent $2\frac{1}{2}$ *n* sodium chloride solution. Solution 2 has greater osmotic pressure than Solution 1. while Solution 3, the sea-water reduced in volume 25 per cent by evaporation, which gives even more satisfactory results than Solution 2, is isotonic with Solution 1. Possibly the optimum duration of time for eggs in Solution 2 is less than the optimum duration of time for eggs in Solution 3.

AN ANALYSIS OF THE INFLUENCE OF THE SODIUM, POTASSIUM, AND CALCIUM SALTS OF THE BLOOD ON THE AUTOMATIC CONTRACTIONS OF HEART-MUSCLE.

By W. H. HOWELL.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

THE relation of the phenomenon of rhythmic contractility to the inorganic constituents of the lymph, blood, or other medium bathing the tissue has attracted much attention in recent years. The relation has been studied most carefully in connection with the typically rhythmic heart-muscle, but investigations have been extended to other tissues exhibiting the same property, among the invertebrates as well as the vertebrates.

Historically the pioneer work in this field was accomplished under the influence of Ludwig. Systematic investigations by Merunowicz, Kronecker, Stiénon, Gaule, Martius, von Ott, and others[1] emphasized in a striking way the importance of the inorganic constituents in all media designed to maintain the rhythmic contractility of the isolated frog's heart. The earlier observers in studying this question seem to have assumed that the influence of the inorganic constituents upon the isolated heart is secondary. The essential or an essential factor was the presence in the circulating medium of some albuminous material. This point of view was unfortunately over-emphasized by Kronecker and his pupils. Most of the work of this school was directed to prove that a circulating medium cannot sustain rhythmic contractility in heart-muscle unless it contains serum albumin, and that the heart-muscle, as regards its rhythmic contractions, depends upon an ever present supply of this material in the surrounding liquid, having no store of available energy within its own substance. This view of the immediate necessity of serum albumin in the bathing liquid has, I believe, been fully demonstrated to be erroneous by the investigations of the author and his pupils. All students of this problem at present will be forced to admit that the contractions

[1] For references see HOWELL. This journal, 1899, ii, p. 81.

of heart-muscle and other rhythmically acting tissues may be maintained for very long periods in solutions containing only inorganic constituents.

Merunowicz analyzed very successfully the influence of the various inorganic constituents and stopped short of reaching the present standpoint only because he was unaware of the essential importance of calcium salts. The credit of first recognizing the peculiar importance of calcium is due to Ringer. This author showed so clearly the especial part taken by Ca and K salts in maintaining the rhythmic contractility of heart-muscle that subsequent investigators have of necessity followed the lines of work as laid down by him. The extraordinary efficacy of the so-called Ringer's mixture of sodium, potassium, and calcium salts in proper proportions in maintaining the contractions of heart-muscle is now a familiar fact. Recent studies have been directed largely to an attempt to discover the nature of their action and the individual influence of each of the elements in question. With regard to the nature of their action only vague hypotheses have been offered. Loeb[1] has imagined the existence of compounds of the metallic ions with the proteids, but, although there is every reason to believe that the proteids may form dissociable compounds with either the kation or the anion or possibly with both (Pauli), the existence of such compounds has not been demonstrated experimentally, and, even granting their existence, no clearer idea is obtained of the connection between the inorganic salts and the periodic katabolisms of rhythmically contracting muscle. If we confine ourselves to experimental facts, it would seem that in this matter of the relation of the inorganic salts to rhythmic contractions we are not justified in going beyond the conservative statement made by Merunowicz — namely, that for the heart contractions both organic and mineral constituents are necessary, the former supplying compounds from which energy is obtained, the latter giving to these compounds such a form that they can be used in the production of contractions.

The particular influence exercised by each of the three salts present in a Ringer's mixture, as well as the effect of other inorganic compounds, may, however, be investigated by the methods available at present, and in this respect much has been accomplished in recent years. The present paper is a report of a series of experiments made

[1] Loeb: Archiv für die gesammte Physiologie, 1900, lxxx, p. 229, and Fick's Festschrift, Braunschweig, 1900.

with this end in view and is a continuation of previous work of a similar character.

The experiments reported were all made upon strips of the ventricular muscle of the terrapin. A longitudinal strip was prepared from the free edge of the ventricle in its lower two thirds, and this strip was then bisected in its long diameter so as to give two strips as nearly similar as possible for comparative experiments. The strips were attached to levers which traced the records of the contractions on slowly revolving drums that made one revolution in from one to twelve hours. The apparatus was so arranged that the strips could be immersed conveniently in baths of liquids of different compositions. It should be borne in mind, as has been shown by work from this laboratory, that strips from this portion of the ventricle do not, as a rule beat spontaneously when freshly taken from the heart and immersed in the animal's own serum or in a Ringer's mixture containing Ca and K in the proportions found in blood. Under what might be called normal conditions they are not automatically contractile. By modifying the proportions of the salts in various ways, or by first altering the conditions within the strip itself, a prolonged series of spontaneous contractions may be obtained. As opposed to this behavior of strips from the apex of the ventricle, strips from the venous end of the heart (vena cava) beat with perfect regularity in serum or an equivalent Ringer's mixture.

Action of Apical Strips of Ventricle in Solutions of NaCl.

NaCl exhaustion. — Ventricular strips placed in an isotonic or approximately isotonic solution of NaCl give a characteristic series of beats that has been described in detail by Greene.[1] I wish to emphasize here only certain peculiarities of this series that seem to be of fundamental significance. In the first place, if the strip has been freshly prepared there is always a certain latent period before the contractions begin. The length of this latent period is very variable. In thirty experiments made during the present series in which this factor was observed the period varied from ten to one hundred and eighty minutes, but the average was fifty-six minutes, which corresponds fairly well with the interval usually to be expected.

In the second place the series of beats begins with contractions which compared with later ones are submaximal and infrequent. The

[1] Greene: This journal, 1899, ii, p. 82.

contractions then increase quickly or slowly to a maximum in rate and amplitude showing often a beautiful "staircase." Afterward the contractions decline gradually to zero, or in some cases after reaching a minute size become somewhat infrequent without decreasing in height for a considerable time. The duration of the entire series of beats varied in the present experiments between fifty and two hundred minutes, with an average of about one hundred and twenty minutes. The characteristic feature after the maximum is passed is the steady decline to a minimum or to zero. The contractions are as a rule well coördinated. The so-called exhaustion is due to changes in the strip since in all cases the bathing solution was relatively large in quantity (25 c.c.) and renewal of the solution at the end of the series was not followed by a revival of the contractions.

In the third place the strip shows a continuous loss of tone during the latent period and throughout the whole series of beats. This loss of tone in NaCl is one of the most distinct effects of this solution and will be referred to again in connection with an attempt to analyze the effect of sodium salts.

With reference to this limited series of contractions in NaCl the questions that naturally arise are: Why do the ventricular strips beat in NaCl and not in serum or an equivalent Ringer's mixture? What is the meaning of the long latent period and why is the series a descending one, ending finally in the state of NaCl exhaustion? A number of facts that have a bearing upon these questions will be described and discussed later. At present, for the sake of clearness it may be said that this series of experiments has led the author to adopt provisionally at least the following hypotheses. 1. That the apex of the ventricle in the terrapin beats for a time in pure solutions of NaCl, owing to the gradual removal of the potassium which under these conditions diffuses out from the heart tissue; whereas in normal serum or an equivalent Ringer's mixture the proportion of potassium present is sufficient to prevent, or if one prefers the expression, to inhibit, spontaneous contractions. 2. That the latent period is an expression of the time necessary for the removal of the potassium to the point at which its inhibitory influence ceases to be felt. Inhibition from mechanical injury probably influences also the duration of this period in excised strips. 3. That the state of NaCl exhaustion is due mainly to the loss of Ca from the tissue as the result of diffusion.

Recovery of contractions after NaCl exhaustion.—After the ventricular strips have ceased to beat in isotonic solutions of NaCl they

are totally insensitive to mechanical or electrical stimulation. They can be made to beat again, however, for a longer or shorter time by altering the composition of the surrounding medium in one of the following ways.

1. *Immersion of the strip in a proper Ringer's mixture of K, Na, and Ca salts.* — The beats thus produced begin in a few minutes and may last for very long periods, for twenty-four hours or more, without any change of solution. When the potassium and the calcium are present as chlorides in the quantities found in serum, that is, 0.03 and 0.026 per cent respectively, the beats are characterized by their long continuance and by their irregular grouping. A perfectly regular rhythm such as occurs in the normal heart-beat is not obtained. A pause in diastole is usually quite distinct and of variable duration, while at irregular intervals the beats fall into groups having a more rapid rhythm.

2. *Immersion of the strip in a proper mixture of NaCl* 0.7 *per cent and* $CaCl_2$.— The recovery caused by this mixture in which no potassium is present varies in character with the amount of Ca used. If present in amounts equal to that found in blood, the revival of contractions may be of short duration and accompanied by a strong development of tone. If, however, the amount of Ca is smaller (0.01 per cent $CaCl_2$) and is subsequently increased, a revival may be obtained for somewhat long periods. It happens that in the present series of experiments the action of the mixture of NaCl and $CaCl_2$ was tested usually when the strip, after exhaustion in NaCl, had been immersed for an hour or more in an isotonic solution of sugar. Under these conditions it may be assumed that the strip had lost most of its diffusible Na as well as K and Ca, and when placed in mixtures of NaCl 0.7 per cent and $CaCl_2$ 0.01 to 0.02 per cent, a series of contractions was obtained lasting for many hours. The characteristic of the revival in NaCl and $CaCl_2$ as contrasted with that occurring in a Ringer's mixture was the perfectly regular rhythm; systole and diastole followed with perfect regularity and with no perceptible diastolic pause and no grouping of beats.

3. *Immersion in isotonic or approximately isotonic solutions of cane sugar or dextrose.* — If the ventricular strip after coming to rest in a solution of NaCl is transferred soon to an isotonic solution of cane sugar or dextrose, it gives always a more or less definite series of beats lasting for a short time. In fifteen experiments of this character the average duration of the series was fifty-five minutes, the extremes lying between thirty and ninety minutes. In this series of

beats the contractions are usually small at first and slow, but both rate and amplitude increase somewhat rapidly to a maximum. After passing the maximum the beats again decrease somewhat in size and become slower, the series ending as a rule with several infrequent beats of good amplitude, instead of dying out gradually as in the NaCl series. Soon after the last spontaneous beat the strip becomes entirely unirritable to mechanical or electrical stimuli. When a strip is brought into this condition by exhaustion first in NaCl and then in sugar and is deprived apparently of all its diffusible ions of Ca,K, and Na, it seems to be in the best condition for a long-lasting revival with Ringer's mixture or with an appropriate mixture of NaCl and $CaCl_2$.

4. *Immersion in isotonic solutions of LiCl.*—The series of beats in this solution is usually shorter than in sugar solutions, but presents the same general characteristics. During the series the tone may increase somewhat, although usually not so much as in the sugar solution. After the cessation of the LiCl series an excellent and long-lasting revival of contractions may be obtained with a Ringer's mixture, while an isotonic solution of NaCl alone is usually entirely ineffective. Some further reference to the action of solutions of LiCl will be made below.

Action of Apical Strips of Ventricle in Solutions of NaCl and KCl.

As Greene has previously stated, fresh strips of the ventricle placed in NaCl 0.7 per cent to which KCl has been added in normal amount, 0.03 per cent, usually remain perfectly quiet except for a marked loss of tone. In some cases a series of beats may occur, but they are of smaller amplitude than the usual NaCl series and the whole series is of briefer duration. In such cases, increasing somewhat the amount of KCl suffices to prevent spontaneous contractions. It is worth noting that when the mixture contains KCl in amounts not much exceeding 0.03 per cent the effect upon the heart muscle is not injurious. Subsequent immersion in Ringer's mixtures gives an excellent series of beats.

Action of Apical Strips of Ventricle in Solutions of NaCl and $CaCl_2$.

In marked contrast to the action of NaCl and KCl, a mixture of NaCl and $CaCl_2$ may cause a series of vigorous contractions. The

duration and characteristics of this series depend largely upon the amount of $CaCl_2$ used. The general action of such mixtures has been described by Greene. I have only to add that in the present series of experiments two points were particularly noted. First, contrary to the statement made by Greene, no distinct shortening of the latent period was observed as compared with the action of solutions of NaCl. It should be said, however, that the $CaCl_2$ was never used above the concentration found in the blood, namely, 0.026 per cent; usually, in fact, the solutions were made up to half this strength. Moreover, the latent period is so variable even in companion strips from the same heart that only a long series of observations would be conclusive.

Second, if the proportion of $CaCl_2$ is kept below 0.026 per cent the series of contractions may continue for four or five hours or more, the size of the beats gradually diminishing in amplitude. The series of beats obtained differs from the series in NaCl alone in two particulars; first, in its greater duration when the proportion of $CaCl_2$ is not excessive; second, in the fact that the condition of tone in the strip follows a different course. There is first a loss of tone, as in the NaCl alone, but subsequently the tone increases, the rise of the curve being more or less rapid according to the amount of Ca used. If the solution is changed to a standard Ringer's mixture before this rise in tone has become too marked, a typical long-continued series of beats may be obtained. If, however, the rise in tone has been rapid because of the amount of Ca used, or if it has been allowed to continue too long, the subsequent use of Ringer's mixture or solutions of NaCl may be without effect. It would appear that under these conditions the muscle goes into a condition which may be designated as Calcium rigor, from which recovery is impossible.

Action of Apical Strips of the Ventricle in Isotonic Solutions of Sugar.

Numerous experiments under varying conditions were made upon the influence of isotonic solutions of cane sugar or dextrose. As the results obtained will be used frequently in the subsequent discussion of the effect of the different salts, they may be briefly summarized here. 1. Immersion of a fresh strip in an isotonic solution of sugar gives as a rule no contractions whatever. The strip shows only certain changes in tone which follow usually a definite course. The

curve at first falls for an hour or less, owing to loss of tone. This first fall in tone, seen also in solutions of LiCl, may probably be explained by the fact that the handling of the strip during its preparation and attachment to the recording lever throws it into a condition of excessive tone from operative violence. From this condition the strip slowly relaxes in the more or less neutral solutions in which it is placed. After this initial fall the curve slowly rises again because of a gradually increasing development of tone. A similar curve is obtained with isotonic solutions of LiCl. The rise of tone is due doubtless to the loss of the diffusible ions in the substance of the strip, particularly those of Na.

2. If one adds to the sugar solution salts of K, Ca, and Na, in amounts equal to those found in the blood or present in Ringer's mixtures, either singly or together the effect is entirely negative so far as spontaneous contractions are concerned. If Ca salts alone are added, especially after the immersion in sugar has continued for some time, the strip shows a tendency to go into excessive tone. If KCl is added alone in small quantities (0.03 per cent) it either exerts no distinct effect, or, like the Ca, causes an increase in tone. If NaCl is added to the sugar solution in amounts equal to 0.7 per cent, giving therefore a hypertonic solution of double the normal osmotic pressure of serum, a loss of tone occurs precisely as when the strip is placed in 0.7 per cent NaCl alone, but no spontaneous contractions result. Such hypertonic solutions are not distinctly injurious to the muscle, since, after an immersion of from three to four hours, transference to a Ringer's mixture is followed by spontaneous beats within a few minutes. If $CaCl_2$ to the amount of 0.026 per cent is added to the sugar solution in addition to the NaCl the effect is the same in so far as spontaneous contractions are concerned. The fact that fresh strips of the ventricle remain quiet in sugar solution plus NaCl to 0.7 per cent or NaCl and $CaCl_2$, but give a definite series of automatic contractions in NaCl alone or NaCl plus $CaCl_2$ indicates that the sugar in the strengths used (nearly 8 per cent cane sugar) is not entirely indifferent but exerts an inhibitory influence. That the quiescence is not due simply to the high osmotic pressure of the solution is indicated by the action of strips in hypertonic solutions of NaCl.

3. *The after-effects of sugar solutions.*—It is noteworthy that after prolonged immersion in isotonic solutions of sugar transferal to 0.7 per cent solution of NaCl is not followed by the usual series of contractions. A loss of tone results as usual with this solution but

no spontaneous contractions are seen. If, however, the strip is transferred from the sugar solution to a Ringer's mixture or a mixture of NaCl and $CaCl_2$ an excellent series of contractions is obtained. That prolonged immersion in sugar solutions is not injurious to the tissue was well illustrated in one experiment in which the strip had remained for twelve hours in a mixture consisting of sugar 8 per cent, 27.5 c.c. and NaCl 0.7 per cent, 2.5 c.c., without giving a single contraction. When transferred to a standard Ringer's mixture a series of beats was obtained lasting over twelve hours.

4. *The action of isotonic solutions of sugar after exhaustion in NaCl 0.7 per cent.* — After a heart-strip has given its typical series of contractions in NaCl and the stage of so-called exhaustion is reached immersion in isotonic solutions of sugar gives usually a more or less definite series of contractions lasting for about one hour. In some cases the series was short and the contractions small. Generally, however, as previously stated, the beats increase somewhat rapidly to a maximum in amplitude and rate and then fall off a little in amplitude as the tone increases, the series ending usually with several separated contractions of nearly maximal amplitude. The precise form of the curve made by the series of beats varied somewhat with each strip.

In these experiments we have examples of a series of spontaneous beats occurring in a medium that contains no Na, Ca, or K ions. It must be remembered, however, that these substances, although not present in the outside medium, may be contained in the tissue itself. In Lingle's[1] paper the author criticises a statement made by Greene to the effect that fresh heart strips give sometimes a short irregular series of beats in isotonic solutions of dextrose. Lingle explains this result on the gratuitous assumption that Greene's dextrose solutions contained some sodium salt, since otherwise "a rhythm was established in a solution of a non-electrolyte, a fact that directly contradicts Loeb's idea." In Greene's experiments the dextrose was prepared carefully from cane sugar according to the method given in Beilstein, and although it may have contained sodium in traces sufficient to give the flame test it certainly did not contain this substance in quantity. The weakness of Lingle's criticism is evident from the fact that addition of NaCl to a sugar solution, to the extent of 0.7 per cent, does not confer upon it the property of causing a strip

[1] Lingle: This journal, 1901, iv, p. 265.

to give spontaneous contractions. And even when the sugar solution is diluted with 0.7 per cent solution of NaCl the addition of as much as $\frac{1}{5}$ of its volume of the saline solution does not, as Lingle himself is at pains to demonstrate, bring about spontaneous contractions.

The experiments described give clear proof of the possibility of a series of automatic beats in a solution entirely free from electrolytes; but that the beats thus produced are probably dependent upon the presence of definite electrolytes in the tissue itself is made probable by the following considerations. First, the definite limitation of the series to about one hour, a fact to be explained possibly by the diffusion of electrolytes out of the tissue. Second, if fresh strips are placed first for from one hour and a half to two hours in a mixture of NaCl and sodium oxalate, the latter being present to the strength of 0.3 to 0.4 per cent and the total osmotic pressure being equal to that for 0.7 per cent solution of NaCl, subsequent immersion in a sugar solution fails to give the series of beats obtained after the action of NaCl alone. The result in such cases is either entire absence of contractions or a feeble series of minute beats, and it is explicable upon the hypothesis that ordinarily after immersion in NaCl for from two to four hours some dissociable Ca compound is still present in the tissue, whereas in the case of the solution containing oxalate this Ca is made insoluble. We may suppose that after the usual completion of the NaCl series of beats, which follows upon an immersion in NaCl of three or four hours, some compounds of Ca and possibly K are still left in the strip since the diffusion out of these substances is slow in fresh strips, and that the series of beats in the sugar solutions continues only so long as they are present. After the cessation of the sugar series the tissue may be considered as practically deprived of electrolytes and in this condition cannot be made to give spontaneous contractions by solutions containing NaCl alone, $CaCl_2$ alone, or KCl alone, but will give a capital series of beats in a mixture of the three salts or in a mixture in proper proportions of only the NaCl and the $CaCl_2$.

5. *The action of isotonic solutions of sugar plus $CaCl_2$ or KCl after previous exhaustion in NaCl 0.7 per cent.*—After exhaustion in NaCl, immersion in sugar containing Ca in doses of from 0.004 to 0.026 per cent is followed by a series of very vigorous contractions superposed on a rapidly rising tone-curve. The contractions are sharp and vigorous in proportion, roughly speaking, to the amount of Ca in the solution, but the duration of the series may be very brief as the strip

soon goes into Ca rigor. Addition of K salts to any amount will not neutralize this effect of the Ca, indeed the K salts alone under these conditions seem often to lead to an increase in tone.

Action of Apical Strips of the Ventricle in Solutions of LiCl.

In solutions of LiCl isotonic with 0.7 per cent NaCl the ventricular strips act much as they do in sugar solutions. Distinct tone changes, but no contractions, occur. With regard to the changes in tone there is first the customary relaxation extending over an hour or more and then a slow shortening, approximately to the original base line. Addition of $CaCl_2$ alone to the solution or in combination with KCl in any proportion fails to evoke automatic contractions, but tends to throw the strip into augmented tone. If the strip is allowed to remain in the LiCl for a long time, for three or four hours,—subsequent immersion in 0.7 per cent NaCl causes no contractions. If, however, the strip under these conditions is changed to a Ringer's mixture, recovery may take place and an excellent series of beats be obtained.

Effect of solutions of LiCl after exhaustion in NaCl. After a strip has ceased to beat in 0.7 per cent NaCl, immersion in LiCl gives a short series of beats the general characteristics of which have already been described. After the cessation of this series, addition of $CaCl_2$ alone or in conjunction with KCl has no effect other than to cause a rapid development of tone. If changed to a 0.7 per cent solution of NaCl the result is a pronounced decrease in tone without any contractions. If, however, the strip is transferred to a Ringer's mixture or to an appropriate mixture of NaCl 0.7 per cent and $CaCl_2$ (0.01 to 0.02 per cent) a good series of beats may be obtained. These reactions are significant from the point of view of the influence of the various salts or their kations. Ca alone added to the LiCl gives no beats but an increased tone, Na alone in 0.7 per cent solution of NaCl gives no beats but diminished tone, but Na and Ca together or with K as in a Ringer's mixture cause spontaneous beats and an intermediate condition of tone. These facts, like many others, indicate the necessity of both Ca and Na for spontaneous rhythmic contractions.

Action of Apical Strips of Ventricle in Solutions of Sodium Oxalate.

The experiments made with this salt were intended to throw some light on the special significance of Ca for rhythmic contractions. They were made in two ways; first, by adding solid sodium oxalate to a 0.7 per cent solution of NaCl in quantities equal to 0.1 to 0.3 per cent of the solution, giving therefore an hypertonic solution; second, by replacing a part of the NaCl in a 0.7 per cent solution by an equivalent amount of sodium oxalate, thus preserving approximately the osmotic pressure of the solution and the total number of sodium ions. The results which will be used subsequently in the theoretical discussion were as follows:

1. Immersion of a fresh strip in a solution of NaCl and $Na_2C_2O_4$ is followed by a relaxation from loss of tone which is apparently more rapid and complete than in NaCl alone, but no contractions occur except possibly a series of very small beats lasting but a short time. Subsequent immersion in NaCl 0.7 per cent or in an isotonic solution of sugar fails to give any contractions. That the strip has not been permanently injured by the oxalate is shown, however, by the fact that immersion in a Ringer's mixture gives a good series of beats. It is interesting, moreover, to find that if the Ringer's mixture is followed by a bath of 0.7 per cent NaCl the usual series of beats from this solution is obtained, although as just stated the NaCl solution without previous immersion for some time in Ringer is not capable of arousing contractions.

2. After a fresh strip has been immersed in a 0.7 per cent solution of NaCl and has gotten well started on its series of beats, addition of sodium oxalate causes a marked diminution in the amplitude of the beats but may not bring them to a complete stop, as would perhaps be expected from the results given under experiment 1. After cessation of such a series of beats immersion in a sugar solution fails to give the usual series of beats such as have been described as following after ordinary NaCl exhaustion.

Action of Apical Strips of Ventricle in Solutions deprived of Oxygen.

It seems certain that a supply of oxygen is necessary for the maintenance of automatic contractions; but fortunately in experi-

ments upon the effect of solutions of the inorganic salts this factor does not have to be considered, since the aqueous solutions used hold sufficient oxygen in solution for the tissues of the cold-blooded animal. A single experiment was performed to ascertain the influence of an inadequate supply of oxygen and the results may be referred to very briefly. A fresh strip was immersed in a solution of NaCl 0.7 per cent which had been boiled thoroughly and then allowed to cool under a layer of oil. This layer was kept upon the solution throughout the experiment. After a latent period of twenty-three minutes the strip gave a series of contractions lasting for sixty-five minutes. The series differed from a normal NaCl series which was obtained from a companion strip from the same heart in unboiled salt solution mainly in the fact that the amplitude of the contractions was small throughout. The deprivation of O_2 in this experiment was of course not complete, as some at least was contained in the strip itself when immersed. The most interesting point, however, was that after the completion of the NaCl series subsequent immersion in a mixture of NaCl and $CaCl_2$ that had been deprived of its oxygen by boiling gave almost no effect in the way of contractions, and changing the strip afterward to a similar unboiled solution was equally negative. The absence of oxygen seemingly prevented the strip from recovering its automaticity in a mixture of NaCl and $CaCl_2$ and permanently altered the tissue.

Theoretical Considerations.

The importance of sodium salts. — Sodium salts exist in the blood and other liquids of the body in large quantities, 0.5 per cent or more, chiefly as sodium chloride. It is obvious therefore that the osmotic pressure of these liquids is dependent mainly upon this constituent. It has been natural to use so-called physiological saline as the basis of the solutions employed in investigating the action of various inorganic salts upon the heart. Experience has shown that solutions of NaCl of from 0.6 to 0.8 per cent are as nearly a normal medium for most living tissues as has yet been obtained by the use of any single substance in solution. The present author therefore in a previous paper ventured the opinion that this salt is essential in the blood and artificial circulating liquids because of its osmotic importance, giving abundant proof at the same time that so-called physiological salt solution is not sufficient alone to maintain the automatic

contractility of heart muscle. While the osmotic importance of NaCl in the blood is evident from the relatively large quantity present, Loeb has called attention to the fact that in addition the sodium ion is of importance in a special way in connection with the causation of automatic contractions. The experiments that have been described in this paper confirm this point of view, although they show equally well that Loeb has been led to attribute an all-importance to the Na ion which is not warranted by his own experiments nor by those of other observers.

Loeb's views of the relations of the Na, Ca, and K ions to the rhythmic activity of heart tissue have not, so far as his own work is concerned, been obtained from experiments performed directly upon this tissue, but are based mainly upon experiments on other rhythmically contracting tissue such as the bell of the gonionemus and the so-called rhythmic contractions of skeletal muscle in certain artificial media. It is a matter of some difficulty to criticise his views upon the importance of Ca, K, and Na to the rhythmic contractions of the heart, since in the numerous papers published by this author his statements of their relative importance have varied somewhat from time to time. In his earlier papers at least Loeb has assumed that his results obtained upon other tissues apply equally well to the heart, an assumption which is not wholly correct, as may be proved by a comparison of the behavior of heart tissue and skeletal muscle tissue in solutions of NaCl and $CaCl_2$, or by the differences in reaction in the same media shown by heart muscle, skeletal muscle, plain muscle (œsophagus) and cilia. In his most recent paper[1] Loeb's theory seems to be that Ca and K are not directly necessary to the automatic contractility of the heart, but only indirectly in that they neutralize the poisonous action of the Na ions. Stress is laid upon the poisonous action of Na as a fundamental fact that explains the necessity of the presence of Ca and K. So far as rhythmic activity is concerned only Na ions are directly concerned, or, to quote from Lingle, they are the producers of rhythmic activity. When, however, only sodium salts are present, when all the Ca and K ions are replaced by Na ions, then the theory assumes that the physical properties of the proteid tissues become so altered that rhythmic activity is impossible. It would seem to follow from this last statement that Ca and K are directly necessary to the maintenance of the normal physical properties

[1] Loeb: Archiv für die gesammte Physiologie, 1900, lxxx. p. 229.

of the heart proteids, a conclusion that it is difficult to reconcile with the hypothesis that they are not directly necessary to the contractility of the heart muscle. All the arguments adduced to show that Ca is only indirectly of importance in rhythmic contraction in that it serves to neutralize the Na might in truth be reversed and used equally well to prove that the Na ions are not directly necessary to heart activity but only indirectly by antagonizing the poisonous action of an excess of Ca.

To lay emphasis upon a poisonous action of sodium salts seems to the present writer to be unfortunate, when one considers that as a matter of fact every other substance soluble in water, when taken alone, is also, so far as known, poisonous to the heart muscle in the same sense. Pure solutions of salts of Ca, K, or other elements, as well as solutions of non-electrolytes such as sugar or urea, are totally unable to support rhythmic activity of the heart muscle. Pure solutions of proteids, fats, or carbohydrates would without doubt show a similar action and would therefore be poisonous to the heart muscle in the same sense as a 0.7 per cent solution of NaCl. To say that pure solutions of NaCl are poisonous to the heart muscle is to say nothing more than that solutions of NaCl alone will not maintain the irritability and automatic contractility of the heart tissue, but the same is true to a more marked extent of every other known constituent of the blood, when taken alone. The really remarkable thing about NaCl is that in pure solution of appropriate concentration it is less injurious to living matter than any other single substance. The universal employment of physiological saline in histological and physiological work upon living tissue bears witness to this fact.

Loeb's interesting discovery that the young fundulus will live indefinitely in distilled water but dies in a short time when placed in a $\frac{5}{8}$ *n* solution of NaCl does not alter this fact at all, so far as the writer can see. The fundulus dies in pure solutions of NaCl, but under such conditions we may suppose that its diffusible Ca, K, etc., are lost, as would be the case if the heart or any other of its tissues were bathed directly in the solution. But, says the author, the fact that the fish lives in pure water shows that neither Ca nor K is directly necessary to the heart's activity or to the act of respiration. How so? The author surely does not mean to assert that the fundulus when placed in pure water loses its store of Ca, K, and Na ions from the liquids and tissues of the body and still continues to live indefinitely. But if the Ca, K, and Na are not lost from the tissues and

liquids of the body, how does the experiment given prove anything regarding the direct or indirect necessity of Ca or K to the heart's activity? It is not necessary to add that heart tissue does not retain its normal properties long in pure water compared with 0.7 per cent solutions of NaCl. If the fundulus can really live indefinitely in pure water we must assume that there is a protective reaction of its superficial epithelium toward the water, which prevents the diffusion from the interior of the animal of the soluble constituents of the body-liquids.

While the writer is unwilling to admit the conclusion of Loeb and Lingle with regard to the sole direct importance of Na ions in rhythmic activity and believes that the experiments reported by Lingle[1] furnish only a superficial justification for this conclusion, he takes pleasure in acknowledging that the work of these authors has been important in directing attention to a special significance of the Na ions. From the experiments reported in some detail in the present paper the author has been led to conclude that the presence of both sodium and calcium salts is absolutely necessary for the production of rhythmic contractions. No experiment that the author has been able to devise, and none that he has seen reported by other observers, has shown in a conclusive way that the ventricular muscle can exhibit spontaneous rhythmic contractions in the absence of either sodium or calcium salts. The long known fact that the ventricle will beat, for a time, in solutions of NaCl is of course not opposed to this conclusion. The fact that there is no Ca in the bathing solution does not mean that there is no Ca in the beating strip. Ventricular strips suspended for many hours in solutions of NaCl still give a calcium reaction with ammonium oxalate. If we remove the calcium, that part at least that is in dissociable form, by the only certain method, namely its precipitation as oxalate, then the strip fails to give any contractions at all unless new calcium is supplied in the bathing liquid.

If one chooses to make the objection to this experiment that the oxalates prevent the contractions, not by precipitating the Ca, but by some direct action of the oxalic acid ion, one cannot prove the contrary directly, since by the nature of the reaction the Ca cannot be removed without an excess of the oxalate and vice versa. The indirect evidence, however, shows conclusively that the effect of the oxalate

[1] Lingle: *Loc. cit.*

is due to its precipitation of the Ca. When a ventricular strip has been immersed for an adequate time in a mixture of NaCl and $Na_2C_2O_4$ it cannot be made to resume spontaneous beats by immersing it for any length of time in a solution of NaCl, or indeed, so far as the writer's experience goes, by immersing it in any other solution except one containing Ca salts in addition to the sodium salts, the only exception to this statement being the similar though less effective reaction of the salts of strontium or barium.

One of the series of experiments described in the first part of this paper would seem at first sight to indicate the possibility of automatic contractions in the presence of sodium salts alone, but a consideration of the conditions makes this conclusion inadmissible. The experiments referred to consist in suspending a fresh strip from the apex of the ventricle in a solution of NaCl 0.7 per cent. After the series of NaCl beats is completed the strip, after being rinsed with water, will give a new series of beats lasting about one hour if immersed in an isotonic solution of sugar or LiCl, the contractions usually being larger and of longer duration in the sugar solution. The bathing solution in this case contains neither Ca nor Na, but it might be assumed that since the strip has been removed from a solution of NaCl the beats continue during the time necessary for the diffusion of the Na from the strip into the solution of sugar.

This explanation, however, is contradicted by the following facts. 1. Ca is still present in such strips, as may be shown by the reaction with ammonium oxalate. 2. In some cases after a very long continued series in NaCl, the subsequent bath in sugar solution gives only a short lasting series of feeble contractions. 3. After immersion in NaCl and $Na_2C_2O_4$ for a time equivalent to that required for a NaCl series, subsequent treatment with sugar solutions gives no contractions at all. 4. After completion of the series of beats in the sugar solution or in LiCl, addition of NaCl in small or large quantities to the bath causes no revival of the contractions. Transferal again to a 0.7 per cent solution of NaCl gives no new series of beats in the case of LiCl. With the sugar solution, reversing the solutions in this way gives in some cases no contractions, in others a brief series of small beats. If the sugar or LiCl series were due simply to the Na while diffusing out of the strip, one should expect a similar series when opportunity is given for diffusion of the same substance into the strip. 5. After completion of the sugar series, immersion in a proper mixture of NaCl and $CaCl_2$ gives usually a beautiful series of

contractions, the ventricular strip under these conditions being in fact in a particularly favorable condition for reaction to appropriate solutions.

The occurrence of a limited series of beats in a solution of sugar or LiCl after previous exhaustion in NaCl may be referred therefore to the presence of both Ca and Na in a usable form in the strip. The whole matter of the diffusion of the ions out of or into the heart strips is of course an assumption, but a permissible, if not necessary one, to explain the action of the various solutions used. Most of the results obtained are in harmony with the hypothesis that the fresh strip gives up its kations slowly, particularly the Ca, under the influence of diffusion, and that the interaction of Na and Ca in the production of rhythmic contractions is possible only when the quantities present in the tissue do not depart too far from a certain proportion. That the diffusion of the K out of the heart strip when suspended in a bath of NaCl is more rapid than that of the Ca is indicated by an analysis of the experiments reported in the first part of this paper, and is in accord with an interesting experiment related by Ringer.[1] According to this author, when a frog's heart is fed with 0.6 per cent NaCl and *dialyzed* serum the contractions obtained resemble those resulting from a mixture of sodium chloride and calcium salts. Addition of potassium salts to the mixture brings the heart-beats back to their normal character.

When potassium is not present in the solutions the proportions of NaCl and $CaCl_2$ which seem to give the most regular and forcible contractions with the ventricular strips are NaCl 0.7 per cent and $CaCl_2$ 0.01 per cent to 0.012 per cent. Any marked increase of the Ca leads to an exaggerated tone ending in a condition of calcium rigor. On the other hand, if the amount of calcium is greatly reduced contractions become impossible. We may suppose that this last condition exists at the end of the usual NaCl series when the stage of NaCl exhaustion is reached. Subsequent immersion in a solution of sugar or LiCl may give a short series of beats, as we have seen. We have given reasons for believing that this last series is dependent upon the presence of some Ca in the strip in dissociable form which becomes effective as the excess of NaCl is reduced by diffusion. That the Ca plays a part in this series is indicated further by the fact that a fresh strip which is immersed for a longer time than

[1] Ringer: Journal of physiology, 1887, viii, p. 288.

usual, about four hours, in a solution of sugar or LiCl, remaining quiet during this time except for changes in tone, and which is then immersed in NaCl 0.7 per cent does not contract, or gives at best a few feeble inconspicuous beats. When the strip, however, is transferred to a proper mixture of NaCl and $CaCl_2$ or to a Ringer's mixture a long-lasting series of beats is obtained.

After completion of its series of beats in NaCl and the subsequent series in solutions of sugar or LiCl, if some $CaCl_2$ or KCl is added in physiological doses to the last solution the only effect is an augmented tone. If on the contrary the strip is transferred to a solution of NaCl 0.7 per cent the only effect is a loss of tone. But transference to a mixture of NaCl and $CaCl_2$ or to a Ringer's mixture is followed by an excellent series of automatic contractions. The only conclusion that one can draw from such experiments is that both the Na and the Ca are necessary for the production of rhythmic spontaneous contractions. The two substances seem to have a distinctly antagonistic effect upon the heart-tissue, — excess of Na leading always to a loss of tone, excess of Ca to a greatly increased tone, while in mixtures of proper proportions the condition of tone is practically unchanged and automatic contractions result.

Ringer laid emphasis upon the antagonistic influence of the K and Ca salts in the matter of tone, an excess of the former leading to a loss of tone and of the latter to an increase of tone. In his experiments, however, NaCl was always present, so that its influence was a constant factor under both conditions. That potassium salts within certain limits tend to counteract the stimulating influence of calcium salts upon tone cannot be doubted, but that this influence is far less marked than the effect of Na salts is shown conclusively by the experiments described. In solutions of sugar, for instance, the very marked influence of traces of Ca in causing an augmentation of tone is scarcely if at all counteracted by adding K salts, whereas the addition of Na salts, provided the Ca is not present in excess, is promptly followed by a relaxation of tone. The fundamental antagonism is between the sodium and the calcium, and in this matter of the condition of tone, as in that of automatic contractions, it is noteworthy that for the preservation of normal conditions the sodium salts must be present in relatively large proportions as compared with the calcium salts.

The rhythmic play of contraction and relaxation in a series of beats under the combined action of sodium and calcium salts may be

compared with the opposing influence of the same substances upon the condition of tone. As I have described in a previous paper the changes in tone may themselves take on a rhythmic character, which indeed is very marked for the tissue at the venous end of the heart. When we compare the rhythmic contractions of various tissues such as the heart, œsophagus,[1] stomach, etc., it becomes in fact a matter of difficulty to say when these changes are merely variations in tone and when definite contractions. For the present it would seem necessary to base any distinction made simply upon some arbitrary standard of the rapidity of shortening. The facts so far as they are known at present would seem to show that the contractions and relaxations in rhythmic beats are due to fundamentally the same causes as lead to rhythmic changes in tone. It is natural to suppose that the energy of the contraction or increased tone is referable to a reaction of the proteid tissues of the heart muscle. The influence of the inorganic salts can only be secondary in making this reaction possible.

This suggested relationship between the proteids and the salts recalls the known facts regarding the dependence of the proteids for their properties upon combinations with inorganic salts, the solubility for instance of the so-called globulin group. Pauli[2] has recently called attention to the fact that the globulins are not soluble in solutions of non-electrolytes such as sugar, and he supposes that a globulin in solution is in chemical combination both with the anion and kation of the salts present. A further fact of possible bearing upon this physiological relationship between the proteids and the inorganic salts is the discovery by Starke[3] that globulins may be precipitated from their solutions by the action of dilute solutions of $CaCl_2$, a fact which may have some bearing upon the phenomenon of Ca rigor so often referred to in this paper.

The special influence of potassium salts. — So far little has been said of the influence of potassium salts on the heart beat, for the reason that the experiments described seem to indicate that the presence of potassium salts is not essential to the production of spontaneous contractions; under proper conditions an appropriate mixture of sodium and calcium salts suffices to initiate spontaneous contractions and maintain them for a considerable period. However, the

[1] Stiles: This journal, 1901, v, p. 338.

[2] Pauli: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 315.

[3] Starke: Zeitschrift für Biologie, 1900, xl, p. 419.

influence of the potassium upon the heart rhythm is most evident, and when used in optimum proportions, as in Ringer's mixture, the spontaneous contractions of the heart strips are maintained for much longer periods than with a mixture containing only sodium and calcium salts.

As far as my experiments have gone the potassium ion seems to influence the rhythm chiefly, its influence in general being comparable to that of inhibitory agents. In mixtures of NaCl and $CaCl_2$ the strips beat with a rapid and very regular rhythm, although the diastolic phase is gradually shortened as the strip slowly goes into a condition of heightened tone. When KCl is added to the mixture in physiological doses its effect, as soon as it is felt at all, is to lengthen the period of diastole or as one might say to increase the duration of the refractory phase. Records of contractions of heart strips obtained in Ringer's mixture of the composition used in this laboratory (NaCl 0.7 per cent—$CaCl_2$ 0.026 per cent—KCl 0.03 per cent) are characterized by a certain irregularity in rhythm; pauses of longer or shorter duration are intercalated between single beats or between groups of beats.

An attempt has been made to determine how completely this influence of KCl on the rate of beat can be controlled experimentally. For this purpose ventricular strips were chosen that had been exhausted by an immersion for three or more hours in NaCl 0.7 per cent and subsequent immersion for an hour or more in sugar solutions. Under these conditions the strip must be practically deprived of its diffusible Na, Ca, and K, but, as has been pointed out, is in an excellent condition to give spontaneous contractions in appropriate mixtures. In one such experiment the strip had been transferred to a Ringer's mixture (NaCl 0.7 per cent, $CaCl_2$ 0.024 per cent, KCl 0.024 per cent) and had been beating well for about eighteen hours. It was then placed alternately in a solution containing only NaCl 0.7 per cent and $CaCl_2$ 0.024 per cent, and in the mixture containing KCl in which it had been beating. In the solution without K the rate was very regular, ten to eleven contractions a minute, while in the solution containing K the rate was much slower, the beats for a time occurring at the rate of five per minute, but finally becoming slower, two per minute, and eventually only two in ten minutes. Changing back to the solution without K the beats within one half minute again returned to the previous rapid regular rhythm. In another experiment the

strip after a similar preparation was placed in a Ringer's mixture (NaCl 0.7 per cent, $CaCl_2$ 0.024 per cent, KCl 0.024 per cent) in which it beat quite regularly at the rate of from twenty to twenty-two beats per minute. KCl was then added to the solution, which consisted of 25 c.c. of the above mixture. Successive additions were made of $\frac{1}{10}$ c.c. of a 1 per cent solution of KCl. The effect upon the rhythm was marked, but it was difficult to hold the strip to a constant rate. That is, the addition of a certain amount of KCl would be followed by a slower beat, but in a little while this effect would pass off and the strip would beat at its former rapid rate. Addition of more KCl would again slow the rhythm, but if too much had been added the strip would gradually pass into a condition of complete inhibition, from which it could quickly be brought back to a rapid beat by changing to the original solution.

In this and in other experiments it was found that increasing the amount of K was followed at a certain point by a more or less sudden cessation of the spontaneous beats. While in this state of inhibition the strip could be made to beat again, either by adding more $CaCl_2$ to the solution or by removing some of the KCl, that is, by changing to a solution containing less KCl; but the latter method always gave a more rapid and regular rhythm than the former. In other words, after sufficient KCl had been added to bring about complete inhibition, increasing the $CaCl_2$ in proportion never completely antagonized the potassium effect. I was not able, therefore, to corroborate fully the statement made by Ringer that the effect of a toxic dose of KCl may be completely removed by adding what, if taken alone, would be a toxic dose of $CaCl_2$. In my experiments the antagonism between the Ca and the K was incomplete, excessive doses of the one not being entirely neutralized by increasing the proportion of the other. The experiments, so far as they go, seem to indicate that there is for each ventricular strip a certain amount of KCl which almost or completely inhibits the automatic contractions in the presence of Ca and Na. This dose of potassium varies somewhat with strips from different hearts or with the same strip under different conditions.

If the amount of potassium salt added is not too great this inhibition is not toxic in character, that is, the tissue is not injured. On the contrary, the strip readily beats again when transferred to a mixture containing less potassium, its condition in fact resembling very much

that of a fresh ventricular strip when immersed in the animal's own serum. In serum, as I have described in a previous paper, the strip usually remains quiet but irritable to artificial stimulation, and it may be made to beat spontaneously by diluting the serum with a 0.7 per cent solution of NaCl, or in some cases by increasing the amount of calcium by the addition of a little of a solution of $CaCl_2$. The latter method is never so effective as the former or the transference directly to a 0.7 per cent solution of NaCl, and in the light of the present series of experiments I should explain the more favorable effect of the latter procedure by the resulting diminution in the percentage of potassium salts.

The NaCl series of beats. — The fundamental phenomenon in all the experiments upon the action of the inorganic salts upon the heart rhythm is the series of beats obtained by immersion of fresh strips in 0.7 per cent solutions of NaCl. It will be remembered that this series shows first an increase to a maximum and then a gradual and very regular diminution to zero. The general effect of NaCl solutions was described by Merunowicz, Aubert, and others, and more recently by Greene. In a former paper I suggested that this effect of NaCl solutions was explained by the fact that under these conditions both the potassium and the calcium salts diffuse out of the strip into the surrounding bath and that the diffusion of the potassium takes place more rapidly, so that the antagonistic influence between the potassium and calcium is removed by a relative preponderance of the latter, the strip eventually ceasing to beat when the dissociable calcium is completely removed or very much reduced in amount.

While still holding to this general explanation, I should be inclined now to give the following theory of the cause and course of this series of beats. When the strip is placed in the solution of 0.7 per cent NaCl, both the potassium and calcium ions begin to diffuse out, and we may suppose that the former pass out more rapidly and completely. After the potassium contents of the strip are sufficiently reduced its inhibitory influence is weakened and under the combined influence of the Na and the Ca still present the strip gives its automatic contractions, which, under the conditions assumed, would naturally increase to a maximum and then slowly decrease as the Ca diffused out, the strip, as the foregoing experiments have indicated, being unable to contract spontaneously in the presence of sodium alone.

Loeb has given a different explanation of this series of NaCl beats.

To quote Lingle, who has applied his views to the ventricular muscle, "the sodium ions act by migrating into the muscle substance and combining with some part of it. And hence when too many sodium ions have combined and taken the place of a number of Ca ions in the muscle, rhythmic beats cease." That is, the series is started by the action of Na ions and subsequently suspended by the toxic action of the same ions when the Ca has been completely, or almost completely, displaced. The cessation of the series of beats, according to Loeb's view, is due directly to the poisonous effect of the NaCl; according to my view, to the loss of Ca, which forms a necessary factor in the production of the contractions.

With regard to the first part of Loeb's theory, that the initiation of the series of beats is due to an excess of Na ions migrating into the strip and replacing the Ca ions, it is to be borne in mind that when the strip is placed in a solution containing both NaCl and $CaCl_2$ it beats as well as, or indeed much better than in a solution of NaCl alone. This fact would seem to contradict the view that the replacement of the Ca in the tissue by the Na is the necessary factor in starting the contractions, while it supports my hypothesis that the removal of the excess of potassium by diffusion is what really liberates the tissue and permits it to beat under the combined influence of the Na and Ca. It is quite certain that when the strip is immersed directly in a solution containing the Ca, Na, and K in the proportions present in serum it does not beat, as a rule, and that a proper mixture without the potassium gives rise to a series of beats.

As for the view that the gradual falling off of the series of beats in NaCl and their final disappearance is due to a poisonous effect of the Na ions, it would seem, if I interpret their views correctly, that Loeb and Lingle believe that this toxic action is exhibited only in the absence or great reduction in number of the Ca ions, and that only as long as Ca ions are present do contractions occur. Looked at objectively, this way of stating the matter amounts to the same thing as saying that the Ca is necessary to the rhythmic contractions, a view in which I heartily concur.

The action of potassium salts and inhibition. — The especial effect of potassium salts upon the heart has been known since the pioneer work of Bernard, and has been made the subject of investigation by a number of observers. Bottazzi [1] particularly has described the effect

[1] Bottazzi: Archives de physiologie normale et pathologique, 1896, p. 882.

of potassium salts upon the heart of cold-blooded animals. Like Ranke, Ringer and others, he has been impressed by the inhibitory character of the standstill produced by these salts. So much so, in fact, that he compares their action directly with that produced by stimulation of the inhibitory fibres of the vagus, and suggests that the action of the two are not only superficially alike, but fundamentally identical. Adopting the hypothesis of Gaskell and Fano that inhibition is an expression of anabolic processes in the heart tissue, he assumes that the potassium salts also bring about cessation of contractions by augmenting the anabolic processes.

There are no facts, so far as I know, that give any real support to this hypothesis, but it is somewhat interesting to recall how many physiologists (Aubert, Ranke, Fano, Meltzer, Bottazzi), have been led to suggest in one form or another that the normal diastole of the heart muscle is at bottom a phenomenon of inhibition. To the present writer it seems unnecessary to assume that the standstill produced by a slight excess of K salts is due to an augmented anabolism. If the properties of the proteids in the heart tissue are dependent upon their union with the inorganic salts of the blood and lymph, a preferable hypothesis would be that the compounds with potassium possess too great a stability to undergo that decomposition which we suppose to be the origin of the liberation of energy in a contraction.

An interesting point with regard to the potassium inhibition, if the phrase may be allowed, is that the heart tissue is not only incapable of spontaneous contraction, but, according to those who have tested the matter (Ringer-Bottazzi) has lost its irritability toward electrical stimuli. In this respect there would seem to be a difference between the standstill produced by potassium salts and vagus inhibition, but the difference may be one of degree only. While the heart inhibited through the vagus is usually irritable toward electrical stimulation, yet, according to Schiff and Eckhard,[1] if the inhibitory stimulus is sufficiently strong this irritability is lacking. On the other hand, although a dose of KCl strong enough to stop the whole heart may remove the irritability of the ventricle toward electrical stimulation it is possible that the dose might be so graduated as to stop spontaneous contractions without loss of irritability to artificial stimuli. If the views advocated in this paper are correct such a condition of affairs exists normally in the living ventricle, the proportion of potassium salts in the blood being sufficient to suspend automatic contractility

[1] Quoted from TIGERSTEDT'S Physiologie des Kreislaufes, 1893, p. 254.

in this part of the heart without destroying its irritability toward artificial stimuli.

The analogy or relationship between potassium standstill and vagus inhibition is very suggestive and need not be complicated by any assumption as to the cause of inhibition. The very potent influence of the kations Ca, K, and Na upon the liberation of spontaneous contractions suggests furthermore the possibility that the opposing influence of inhibitory and augmentor nerve impulses on the heart's contractions may eventually be traced to an influence upon the relations of these elements to the living proteids of the heart tissue.

Summary.

The chief conclusions arrived at in this paper may be stated briefly as follows:

1. Spontaneous contractions of the ventricular muscle of the terrapin's heart are dependent upon the presence in the tissue of dissociable compounds of both calcium and sodium. If either the Ca or the Na is absent automatic contractions are impossible.

2. Sodium salts (NaCl) in the liquid surrounding the heart tissue tend to produce a relaxation from loss of tone. Calcium chloride on the contrary causes a shortening from increase of tone which may pass into a permanent rigor. Potassium chloride exhibits an antagonistic effect toward the action of calcium chloride, but only to a marked extent when sodium salts are also present in approximately normal proportions.

3. The influence of the potassium ion when present in physiological proportions is shown by a slowing of the rhythm or a lengthening of the refractory phase. Under the combined influence of Ca, Na, and K the automatic contractility is maintained for longer periods than by the action of Na and Ca alone.

4. The fact that ventricular strips do not contract spontaneously as a rule in the animal's own serum or in an equivalent Ringer's mixture is due to the inhibitory influence of the potassium salts.

5. The characteristic series of beats obtained by immersing a fresh strip of ventricle in a 0.7 per cent solution of NaCl is referable to the following changes. *a.* The latent period and the beginning of the series of spontaneous beats is due to the gradual loss of dissociable potassium from the strip by diffusion. *b.* The gradual diminution and final disappearance of the beats is due to the loss of the dissociable calcium by diffusion.

THE ACTION OF PILOCARPINE AND ATROPINE ON THE EMBRYOS OF THE STAR-FISH AND THE SEA-URCHIN.

By ALBERT P. MATHEWS.

[*From the Marine Biological Laboratory, Woods Holl, Mass.*]

THE work of Heidenhain and Langley has seemed to show that pilocarpine and atropine do not affect gland-cells, but only the endings of the hypothetical secretory nerves. The chief evidence of this is the observation that atropine stops the secretion which normally occurs in the submaxillary gland of the dog upon stimulation of the chorda tympani, but not that which follows stimulation of the cervical sympathetic. This fact indicates either that the secreting cells are not poisoned by atropine or that the sympathetic causes secretion in some other way than by acting on these cells. There is no evidence except in the salivary glands that atropine has not poisoned the secreting cells, for in no other glands have two secretory nerves been found, one of which is paralyzed by the drug, while the other is not affected.

The possibility that the sympathetic produces its secretion by acting in some other way than upon the secreting cells has not recently received the attention which it deserves. Langley, in Schaefer's text-book, dismisses it as improbable. The alternative has been so generally accepted that if atropine paralyzes a gland's secretion it is considered strong evidence of the existence of secretory nerves in this organ. In spite of the general acceptance of the view just stated that the sympathetic acts directly on the secreting cells, there is reason for doubting whether this is the case. I have elsewhere put together evidence pointing to the action of the nerve on contractile tissue in the gland. There appears to me to be no difficulty in understanding all the phenomena of the sympathetic secretion on this hypothesis, and several facts exist which are, I think, incompatible with any other explanation. The presence in the salivary glands of contractile cells corresponding to the contractile sheath about the sweat glands is now abundantly demonstrated. These, the so-called "basket-cells," closely resemble the similarly situated cells about the salivary glands of the molluscs, and in these animals their contraction

has been observed under the microscope by Saint-Hilaire.[1] Miss Hyde's[2] work, also, on the physiology of the salivary glands of Cephalopods leaves no doubt that these glands contract and thus expel most, if not all, of the secretion observed upon nerve stimulation. Furthermore, suprarenal extract, which causes marked contraction of smooth muscle, causes a secretion from the salivary glands, but not, so far as I have observed, from the pancreas, where contractile cells are absent. These facts, showing a probability that the sympathetic does not cause secretion by acting on gland cells, undermines the main evidence that atropine does not act on the gland cells. The meaning of the fact observed by Bunch[3] that stimulation of the sympathetic may cause a slight preliminary increase in volume of the submaxillary gland is not sufficiently clear to offset nearly all other phenomena of sympathetic secretion, which point clearly to a contraction of the gland upon sympathetic stimulation.

The following objections may also be advanced against the assumption that atropine does not act on these cells. The same argument applies, for instance, to quinine. Quinine, if injected into the duct, paralyzes the chorda secretion but not the sympathetic. If the sympathetic produces its secretion by action on the gland cells this demonstrates that the chorda is paralyzed at a time when the gland cells are not paralyzed, for the sympathetic is then still active and there is no reason for believing that the gland-cells connected with the sympathetic differ from those connected with the chorda. We are hence driven to the very improbable conclusion that a general protoplasmic poison, such as quinine, does not act on the cells of the salivary glands, but only on the ends of the nerves, and particularly the ends of the chorda secretory fibres. The inhibitory action of atropine on the spontaneous secretion of saliva is also hard to understand on the nerve-end hypothesis.[4] There are also well substantiated observations that in cats in which the sciatic nerve has been severed for two weeks, pilocarpine will still cause sweat secretion in the hind foot, thus indicating that the drug acts in part at least on the gland cells.

These facts made it desirable to test the action of the drugs on embryos in which there are no nerve cells. I used embryos of the

[1] Saint-Hilaire: Anatomischer Anzeiger, xix, 1901, p. 478.
[2] Hyde, Ida H.: Zeitschrift für Biologie, 35, 1897, p. 459.
[3] Bunch: Journal of physiology, 1900, xxvi, p. 1.
[4] Mathews: This journal, iv, 1901, p. 483.

star-fish, Asterias Forbesii, and the sea-urchin, Arbacia punctulata. The eggs were transferred immediately after fertilization into 100 cubic centimetres of sea-water to which had been added from one tenth of a cubic centimetre to two cubic centimetres of a one half per cent solution of pilocarpine hydrochlorate or atropine sulphate. They remained in these solutions during development. The drugs were dissolved either in sea-water or fresh water, the result being the same in either case. The eggs from each female were kept separate and were invariably compared with the control eggs in sea-water.

Following are the protocols of some of the experiments:

No. of experiment.	Time.	No. of c.c. of ½% pilocarpine added to 100 c.c. of sea-water.			Normal.	No. of c.c. atropine sulph. ½% added to 100 c.c. sea-water.		
		1 c.c.	0.5 c.c.	0.3 c.c.		0.3 c.c.	0.5 c.c.	1 c.c.
14 Asterias. Fertilized August 3, 3.30 P. M.	August 4, 9 A. M.	Large, active gastrulæ. All alive and actively swimming.	Gastrulation well advanced.	Smaller than preceding. Gastrulation not so advanced.	Free swimming blastulæ. Invagination just beginning.	Blastulæ. A portion only beginning to invaginate.	Blastulæ just beginning to swim. Opaque and small.	All dead.
	August 5, 9 A. M.	Changing to bipinnaria.	About the same as preceding.	....	Gastrulæ.	Gastrulæ.	Gastrulæ smaller than normal.	
	August 7, 9 A. M.	Very large bipinnaria.	Bipinnaria twice as large as the atropine specimens and half again as large as normal.	Large bipinnaria.	Bipinnaria, but much smaller than pilocarpinized.	Gastrulæ nearly bipinnaria but small and opaque.	Gastrulæ smaller than preceding.	

No. of experiment.	Time.	No. of c.c. of ½% pilocarpine added to 100 c.c. of sea-water.			Normal.	No. of c.c. of ½% atropine added to 100 c.c. sea-water.		
		1 c.c.	0.5 c.c.	0.3 c.c.		0.2 c.c.	0.5 c.c.	1 c.c.
100 Asterias. Fertilized Aug. 14, 4 P. M.	Aug. 15, 2 P. M.	Very large gastrulæ. Far advanced.	Large gastrulæ. Far advanced.	Gastrulæ smaller than preceding.	Gastrulation begun. Many abnormal and smaller than pilocarpinized.	Gastrulæ smaller and more opaque than normal.	Blastulæ. A few just beginning to invaginate. Small and opaque	Blastulæ. No invagination. Opaque and very small.
	Aug. 16, 2 P. M.	Enormously large and transparent bipinnaria, all swimming. Measured by eye-piece micrometer. Average size: 384 μ long × 208 μ wide.	Large, transparent bipinnaria. Most are swimming but some are at bottom. 320 μ × 176 μ.	Some bipinnaria, but most are gastrulæ at bottom of the dish. Many are opaque. 240 μ × 160 μ.	Most are abnormal gastrulæ at the bottom of the dish. Some are bipinnaria much smaller than the pilocarpine embryos. 224 μ × 160 μ.	Gastrulæ opaque and small. Nearly all at the bottom. 192 μ × 128 μ.	Gastrulation just beginning. Small and opaque at bottom of the dish. 80 μ × 160 μ.	Blastulæ. All at bottom. Small and opaque. 80 μ × 160 μ.
	Aug. 18.	Large, living bipinnaria.	Dead.	Large, clear bipinnaria.	Dead.	Small, opaque ill-formed bipinnaria.	Dead.	Dead.
269 Fertilized Aug. 22, 11.45 A. M.	Aug. 23, 11 A. M.	Large, clear gastrulæ. Larger than normal.	Gastrulæ larger than normal.	....	Gastrulation just beginning.	....	Blastulæ. A few gastrulæ.	Dwarf blastulæ.
	Aug. 24, 9 A. M.	Large, swimming bipinnaria, smaller than the next. Average size: 297.6 μ × 167.8 μ.	Largest, clear bipinnaria. 332.8 μ × 176 μ.	....	Gastrulæ. Bipinnaria stage just beginning. Many dead. 283 μ × 160 μ.	....	Gastrulæ. No bipinnaria. All living. 212.8 μ × 156.8 μ.	Dwarf swimming blastulæ. 126.4 μ × 126 μ.

No. of experiment.	Time.	No. of c.c. of ½% pilocarpine added to 100 c.c. of sea-water.		Normal.	No. of c.c. of ½% atropine added to 100 c.c. of sea-water.	
		2 c.c.	1 c.c.		1 c.c.	2 c.c.
297 Arbacia. Fertilized Aug. 24, at 10 A. M.	August 24, 10.50 A. M.	2 cells.	2 cells.	2 cells.	2 cells.	2 cells.
	3.15 P. M.	A few swimming.	Few swimming.	Few swimming.	None swimming.	None swimming.
	3.25 P. M.	Almost all swimming.	Almost all swimming.	Almost all swimming.	Few swimming.	Few swimming.
	3.37 P. M.	All swimming.	All swimming.	All swimming.	About ⅓ swimming.	About ⅕ swimming.
	4.50 P. M.		More are swimming near top than in any other dish.			
	August 25, 8 A. M.	Plutei. Average size of longest arm : 120 μ. Average greatest length : 169.2 μ.	Plutei. 124 μ.	Plutei. 116.4 μ.	Plutei just beginning. 109.2 μ.	Beginning to form plutei. 91.2 μ.
	3 P. M.		170.4 μ.	170.4 μ.	145.2 μ.	116.4 μ.
	August 26, 9 A. M.	238.0 μ.	209.8 μ.	220.8 μ.	175.2 μ.	167 μ.
21 Arbacia. Fertilized Aug. 3, at 12.30 P. M.	August 4, 9 A. M.	Plutei.	....	Beginning to change to plutei.	....	Small gastrulæ, just beginning to change to plutei.
	August 7, 9 A. M.	Large plutei. All alive.	....	All dead.	....	Small opaque plutei and gastrulæ.

The foregoing experiments show that the addition of small amounts of pilocarpine to sea-water hastens the development of the embryos of Asterias and increases their size above normal; that the addition of atropine delays development both of Asterias and Arbacia and gives rise to dwarf embryos. In all experiments on Asterias, well developed gastrulae were found among the pilocarpinized embryos at a time when the control larvæ were just beginning to invaginate and the atropinized larvæ were still blastulæ. The control embryos were always intermediate in size between the pilocarpinized embryos and those in atropine. On the sea-urchin, pilocarpine generally has little action except that in large doses it slows development, but occasionally small doses accelerate development. I believe the action of the drug is less obvious in Arbacia because the normal development is already exceedingly rapid and the early formation of the skeleton interferes with the determination of any change in bulk. The measurements cited express but poorly the effect of the pilocarpine on the developing starfish. The pilocarpine embryos in some instances appeared fully twice as large as the control and were more transparent, implying a bulk almost eight times the normal.

The experiments just cited show beyond doubt, I think, that these drugs act directly on cells and that they are not confined in their activity to highly specialized protoplasm such as the hypothetical secretory nerve ends.

In their action on development these drugs closely parallel the effects of hydrogen and hydroxyl ions as already observed by Loeb.[1] Loeb found that a slight increase of hydroxyl ions hastened Arbacia's development, while hydrogen ions delayed it. He interpreted this to mean that in the former case the oxidative syntheses of the protoplasm were increased, in the latter diminished.

This explanation appears probable and implies that pilocarpine, like the hydroxyl ions, hastens oxidation in protoplasm, while atropine, like the hydrogen ion, diminishes it. There are facts which seem to me to bear out this hypothesis. For example, if oxygenated blood is cut off for a short period from the submaxillary gland, the kidney, or the pancreas, the secretion from these organs is temporarily interrupted for some time after the circulation is re-established. The glands act somewhat as if poisoned by atropine. Or if hydrochloric acid is injected into the duct of the salivary glands secretion is also stopped.

[1] Loeb: Archiv für Entwickelungsmechanik der Organismen, 1898, vii, p. 631.

Barcroft's[1] very recently published observation that the atropinized submaxillary gland of the dog no longer takes up oxygen, although vasodilation occurs on stimulation of the chorda tympani nerve, will also bear the interpretation just given, namely, that the drug interferes with the oxidative processes.

In place of the theory that these drugs act on the hypothetical secretory-nerve endings, the following hypothesis is suggested by the facts just cited.

Pilocarpine causes secretion by increasing the oxidative decompositions in the secreting cells, thus increasing the number of molecules, diminishing their size, increasing the osmotic pressure and causing thus the imbibition of water. This increases the turgor of the cell beyond the resisting point of its inner end and the secretion flows out. Possibly it may also cause contraction of the basket cells.

Atropine acts on the gland cell in an opposite manner. It stops the oxidative processes so far that even though vasodilation occur and an abnormal supply of oxygen be present, the oxidations do not occur, the osmotic pressure of the cell is not increased and the cell does not secrete at all, or only at a slow rate. Atropine acts also, probably, on the nerve cells, or interrupts connection between successive neurons. And, further, it may prevent in some manner the action of the chorda on the basket-cells, assuming such action to exist.

When it is remembered that the chief reason for supposing that atropine does not act on the gland cells is a possibly erroneous interpretation of the method of action of the cervical sympathetic nerve in the dog, it appears to me that the facts presented in this paper showing that the drug certainly acts on cellular beings having no nerves at all and the facts already cited in the introduction and in my paper on spontaneous salivary secretions, deprive this evidence of secretory nerves to the gland cells of most of its value.

As in the abstract of my paper on the spontaneous secretion of saliva published in the Centralblatt für Physiologie[2] the author apparently misunderstood my explanation of the chorda secretion, I would like to repeat that explanation here. The facts of the chorda secretion appear to me explicable on the assumption that the chorda innervates some of the basket-cells of the gland, while the sympathetic innervates the others. When the chorda is stimulated a flow of saliva

[1] BARCROFT: Journal of physiology, 1901, xxvii, p. 31.

[2] Centralblatt für Physiologie, 1901, xv, p. 17.

is produced in two ways: first, by the contraction of the basket-cells, and second, by the results of vasodilation. The former action accounts for the chorda secretion in the absence of blood supply, the diminution in volume which the gland undergoes during stimulation even though vasodilation takes place, and the fact that post-mortem secretion is observed only in glands with contractile tissue. By its vasodilator action, on the other hand, the nerve suddenly overwhelms the cells with oxygen and the oxidative decompositions cause an increase in osmotic pressure and secretion. This causes the flow of more limpid saliva, accounts for the difference in composition of chorda and sympathetic saliva and explains the fact that in almost all glands vasodilation accompanies secretion. On this hypothesis atropine would paralyze the chorda secretion not because it paralyzes the endings of the secretory nerves, but because it prevents the oxidations which normally occur when the cells are overwhelmed with oxygen. It may be asked why the drug paralyzes the chorda before the sympathetic. If the hypothesis just stated is true, the most probable explanation which occurs to me is that atropine acts on the relay between the chorda and the neurons in the gland itself or in some other way interrupts the connection between the chorda and the basket-cells. The sympathetic has no relay. The only evidence in favor of this suggestion which occurs to me is the fact that in the cat, in which the sympathetic is paralyzed by atropine though somewhat later than the chorda, Langley has shown that the drug certainly acts on the relay of the sympathetic in the superior cervical ganglion. He found that stimulation of the nerve below the ganglion was ineffective after atropine, whereas beyond the ganglion the nerve was still active. Relaying in the gland the action of the chorda on the basket-cells is lost, while that of the sympathetic is not. This hypothesis, however, necessitates the further assumption that the relay of the chorda in the gland is more sensitive than that of the sympathetic in the cervical ganglion. It is possible that the greater blood supply of the former might account for its early poisoning.

Summary.

1. Atropine sulphate in small doses hinders the development of Arbacia and Asterias larvæ and gives rise to dwarf embryos.

2. Pilocarpine hydrochlorate hastens development of Asterias larvæ and gives rise to abnormally large embryos.

3. This action of atropine resembles that of hydrogen ions; the action of pilocarpine that of hydroxyl ions.

4. Hence atropine and pilocarpine act on animal cells directly and they are not confined in their action to secretory nerve ends.

5. The nature of their action suggests that atropine inhibits the oxidations taking place in the cells, while pilocarpine increases those oxidations.

6. Atropine may stop secretion by checking the oxidative decompositions in protoplasm so that there is no normal increase in osmotic pressure during vasodilation and secretion does not occur. Pilocarpine by hastening these decompositions increases osmotic pressure and thus secretion. Both drugs probably act directly on the gland cells. The evidence that they do not act on secretory cells, but on the ends of the hypothetical secretory nerves is insufficient.

THE SO-CALLED CROSS FERTILIZATION OF ASTERIAS BY ARBACIA.

By ALBERT P. MATHEWS.

[*From the Marine Biological Laboratory, Woods Holl, Mass.*]

IN 1893 Morgan[1] described what he believed to be the fertilization of the eggs of the star fish, Asterias Forbesii, by the sperm of the sea-urchin, Arbacia punctulata. The eggs were vigorously attacked by the sea-urchin sperm, the sperm entered and a few eggs developed into abnormal-looking blastulæ and gastrulæ, which lived for forty-eight hours. These results were so interesting that I determined to repeat them, particularly as the more recently acquired knowledge of the ease with which parthenogenetic development may be started renders it not impossible that the larvæ described may have been parthenogenetic. Moreover, it has been recently affirmed by Von Dungern [2] that the eggs of Asterias kill sea-urchin sperm.

My experiments have entirely corroborated Morgan's observations, with the possible exception of the penetration of the sperm into the egg; but I believe they show that the resulting larvæ are not due to the fertilization of the egg by the sperm, but are parthenogenetic in consequence of agitation of the eggs by the experimenter. In a preceding number of this journal,[3] it was shown how easily slight agitation of the eggs, such as occurs in squirting them into sea-water or mixing them with the sperm with a pipette, will start parthenogenetic development. The gastrulæ observed by Morgan were, in my opinion, in all probability parthenogenetic gastrulæ produced by agitation, but I do not wish to affirm absolutely that the impregnation of the Asterias eggs by Arbacia sperm may not under other circumstances occur. I have not been able to get entirely ripe female star-fish and it is possible that their eggs would be more easily entered. But as Morgan worked on the same star-fish at about the same period of the year my results ought to be comparable with his. The experi-

[1] MORGAN: Anatomischer Anzeiger, 1893, ix, p. 141.

[2] VON DUNGERN: Centralblatt für physiologie, 1901, xv, p. 1.

[3] MATHEWS: This journal, 1901, vi, p. 142.

ments were performed with the care already described to prevent any infection with star-fish sperm.

Series I. The eggs were mixed with Arbacia sperm before maturation was complete and were allowed to remain quiet after mixing.

In twenty-five experiments extending from July 31 to August 19 on the eggs from fourteen different star-fish, the result in every case but one was negative. In this experiment one embryo was found in two thousand eggs from a lot which had been shaken vigorously the day before.

Series II. The eggs after maturation was far advanced or complete, were transferred with great care to the water containing Arbacia sperm and were then kept undisturbed. Twelve experiments yielded no embryos. In one experiment in which the eggs were transferred after they had remained four hours in sea-water, two swimming blastulæ were found the next morning in two thousand eggs.

Series III. The eggs were transferred carelessly or after intentional agitation, to sea-water with Arbacia sperm after maturation was complete.

Experiments 49 and 50. A few abnormal swimming blastulae develop, while many eggs begin segmentation.

Experiment 52. Eggs shaken first. Several ciliated blastulae found in each pipette full of eggs. The control eggs shaken, but not exposed to fertilization, show a similar number of embryos.

Experiments 62, 111, 117, 188. A few swimming blastulae develop in each.

Experiment 63. No development.

Series IV. Each lot of eggs was divided into two portions. Both were shaken, or transferred carelessly or carefully. One was mixed with Arbacia sperm, the other not.

See also Table on page 218.

The foregoing experiments show: first, that if the eggs are mixed with Arbacia sperm before maturation is far advanced, embryos do not develop either with or without agitation ; second, that if the eggs are gently mixed with the sperm after maturation is complete embryos are hardly ever obtained ; and third, that careless transference or intentional shaking of the eggs after maturation is complete almost always causes the development of embryos, but the number obtained from the eggs mixed with the sperm is no larger than that obtained from the eggs without sperm.

These facts lead to the conclusion that no true crossing or fertilization occurred and that Morgan's embryos were really parthenogenetic and not crosses. To what extent other reported crossings may ultimately be shown to be of the same nature cannot of course be

said, but this source of error has not been hitherto guarded against. I was unable to satisfy myself by examination of the living egg whether the Arbacia sperm really enter the star-fish egg as they

No. of exp.		No. of embryos in 1500 eggs when						
		Transferred carefully.	Squirted into water.	Squirted twice.	Squirted four times.	Squirted eight times.	Squirted sixteen times.	Shaken hard.
158	With sperm	0	0	..	18	17	14	
	Without sperm	0	0	0	12	23	24	107
170	With sperm	0	0	2	4	14	..	0
	Without sperm	0	0	3	12	20	6	0
205	With sperm	1	..	6	4	4		
	Without sperm	2	17	8	15	10		
231	With sperm	1	1	0	1	15	..	12
	Without sperm	1	0	0	1	7	..	7
287	With sperm	0	..	..	..	..	..	750
	Without sperm	0	..	..	..	..	..	750

appear to do, or whether they merely stick to the outer surface. It appeared in some instances as if fertilization membranes were thrown out in larger numbers in the eggs exposed to the sperm, than in those to which no sperm was added, but no more embryos developed in the one case than in the other. No other physiological evidence of the penetration of the sperm was observed.

NOTE. — In my paper entitled "Artificial Parthenogenesis by Mechanical Agitation," published in this journal, vi, p. 150, Nereis was placed by a regrettable oversight among the forms in which Loeb and Fischer secured development by agitation. In Chætopterus the development is apparently dependent on the length of time the eggs have been in the sea-water.

THE CHEMICAL CONSTITUENTS OF TENDINOUS TISSUE.[1]

By LEO BUERGER and WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

IN a previous paper from this laboratory[2] the results were given of some analyses of yellow elastic tissue, represented by the ligamentum nuchæ of the ox and calf. So little attention has been given by chemists to structures such as tendon, which possess mainly mechanical functions, that it seemed to us desirable to investigate in a similar study the general composition of white fibrous connective tissue.

Historical.

Early in the last century, when it was assumed that elementary composition determined not only definite chemical relationships, but indicated similarities and differences in development as well as function, the tissues were carefully subjected to elementary analysis. Like a number of the other parts of the body, tendon, in the fresh condition, was looked upon as consisting of practically a single organic substance (collagen) holding water mechanically, and admixed with slight quantities of saline matter and other blood and lymph constituents.[3]

Scherer[4] analyzed several forms of gelatin-yielding fibrous tissues. On the next page we give the results of his elementary analysis of calf-tendon. The tissue was prepared for analysis by preliminary maceration and extraction in dilute saline solution. Subsequently the residue was washed in water and then in boiling alcohol and ether. To this residue, "collagen," Scherer ascribed the formula $C_{48}H_{82}N_{15}O_{18}$.

[1] Some of these results were given at the New York meeting of the American Association for the Advancement of Science, June, 1900: Proceedings, 1900, p. 123.

[2] Vandegrift and Gies: This journal, 1901, v, p. 287.

[3] See references to collagen content on page 230.

[4] Scherer: Annalen der Chemie und Pharmacie, 1841, xl. p. 46.

Marchand,[1] who pointed out a number of defects in Scherer's work, subjected dried tendons from the foot of the calf to similar analysis. The results given below for ash-free substance led him to ascribe to this "collagen" the formula $C_{40}H_{62}N_{12}O_{15}$. He also calculated its molecular weight from this formula, expressing it with the figures 5937.5. The composition of the ash-free hydrated tendon ("gelatin"), taken from the same source, was found by Marchand to accord very well with the average analytic results of similar products, from bone and other tissues, obtained by Mulder.[2] The latter observer gave the gelatin-yielding tissues (dry) the formula $C_{13}H_{20}N_4O_5$.

Winkler's[3] analysis of the tendon of the cow, after extraction in cold water and later in boiling alcohol and ether, led to similar results.

The following summary gives the analytic averages referred to above:[4]

		C	H	N	O
Scherer.	Crude tendon collagen	50.51	7.16	18.37	23.96
Marchand.	Dry calf tendon	50.27	6.77	17.88	25.08
Marchand.	Crude tendon gelatin	50.02	6.82	18.00	25.16
Mulder.	Crude bone gelatin	50.37	6.33	17.95	25.35
Winkler.	Crude tendon collagen	49.68	6.64	17.94	25.74
	Average	50.17	6.74	18.03	25.06

These close agreements in analytic figures naturally suggested to the earlier observers that the chief organic substance of bone, tendon, and related forms was the same in each; further, that "gelatin" and "collagen" were very nearly if not altogether isomeric.[5] In the light of modern chemical knowledge, however, these analytic harmonies emphasize the lack of information which elementary analysis of tissues furnished on the characters and qualities of the various constituents. Definite separation of the tissue-forming substances, however, and subsequent detailed analysis of them individually has increased our appreciation of the important parts the numerous constituents of the body play in the maintenance of its functions.

[1] Marchand: Lehrbuch der physiologischen Chemie, 1844, p. 166.

[2] Mulder: Versuch einer allgemeinen physiologischen Chemie, erste Hälfte, 1844–51, p. 333.

[3] Winkler: Quoted by Mulder, *loc. cit.*, zweite Hälfte, p. 583.

[4] The small amounts of phosphorus and sulphur detected in these substances at this time were attributed to inorganic impurity. Oxygen was calculated by difference, and the figures for it therefore include organic phosphorus and sulphur.

[5] Hofmeister has since shown, and it is now generally understood, that gelatin is the hydrate of collagen: Zeitschrift für physiologische Chemie, 1878–79, ii. p. 299.

Aside from the above elementary analyses, and a few others of similar character in close agreement with them,[1] practically nothing has been done to determine quantitatively the composition of tendinous tissue. Several observers have determined the proportion of ash.[2] Gorup-Besanez[3] states that a few determinations of water and solid matter in connective tissues, containing collaginous fibres in abundance, have been made, which show a variable content of water ranging between 57.5 and 78.9 per cent of the fresh tissue.[4] Beaunis, in the table presented by Halliburton,[5] gives the average proportion of water in "connective tissue" as 79.6 per cent; but this does not refer to tendon.[6]

Analyses of Tendo Achillis.

Material and methods of analysis. — In the work described in this paper the Achilles tendons of the ox and the calf were employed. The Achilles tendon is easily separated from extraneous matter. It is more completely collaginous and contains relatively less elastin than is found in any other tendinous tissue available for such work. It may be regarded as the best representative of white fibrous connective tissues.

This research followed so closely the plan of our previous study[7] that it is needless to describe in detail the methods of analysis. The details of procedure not mentioned here may be understood to correspond with those given by Vandegrift and Gies.

The main shaft of the tendon was used in each experiment. Occasionally small portions of the bifurcations were employed with parts of the former.[8] Only perfectly white tendons were analyzed. Any tendons showing bloody lines superficially or internally were rejected. Usually the tendons were rapidly cut into very thin cross sections of

[1] Gorup-Besanez: Lehrbuch der physiologischen Chemie, 1878, p. 142.

[2] See page 223. Also foot-note, page 225.

[3] Gorup-Besanez: *Loc. cit.*, p. 649.

[4] See Chevreul's results; given by Marchand: *Loc. cit.*, p. 164.

[5] Halliburton: Text-book of chemical physiology and pathology, 1891, p. 58.

[6] Results of analyses of various non-tendinous tissues containing collaginous fibres, such as the cornea, are not strictly comparable in this connection and are therefore not given here.

[7] Vandegrift and Gies: *Loc. cit.*

[8] See Cutter and Gies: This journal, 1901, vi, p. 157.

GENERAL COMPOSITION.

No.	Tendon used.	Percentage of fresh tissue.				Percentage of solids.	
	Grams.	Water.	Solid matter.			Organic matter.	Inorganic matter.
			Total.	Organic.	Inorganic.		
Ox Tendon.							
1	5.03	61.55	38.45	37.97	0.48	98.74	1.26
2	7.05	63.20	36.80	36.20	0.60	98.38	1.62
3	5.65	62.34	37.66	37.16	0.50	98.67	1.33
4	5.80	63.58	36.42	35.92	0.50	98.62	1.38
5	5.91	62.02	37.98	37.58	0.40	98.54	1.46
6	4.49	65.05	34.95	34.40	0.55	98.43	1.57
7	5.70	62.92	37.08	36.69	0.39	98.94	1.06
8	2.69	61.32	38.68	38.27	0.41	98.94	1.06
9	4.02	64.76	35.24	34.76	0.48	98.65	1.35
10	2.54	62.69	37.31	36.83	0.48	98.71	1.29
11	3.82	64.32	35.68	35.25	0.43	98.79	1.21
12	2.72	62.64	37.36	36.96	0.40	98.94	1.06
13	4.21	60.93	39.07	38.64	0.43	98.91	1.09
Aver.	4.59	62.87	37.13	36.66	0.47	98.71	1.29
Calf Tendon.							
1	2.21	65.39	34.61	33.98	0.63	98.18	1.82
2	3.96	66.54	33.46	32.89	0.57	98.30	1.70
3	5.17	68.75	31.25	30.60	0.65	97.91	2.09
4	4.32	68.32	31.68	31.06	0.62	98.04	1.96
5	4.12	67.23	32.77	32.33	0.44	98.68	1.32
6	2.68	68.84	31.16	30.42	0.74	97.63	2.37
Aver.	3.74	67.51	32.49	31.88	0.61	98.12	1.88

sufficient quantity for the determinations. Sometimes they were cut into strips with a knife and the strips finely divided with scissors. All preparations were conducted rapidly and with due regard to the usual precautions to prevent loss of moisture, etc.

Proportions of water, solids, organic and inorganic matter. — In these determinations the finely divided substance was dried at 100–110° C. to constant weight. Incineration was carefully conducted over a very low flame until all carbon was burned out and the ash was constant in weight.

The general summary on the opposite page gives the results of these determinations for the tendo Achillis from both the ox and the calf. It will be seen from the general averages that the tendon of the calf contains relatively more water and inorganic matter than that of the mature animal. The tissue of the full grown ox on the other hand contains larger proportions of solid substance and organic matter.

In his determinations of the composition of dry tendon from the foot of the calf, Marchand [1] also weighed the ash. In three separate determinations he found the ash to be 1.72, 1.82 and 1.89 per cent — an average of 1.81 per cent of the dry tissue.[2] These results accord very closely with our own, if it be assumed that the tendons of the calf which Marchand analyzed contained approximately the same amount of water found in these experiments — 67.5 per cent. At this rate, the fresh tendons analyzed by him contained 0.59 per cent of ash.[3]

The facts brought out by the figures in the table on the opposite page harmonize with comparative analytic data for other tissues of fully developed as well as immature animals. On the next page we present a summary giving percentage figures for the general composition of morphologically related parts. Attention may be called to the general similarity in the results for tendon and ligament. Costal cartilage is somewhat similar to these two in general composition, the analytic differences being mainly due to its larger content of water and inorganic matter.

Inorganic matter. — Ash in suitable quantity was prepared by gradual combustion in a nickel crucible over an alcohol burner and then by complete incineration over a very low flame in a platinum

[1] See page 220.

[2] See foot-note, page 225: also, summary on page 230.

[3] The ash of tendons containing ossa sesamoidea would naturally be much greater than any of the amounts here recorded for the normal tissue.

dish. The qualitative characters of the ash of the Achilles tendon are much the same as those of the inorganic matter in many other parts of the body. Solutions of the ash were strongly alkaline in reaction. We detected in it chloride, carbonate, sulphate, and phosphate. Of the basic elements sodium, calcium, magnesium, potassium, and iron were particularly prominent. It is probable that the iron came from traces of haemoglobin in the capillaries. Some of the

COMPARATIVE COMPOSITION.

	Tendon.		Ligament.[1]		Vitreous humor.[2]	Costal cartilage.[3]	Bone with marrow.[4]	Adipose tissue; kidney fat.[5]
	Calf.	Ox.	Calf.	Ox.				
Fresh tissue.								
Water	67.51	62.87	65.10	57.57	98.64	67.67	50.00	4.30
Solids	32.49	37.13	34.90	42.43	1.36	32.33	50.00	95.70
Organic	31.88	36.66	34.24	41.96	0.48	30.13	28.15	95.51
Inorganic	0.61	0.47	0.66	0.47	0.88	2.20	21.85	0.19
Dry tissue.								
Organic	98.12	98.71	98.10	98.90	35.29	93.20	56.30	99.80
Inorganic	1.88	1.29	1.90	1.10	64.71	6.80	43.70	0.20

[1] VANDEGRIFT and GIES: *Loc. cit.*

[2] Representing jelly-like connective tissue. Analyses by LOHMEYER, source of material not specified. See GORUP-BESANEZ: *Loc. cit.*, p. 401.

[3] Human. Analyses by HOPPE-SEYLER. See KÜHNE: Lehrbuch der physiologischen Chemie, 1868, p. 387.

[4] Average of many analyses of various human bones before removal of marrow. HOPPE-SEYLER: Physiologische Chemie, 1881, p. 625.

[5] From the ox. ATWATER: Methods and results of investigations on the chemistry and economy of food, 1895, p. 34.

carbonate doubtless arose from the proteid in the process of oxidation. Much of the sulphate came from the acid radicle of the tendon mucoid. The proportion of ash in tendon, as in ligament, is unusually small.

Schulz[1] has recently detected silicic acid in a number of the forms of connective tissue. The average amount of silicic acid in 1 kilo of

[1] SCHULZ: Archiv für die gesammte Physiologie, 1901, lxxxiv, p. 67.

dry ox tendon was found to be 0.1086 gram (0.01 per cent of the solid matter). In the same quantity of dry human tendon silicic acid amounts on an average to 0.0637 (0.006 per cent of the solid matter).[1]

Soluble and insoluble portions. Several direct determinations of the amount of insoluble matter in the ash were made. Ash which had been reheated in a platinum crucible was cooled in a desiccator. Quantities of this perfectly anhydrous material, from one to two grams in weight, were treated with 500 c.c. of distilled water per gram of substance. The mixture was repeatedly stirred for forty-eight hours, then filtered on weighed papers and the amount of insoluble substance directly determined gravimetrically in the customary way. The appended percentage results were obtained on three different preparations:

	1	2	3	Average.
Substance *insoluble* in cold water	27.1	27.4	26.6	27.0
Substance *soluble* in cold water	72.9	72.6	73.4	73.0

Similar determinations were made by us on samples of the ligament ash prepared by Vandegrift and Gies. 24.3 per cent of the same was found to be insoluble, 75.7 per cent soluble, in cold water. In Pickardt's [2] analyses of the ash of laryngeal cartilage 37.2 per cent was insoluble in water, 62.8 per cent soluble.

Sulphate. — The ash gave striking sulphate reactions with $BaCl_2$ in the presence of free HCl. In some preliminary experiments samples of ash which had been prepared quickly by incineration in a platinum dish over a Bunsen gas burner contained from 9.56 to 14.92 per cent of SO_3.[3] As these results were obviously affected by sulphur products in the gas, we next made several preparations of the ash in platinum dishes over alcohol burners. The following results for SO_3 content in ash prepared in this way were obtained by the usual $BaCl_2$ method,

[1] In these determinations SCHULZ also estimated the percentage of ash in the dry substance. In tendons of the calf it amounted to 3.19 per cent. In the older animals it was as low as 2.07 per cent. In human tendon it was as high as 3.88 per cent. The amount of silicic acid in the ash of the tendons from cattle ranged from 0.23 to 0.66 per cent. In the ash of human tendon it varied between 0.11 and 0.49 per cent. SCHULZ'S results indicate that the older the animal is the larger is the percentage of silicic acid in its connective tissues.

[2] PICKARDT: Centralblatt für Physiologie, 1892, vi, p. 735.

[3] Compare with results for ligament ash, under similar conditions of preparation, given by VANDEGRIFT and GIES, *loc. cit.*, p. 291. See also, BIELFELD: Zeitschrift für physiologische Chemie, 1898, xxv, p. 352.

in 0.25–0.71 gram portions, after solution in hot dilute HCl and subsequent filtration:

PERCENTAGE OF SO_3 IN TENDON ASH.

	1	2	3	4	Average.
A	6.72	6.62	6.68	....	6.67
B	6.70	6.60	....	....	6.65
C	6.60	6.58	6.63	6.61	6.60
D	6.63	6.84	6.74	6.69	6.72
E	6.55	6.63	....	....	6.59
				General average . .	6.65

The relation of tendon ash to the ash of other tissues and various fluids, with respect to SO_3 content, may be seen at a glance in the following summary of SO_3 percentages [1]: —

Bone . .	0.02	Liver . .	0.92	Serum .	2.10	Ligament .	5.64
Muscle .	0.30	Lungs . .	1.40	Spleen .	2.54	Bile . . .	6.39
Brain . .	0.75	Blood . .	1.67	Milk . .	2.64	Cartilage .	37.47

There can be little doubt that most of the SO_3 in tendon ash arises from an organic source, just as in the case of bile, cartilage, and ligament. It could not have come from blood or lymph. Bile contains combined SO_3 in salts of taurocholic acid. Cartilage contains salts of chondroitin sulphuric acid, as well as chondromucoid.[2] Ligament contains mucoid[3] and possibly, also, chondroitin sulphuric acid.[4] Tendon contains considerable mucoid, as we shall see, but, according to Mörner,[5] no chondroitin sulphuric acid can be separated from the Achilles tendon. Tendo mucoid, however, contains a radicle similar to, if not identical with chondroitin sulphuric acid,[6] and it is probable

[1] Vandegrift and Gies: *Loc. cit.*, p. 292.

[2] C. Th. Mörner: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.

[3] Richards and Gies: Proceedings of the American Physiological Society. This journal, 1900, iii, p. v; also, *Ibid.*, 1901, v, p. xi.

[4] Krawkow: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xl, p. 195.

[5] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1895, xx, p. 361.

[6] Levene: *Ibid.*, 1901, xxxi, p. 395.

that the SO_3 liberated during its combustion unites in part with the basic elements of the ash.[1]

Phosphate and chloride. — No extended quantitative analysis of the ash was made because of the large amount of derived sulphate in it. Figures for the percentage content of other constituents under the circumstances would afford only approximate values. Phosphate and chloride, the chief salts in the ash, were present in large proportion, as the following results for percentage content of P_2O_5 and Cl will indicate:

	1	2	3	4	Average
P_2O_5 . . .	8.38	8.53	8.30	8.16	8.34
Cl	31.73	30.99	31.26	31.52	31.37

The average quantity of chlorine in ligament ash was found by us to be 7.39 per cent. P_2O_5 was equal to 28.95 per cent of the ligament ash.

Fat (ether-soluble matter). — Although the Achilles tendon does not appear to hold as much admixed adipose tissue as ligamentum nuchæ, it seems to contain almost as much extractive substance. The following percentage results in this connection, calculated for fresh tissue in each case, were obtained by Dormeyer's method:

	1	2	3	4	5	6	7	Average
Fresh tissue . .	0.87	1.10	1.21	1.16	0.98	1.05	0.93	1.04

The proteid constituents. — It has been known for a long time that tendon consists mostly of collagen. As we have already indicated the earlier observers considered tendon to be almost pure collagen. Rollett's[2] researches on the structure and composition of connective tissues demonstrated the presence in tendon not only of such soluble proteids as might be constituents of contained lymph, but also of mucoid. Numerous histologists have shown the presence also of elastic fibres in tendinous tissue.

Coagulable proteid (*albumin, globulin*). — Rollett detected only traces of coagulable proteid in aqueous extracts of the Achilles tendon of the horse. Loebisch[3] called attention to the fact that

[1] Levene's result does not harmonize with Mörner's. The latter's method for the detection of chondroitin sulphuric acid in tendon should have revealed the presence of the acid substance in tendo mucoid identified by Levene. See Hawk and Gies: This journal, 1901, v, pp. 398–399.

[2] Rollett: Untersuchungen zur Naturlehre des Menschen und der Thiere (Moleschott), 1859, vi, p. i; also, *Ibid.*, 1860, vii, p. 190.

[3] Loebisch: Zeitschrift für physiologische Chemie, 1886, x, p. 43.

aqueous extracts of the same tendon of the ox contain slight quantities of coagulable proteid — "serum globulin" and an albumin coagulating at 78° C. Richards and Gies[1] recently observed that aqueous extracts of this tendon from the ox contain minute proportions of two coagulable proteids; one, a globulin, coagulating at 54°–57° C., the other, an albumin, coagulating at 73° C.

In this work we experienced great difficulty in making satisfactory quantitative estimations. The quantity of coagulum for 100–200 grams of tissue was always very slight. Frequently it was impossible to obtain the coagulum in a perfectly clear fluid. The results were the same in aqueous and in sodium chloride extracts. One or two indirect methods gave no more satisfactory results. Tendo mucoid is somewhat soluble in the aqueous and saline extracts of the tissue, and possibly the observed interference with perfect coagulation of the simple proteids was due to the presence of larger or smaller amounts of this glucoproteid.

The following percentage results were obtained in extracts from tissue which had been cut into narrow strips and then very finely divided with scissors: —

	1	2	3	4	5	6	7	Average
Fresh tissue .	0.231	0.184	0.191	0.274	0.177	0.219	0.262	0.220

It is possible that not only a small quantity of coagulable proteid was lost in each determination, but also that a small proportion of mucoid was admixed with the coagulum as a result of the addition of the dilute acid ordinarily employed to complete coagulation. We feel satisfied, however, that the above average amount is very nearly that contained in this tissue. Much of it doubtless is a part of contained lymph. The average quantity in ligamentum nuchæ is 0.616 per cent.

Mucoid.[2] — The proportion of mucoid in tendon is comparatively large. Halliburton states that the average amount for normal connective tissues is 0.521 per cent.[3] The amount in the human tendo Achillis he found varied under normal conditions between 0.298 and 0.770 per cent. Chittenden and Gies[4] obtained as much as 1 per cent of chemically pure mucoid from the tendo Achillis of the ox, al-

[1] Richards and Gies: *Loc. cit.*

[2] See Cutter and Gies: *Loc. cit.*, foot-note, p. 155.

[3] Halliburton: *Loc. cit.*, p. 477.

[4] Chittenden and Gies: Journal of experimental medicine, 1896, i, p. 186.

though their experiments were not designed for quantitative determinations. The amount in ligamentum nuchæ was found by us to average 0.525 per cent. Our percentage results for the Achilles tendon of the ox were the following:

	1	2	3	4	5	6	7	Average
Fresh tissue .	1.361	1.420	1.332	1.220	1.043	1.228	1.380	1.283

In these determinations we profited by the experience of Cutter and Gies that repeated treatment with excess of dilute alkali is necessary to extract completely mucoid from tendon.[1]

Halliburton[2] gives a record of determinations of mucoid in human tissues under abnormal conditions. In one case the Achilles tendon contained as much as 1.42 per cent. The tendons of the heart under similar conditions contained 1.65 per cent mucoid.

Elastin. — When tendon pieces are boiled in water they rapidly diminish in size and only a small quantity of elastin-like material is left behind. This residual material is not as resistant to the action of dilute acid and alkali as is the elastin of ligamentum nuchæ, although it appears to be true elastin.[3] The following results for percentage content were obtained in our quantitative determinations:

	1	2	3	4	5	Average
Fresh tissue	1.561	2.130	1.634	1.100	1.740	1.633

Münz[4] separated this substance, studied some of its reactions and decomposition products, and made a few analyses of it. He found its nitrogen content to vary between 14.31 and 14.48 per cent. The accuracy of these analytic results has been doubted, since the nitrogen content of all elastins has been found to be above 15 per cent. One of our own specially prepared samples of tendon elastin, after it had been extracted with alcohol and ether, gave the following percentage results on analysis: (a) Nitrogen — by the Kjeldahl method — 15.42, 15.49, 15.45; average, 15.45. (b) Sulphur — by the fusion method over alcohol burner — 0.48, 0.54; average, 0.52. (c) Ash — 1.32, 1.28; average, 1.28. These results agree fairly well with those for aorta elastin obtained by Bergh[5]: N, 15.20; S, 0.66; Ash, 0.51.

[1] Cutter and Gies: *Loc. cit.*, p. 161.

[2] Halliburton: Jahresbericht über die Fortschritte der Thier-Chemie, 1888, xviii, p. 324.

[3] Kühne: Lehrbuch der physiologischen Chemie, 1868, p. 356.

[4] Münz: Quoted by Gorup-Besanez, *loc. cit.*, pp. 143 and 645.

[5] Bergh: Zeitschrift für physiologische Chemie, 1898, xxv, p. 341.

Collagen. — The great bulk of the solid matter of tendon is collagen. We made five quantitative determinations by the indirect method,[1] with the following percentage results:

	1	2	3	4	5	Average
Fresh tissue	30.63	32.47	30.98	32.27	31.59	31.59

The proportion of collagen in the fresh tendo Achillis is almost exactly the same as that of elastin in ligamentum nuchae.

Recently, in testing his method for the determination of collagen in connective tissue containing little soluble proteid, Schepilewsky[2]

COMPOSITION OF TENDO ACHILLIS.

Constituents.	Fresh tissue.		Dry tissue.		Ash.
	Calf.	Ox.	Calf.	Ox.	Ox.
Water	67.51	62.870			
Solids	32.49	37.130			
Inorganic matter	0.61	0.470	1.88	1.266	
SO_3	....	0.031	....	0.084	6.65
P_2O_5	....	0.039	....	0.106	8.34
Cl	....	0.147	....	0.397	31.37
Organic matter	31.88	36.660	98.12	98.734	
Fat (ether-soluble matter) .	....	1.040	....	2.801	
Albumin, globulin	....	0.220	....	0.593	
Mucoid	....	1.283	....	3.455	
Elastin	....	1.633	....	4.398	
Collagen (gelatin)	....	31.588	....	85.074	
Extractives and undetermined substance . . .	....	0.896	....	2.413	

found 80.86 per cent of collagen in dry tendon. The particular tendon he used is not mentioned. In the dry Achilles tendons of the ox analyzed by us the collagen amounted on an average to 85.074 per cent.

[1] See VANDEGRIFT and GIES: *Loc. cit.*, foot-note, p. 295.

[2] SCHEPILEWSKY: Archiv für Hygiene, 1899, xxxiv, p. 351.

Crystalline extractives. — Our results for extractives were only qualitative. Creatin and nuclein bases could readily be detected. The proportion of extractive matter was small. Our results were similar to those previously obtained in this laboratory for ligament. In the table on the opposite page the extractives are included in "Extractives and undetermined substance," the figures for which were obtained by difference.

Average Composition. — The data of all our analyses are brought together in the summary on the opposite page, which gives the average percentage composition of fresh tendo Achillis and of the dry solid matter in it, together with the results of partial analysis of the ash.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. VIII. — ON THE REACTIONS OF INFUSORIA TO CARBONIC AND OTHER ACIDS, WITH ESPECIAL REFERENCE TO THE CAUSES OF THE GATHERINGS SPONTANEOUSLY FORMED.

By H. S. JENNINGS and E. M. MOORE.

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IT is well known that when certain infusoria are left undisturbed they do not remain scattered, but gather in more or less dense groups. Thus, if they are mounted on a slide in a thin layer of water, soon dense aggregations will be formed in certain areas, while the remainder of the slide will be nearly deserted. One of the first investigators to describe this phenomenon was Pfeffer.[1] He observed its occurrence in Glaucoma scintillans, and less markedly in Colpidium colpoda, Stylonychia mytilus, and Paramecium. Pfeffer was inclined to believe that these aggregations were due, partly at least, to a contact stimulus, resulting from a striking of the organisms against small solid bodies, and especially against each other.

In the first of this series of studies,[2] this phenomenon in the case of Paramecium was subjected to a thorough examination. It was demonstrated that while the contact stimulus plays a certain part in the production of these aggregations, the chief factor involved is a reaction to carbon dioxide. The Paramecia tend to gather into regions where the water is impregnated with this substance. Since the animals themselves produce carbon dioxide in their respiratory processes, any spot where a few have gathered (owing to the contact stimulus or for any other reason), becomes a centre for the production and diffusion of this substance. Therefore other Paramecia collect here; more carbon dioxide is produced; more Paramecia collect, and in time a dense aggregation is formed. It was farther shown that this

[1] Pfeffer: Untersuchungen aus dem Botanischen Institut, Tübingen, 1888. ii. p. 618.

[2] Jennings: Journal of physiology, 1897, xxi, pp. 258–322.

effect of carbon dioxide is due to the fact that it forms in water an acid solution ("carbonic acid") — the Paramecia collecting in the same way in any substance having a weakly acid reaction.

As the other infusoria which form similar aggregations of course likewise produce carbon dioxide in their respiratory processes, it seems very probable, as was pointed out in the paper just referred to, that the same factors are at work here as in the case of Paramecium; that the spontaneous aggregations formed are due to the tendency of the organisms to collect in carbonic acid. This probability has been set forth also by later investigators, as for example in the recent paper of Rothert.[1] But no one has hitherto undertaken to determine by experiment in how far this may be true.

This is the problem which the study here presented has attempted to solve for a certain number of infusoria (sixteen species). The primary question to be answered is therefore as follows: — Are the spontaneous aggregations formed by certain species of infusoria due to their gathering in carbon dioxide excreted by themselves? The investigation involved a test of the reactions of the organisms studied both to carbon dioxide and to acids in general, and at the same time brought out a number of points as to the method in which the reactions of the organisms are produced; these secondary matters are likewise set forth briefly in the following paper.

Methods.

The method of experimentation most used was that described in the first and second[2] papers in this series of studies. The organisms were studied in a thin layer of water, by mounting them on a slide covered with a large cover glass supported near its ends by slender glass rods. Their reactions were tested by introducing with a capillary pipette a drop of the substance in question beneath the cover glass, or in some cases by allowing it to diffuse inward from the side of the cover glass. In the case of gases, as carbon dioxide, it was found very convenient to proceed as follows. The gas is introduced into a large rubber bulb, such as is used with syringes or atomizers. To this is attached by the rubber tube a glass tube drawn out to a fine point. By inserting the point beneath the cover glass and pressing the bulb, a bubble of gas is introduced into the preparation. Where

[1] Rothert: Flora, 1901, lxxxviii, p. 402.

[2] Jennings: This journal, 1899, ii, pp. 311–341.

still different methods were used, these are mentioned in the account of results.

In attempting to determine the reactions of organisms to carbon dioxide, it is of course absolutely necessary that there should be no considerable quantity of this substance already present in the water. And since the organisms are continually producing carbon dioxide in appreciable quantities, some method of getting rid of the gas is a practical requirement of the highest importance. The simplest method is to aerate the water thoroughly immediately before each test. This may be done as follows. Place a few drops of the fluid containing the organisms, — as much as will be placed on the slide at once, — in a watch glass ; then with a clean pipette inject it repeatedly over the surface of the watch glass, force bubbles into it, and mix it thoroughly with the air. Then place on the slide, cover, and perform the tests at once. Repeat the aeration before every test, as it requires only a very short time for water crowded with organisms to become impregnated with carbon dioxide. Of course it is not reasonable to expect organisms to gather in carbon dioxide when the water in which they are found already contains this substance in the optimum concentration. This precaution is equally necessary in testing other acids, as it is the common factor in all acids to which the effects of the carbon dioxide are due.

This or an equally efficacious method of aeration is an absolute necessity, if clear cut and constant results are to be obtained with carbon dioxide or acid solutions in general. This cannot be too much insisted on. Sometimes definite reactions will be obtained without aerating the water, in case it happens not to be already impregnated with carbon dioxide, but a little later the same organisms may give negative results.

A second precaution worthy of mention is the necessity of having the water containing the organisms relatively free from débris : — filamentous bacteria, and the like. Most of the infusoria are markedly thigmotactic, tending to come to rest upon coming in contact with small solid bodies. If a preparation contains a network of fine bacterial filaments, frequently the infusoria will not gather in the acid at all, but remain at rest on the filaments, while if the filaments are removed, as by straining through coarse cloth, marked positive reaction is at once obtained.

No attempt was made to determine quantitatively the exact strength of solution to which the organisms react. The purpose of the work

was to determine whether the organisms do or do not give at any concentration a certain reaction to the substances in question. This was accomplished by beginning with a concentration so slight that the organisms did not react to it at all, and gradually increasing the strength till the solution is destructive. Somewhere between these limits will be found the characteristic reaction of the organisms. The value of quantitative determinations of the exact concentrations of acids to which the organisms react is largely illusory, in the majority of cases, as this varies with the amount of carbon dioxide present in the water, — a factor not under exact control. It varies also apparently with organisms from different cultures, and with the thickness of the layer of water in which the infusoria are confined. In experimenting with carbon dioxide especially, it is impracticable to attempt the use of solutions of known strengths; the introduction of a bubble of gas into the preparation gives all concentrations, from saturation next to the bubble to zero at some distance from it.

In the following account of the work, the organisms will be taken up in the order suggested by the nature of the results obtained.

A. Organisms which collect in Solutions of Carbonic and other Acids.

Chilomonas paramecium. — This small flagellate is perhaps the commonest and most abundant member of the group to which it belongs. It is therefore the most accessible form for experimentation on the Flagellata, and it will probably usually be employed when work on this group is undertaken. It is therefore important that the fundamental facts as to its reactions should be well established. An extensive piece of work has already been done by Garrey[1] on the reactions of this organism to chemicals, especially to acids. To our great regret we were compelled to come to results essentially different in some respects from those set forth by Garrey. It is unfortunate that there should be such disagreement, as this is likely to result in leaving the subject doubtful in the minds of other investigators. We believe however that we are able to point out exactly the factor to which the differing results are due, and to show that Garrey's results would probably not have differed from our own if this factor had been taken sufficiently into consideration. This factor is the normal pres-

[1] Garrey: This journal, 1900, iii, pp. 291–315.

ence of an acid, — a solution of carbon dioxide excreted by the organisms, — in the fluid in which Chilomonas occurs.

Reaction to carbon dioxide. — Water containing Chilomonas is aerated in the manner above directed, and any bacterial filaments are removed by straining through coarse cloth. It is then placed on a slide, covered, and a bubble of carbon dioxide introduced. At first there is no gathering of the organisms, but soon they begin to collect about the bubble of gas, and gradually a dense ring is formed. Fig. 1 gives the general appearance of the progress of the experiment; it was taken from an actual preparation.

This experiment we have repeated many times, always (when the conditions were properly fulfilled) with the same results. The ex-

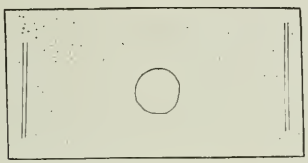

FIGURE 1, *a*.

FIGURE 1, *b*.

FIGURE 1. — Reaction of Chilomonas to a bubble of CO_2. *a*, Preparation immediately after the introduction of the bubble, before the organisms have collected. *b*, The same preparation a few minutes later, showing the dense collection of infusoria about the bubble.

periments succeed equally well in the apparatus used by Garrey, and figured on page 294 of his paper. With a long capillary pipette a bubble of carbon dioxide can be introduced into the chamber beneath the cover glass. The flagellates at once gather about it in a dense ring, while they do not thus gather about bubbles of air similarly introduced.

Chilomonas thus gathers about bubbles of carbon dioxide in dense collections, just as Paramecium does. The conditions above referred to as necessary of fufilment are (1) that the carbon dioxide should be properly removed from the water just before making the experiments; (2) that the bacterial filaments and other débris in the water should be largely removed.

The justification of the first condition is at once seen. It is idle to test the organisms with carbon dioxide when they are already immersed in a solution of that substance. It is undoubtedly to a neglect of this precaution, which is nowhere so much as referred to by Garrey, that the negative results of this investigator are due.

The necessity for the removal of the débris is evident on examining the behavior of the organisms. Chilomonas is very strongly thigmotactic; if when swimming through the water it comes in contact with a bacterial filament or bit of débris of any sort, it at once attaches itself by one of its two flagella, and comes to rest. Thus, in a preparation containing such filaments, all the individuals will soon be found quietly attached, which of course prevents their collecting anywhere.

Reaction to other acids.—To what factor is the collecting in the solution of carbon dioxide due? Is it, as in the case of Paramecium, due to the acid qualities of this solution (to the H ions, according to the dissociation theory)? To answer this question, tests were made

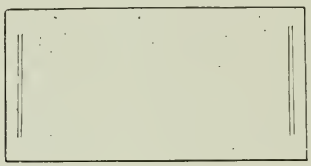

FIGURE 2, *a*.

FIGURE 2, *b*.

FIGURE 2 — Reaction of Chilomonas to a drop of $\frac{1}{50}$% HCl. *a*, Preparation immediately after the introduction of the drop (no organisms either within or gathered about the drop). *b*, The same preparation a few minutes later.

with other acids, and the organisms were found to collect in weak solutions of these exactly as in the carbon dioxide solution. These results were clear cut and unmistakable; they were obtained with hydrochloric, nitric, sulphuric, acetic, formic, butyric, propionic, citric and oxalic acids.

As a control, the organisms were tested with distilled water; no gathering was formed about or in it at any time, but the organisms remained quite neutral in their behavior toward it.

The details of the phenomena vary with the strength of the acid solution used. With a stronger solution (say $\frac{1}{50}$ per cent HCl), the animals gather in a dense ring about the margin of the drop, leaving vacant the area within, containing the stronger acid (Fig. 2). With a weaker solution the organisms gather into the interior of the drop, leaving no part of it vacant (Fig. 3).

No characteristic difference was to be observed between their behavior to inorganic acids, such as HCl and HNO_3, and that toward organic acids, such as acetic and butyric, save that of course

different concentrations were required to produce the same result. In Garrey's work, the results obtained with inorganic acids differed from those obtained with certain organic acids. Garrey studied the organisms in a much thicker layer of water, and introduced the acid through a tube-like opening at one side of the preparation. He observed that a dense gathering was formed about hydrochloric acid, but explained this as follows: When the strong acid reaches the organisms they begin at once to swim violently. This soon takes them outside of the area of acid, leaving it clear. On reaching the outer boundary, they stop, since there is no further cause for movement, thus forming a dense aggregation just outside the drop. "That in the zone surrounding the area there is a dense gathering (in other

FIGURE 3.

FIGURE 3. — Collection of Chilomonas within a drop of $\frac{1}{100}\%$ HCl.

words that there is a ring formation) is in my opinion due to the fact that those individuals which were in the clear area are now gathered in the space immediately surrounding it" (*loc. cit.*, p. 296). The result is thus in a sense accidental, and would occur with any chemical which had the property of setting the organisms in violent movement. In Garrey's experiments no gathering was ever formed in the centre of a drop of inorganic acid, no matter how weak it was.

In our own work, the gatherings which occurred in drops of inorganic acids, were clearly not explicable in this manner. (1) When the drop was first introduced (Fig. 2 *a*) there were no individuals either within the drop or forming a ring about it. Later a dense ring was slowly formed (*b*). As there had been no individuals within the drop, of course the ring was not formed by individuals moving out of the drop and then stopping. Moreover, the process of ring formation is clearly evident to observation; it is due to the swimming of organisms into the ring from outside. (2) If a weak solution of acid is used, it is at first empty, but later becomes completely filled with organisms (Fig. 3). This of course could not possibly take place in the way assumed by Garrey. Collections

of this sort were observed in the case of all inorganic acids studied. They were not formed when distilled water alone was used. (3) The gatherings about bubbles of carbon dioxide of course could not occur in the manner described by Garrey, as the bubble contained no individuals to move out and form a ring.

In the case of the organic acids, the phenomena observed by us were precisely parallel to those occurring in inorganic acids. It is possible that the gatherings formed last longer in the case of some organic acids, though to us this seemed not usually very marked.

The results obtained by Garrey with organic acids varied much in different cases. With oxalic, formic, citric, succinic and valerianic acids the phenomena were the same as with inorganic acids; *i. e.*, a clear area was formed, sometimes with a ring of organisms surrounding it (if the acid was strong); this ring formation Garrey explained as in the case of inorganic acids.

" Malic, tartaric and mandelic acids produce a clear area, often with a ring about it. In the formation of the ring, the phenomena were so inconstant that I was unable to say that it was or was not due to a migration of the organisms from without to it " (*loc. cit.*, p. 307).

Finally, with acetic, butyric and lactic acids, a clear area surrounded by a dense ring was formed, and Garrey was able to assure himself that the ring formation was due to a migration of the organisms from the outside.

Thus Garrey's results with acids can be placed in three categories. (1) Some showed a ring formation, which in the author's opinion was due merely to the driving of the organisms out of the area in which the acid was found. (2) Some gave such inconstant results that the author was not certain what he should conclude about them. (3) Some showed a ring formation of such density and clearness that it was evident that the organisms came from outside of the acid area.

Now this inconstancy and uncertainty in the results with acids is exactly what is obtained when the carbon dioxide is not removed from the water by thorough aeration before each experiment. The culture water contains varying amounts of carbon dioxide and in some cases a part of it is accidentally driven off in the manipulations preparatory to the experiments, in other cases not. The presence of carbon dioxide means also the presence in the water of whatever it is that gives acids their characteristic qualities. Hence in such a fluid the organisms are already in an acid solution and naturally do not

react with any precision when an acid is introduced, while in cases where the carbon dioxide is partly or entirely driven off, distinct reactions are obtained; the results thus become inconstant and uncertain. After careful removal of the carbon dioxide before every experiment, the results with all acids are, according to our observations, essentially the same, — *i. e.*, a ring or group is formed by immigration of the organisms from the outside. It was this same neglect to remove the carbon dioxide from the water that led Garrey to deny the results with Paramecium, though these are demonstrable with ease.

In view of the contrasted results obtained by Garrey on the one hand and by ourselves on the other, it is much to be desired that some third person should reinvestigate the reactions of Chilomonas, testing the various methods used, observing the precautions set forth, and perhaps taking counsel by correspondence or conversation with both sides, that there may be no omission which might seem to vitiate the work. It remains of course possible that Chilomonas from different cultures reacts differently, though we have used dozens of different cultures and have observed no such difference.

Our own results on Chilomonas may now be summarized. This organism reacts to carbonic and other acids just as Paramecium does, forming dense collections in localized areas, where carbon dioxide is present. The spontaneous aggregations sometimes formed by Chilomonas may therefore be due to their collection in carbon dioxide excreted by themselves.

Cyclidium glaucoma. — This ciliate infusorian likewise gathers in carbon dioxide and in solutions of acids in general. The collections thus formed are dense and lasting. Cyclidium was not observed to form spontaneous collections, though this may occur.

Colpidium colpoda. — This is one of the infusoria which was described by Pfeffer as collecting spontaneously into groups. It reacts to solutions of carbon dioxide and other acid solutions, just as Paramecium and Chilomonas do, gathering in dense aggregations about a bubble of CO_2, or in a drop of weak acid. It is therefore probable that the spontaneous groups are due to carbon dioxide. If Paramecium and Colpidium are mounted together, they will gather spontaneously into groups, each group containing both kinds of infusoria, the boundary of the groups being practically the same for each. The cause of the grouping is thus evidently the same in the two cases.

B. Organisms which form Spontaneous Gatherings, but do not collect in Solutions of Carbonic or other Acids.

Oxytricha aeruginosa. — This organism, when mounted on a slide, forms spontaneous groups which are similar in every respect to those formed by Paramecium. The method of reaction to a stimulus in Oxytricha is by backing, and turning to the aboral (or right) side, — the side which is not notched. If the organisms are at first scattered uniformly throughout the preparation, they will soon be found to be forming groups in one or more regions. If the individuals within the groups are observed, they are found to be swimming hither and thither in all directions. But when one comes to the outer boundary of the group, it at once swims backward a short distance, turns toward the aboral side, and then starts forward again. As this happens every time the boundary of the group is reached, the animal remains within it. Individuals outside, whose course carries them by chance into the areas where a group is forming, do not react at all as they enter the area. But after swimming across, they do react as above described upon coming to the outer boundary of the area. Hence every Oxytricha that enters a group remains within it, and after a time a dense aggregation is formed. The groups thus produced increase in area, spreading out regularly, but maintaining a definite boundary.

The phenomena seem thus in every way identical with those observed in the case of Paramecium (see the first and second of these studies). It might therefore be reasonably expected that the cause would be found to be the same. But experiment shows that this is not the case; Oxytricha aeruginosa does not collect in regions where carbon dioxide is present, nor in other acid solutions. If a bubble of carbon dioxide is introduced into the preparation, the Oxytrichas do not gather about it, but on the contrary give their "motor reaction" when they come into its neighborhood, — reversing the direction of movement, and turning toward the aboral side. They thus leave the space about the carbon dioxide empty. Toward drops of acid solutions of all sorts they react in the same manner.

If Oxytricha and Paramecium are present in the same culture, or if the two are mixed together and experimented upon in the usual way, the results are as follows. The Paramecia collect about the bubble of carbon dioxide, or in the drop of acid, at once; the Oxy-

trichas do not. Thus a separation of the two kinds of infusoria is soon brought about.

If Oxytricha and Paramecium are mounted together and the slide is allowed to stand for a time, both kinds of infusoria will form spontaneous groups, but the two groups are quite separate. The Paramecia gather in one region, the Oxytrichas in another. Individuals of either kind may pass directly across the groups formed by the other, or swim in and out of the area where the other group occurs. The groups are thus clearly due to different causes in the two cases.

Oxytricha therefore forms spontaneous gatherings similar to those of Paramecium, but not due to the same cause. It seems evident that Oxytricha must excrete some other substance, not an acid, which acts upon it in the same way that the excreted carbon dioxide acts upon Paramecium. The nature of this substance remains to be discovered.

Loxocephalus granulosus. — In the case of this organism the facts are closely parallel to those described for Oxytricha aeruginosa. It forms spontaneous gatherings, but does not collect about bubbles of carbon dioxide nor in acid solutions in general. Mounted on the same slide with Paramecium, the two organisms form separate groups in different regions of the preparation. Clearly, Loxocephalus, like Oxytricha, excretes some substance which brings about the collections, but this substance is not carbon dioxide.

In preparations containing both Paramecium and Loxocephalus, the following may be observed as to the relations of the two organisms. Loxocephalus swims in and out of the groups of Paramecia, paying no attention to the limits so strictly observed by the Paramecia. In the same way Paramecium swims indifferently in and out of the groups of Loxocephali, when the latter groups are first forming. But after a group of Loxocephali has become well established and contains very large numbers of individuals, a Paramecium passing accidentally into the group usually remains there. Thus after a time a considerable number of Paramecia may be mingled with the Loxocephali. The Paramecia swim about freely within the group, but turn back on coming to an outer limit. It is to be noted that this outer limit is not the same as that which turns back the Loxocephali, but lies a little outside of it, so that the area in which the Paramecia are confined is larger than that which limits the Loxocephali, inclosing the latter.

These phenomena are probably to be explained as follows. Loxocephalus is not affected by carbon dioxide, therefore does not

gather in the groups formed by the Paramecia, but swims in and out of them indifferently. But it does excrete some other substance, not of an acid nature, into which it gathers; hence the spontaneous collections formed. To this substance Paramecium is indifferent, hence it swims indifferently in and out of the groups of Loxocephali, at first. But of course Loxocephalus produces carbon dioxide in its respiratory processes, hence after a group of these organisms has been formed for some time, the water becomes impregnated with carbon dioxide, as well as with the other (hypothetical) substance. Paramecia now passing into the group remain, owing to the carbon dioxide. The areas over which the carbon dioxide and the hypothetical substance are effective are not identical, that for the carbon dioxide being a little larger; hence the limit of the excursions of the Paramecia is outside that for the Loxocephali.

C. Organisms which do not collect in Carbonic or other Acids, and which were not observed to form Spontaneous Gatherings.

The following organisms were tested with carbon dioxide and with solutions of various acids in the same manner as those hitherto described. Every precaution was taken to remove the carbon dioxide from the water before making the tests, and the experiments were repeated under various conditions, with uniform results. None of these organisms gather about bubbles of carbon dioxide or in solutions of acids. They are as follows: Oxytricha fallax, Euplotes charon, Stylonychia pustulata, Colpoda cucullus, Spirostomum teres, Stentor cæruleus, Enchelys farcimen, Halteria grandinella, Didinium nasutum, Euglena viridis, and Heteromita globosa.

Some of these organisms, on coming in contact with a solution of carbon dioxide, at once give their characteristic "motor reaction," backing and turning toward a structurally defined side; thus they turn away from the area in question, leaving it empty. Others do not react at all to carbon dioxide, and to other acids only when very strong. Those that were indifferent were Oxytricha fallax, Stentor cæruleus, Didinium nasutum, Euglena viridis, and Heteromita globosa.

D. The Method by which the Gatherings are Brought About.

Throughout the work attention was given to the method by which the infusoria gather together. The point which was especially studied was the question of orientation. Do the organisms collect in the region where a certain chemical is present because they become oriented in the lines of the diffusing ions? Or are the collections brought about in the manner described for Paramecium, in the first and second of these studies?

The phenomena were carefully examined in all the infusoria in which collections were observed, — in Colpidium colpoda, Oxytricha aeruginosa, and Loxocephalus granulosus, and additional observations were made on Paramecium and Chilomonas. All the ciliates mentioned are of sufficient size so that their movements can be exactly observed with the Braus-Drüner stereoscopic binocular, and there can be no doubt as to the method in which the gatherings take place. They collect in essentially the same manner as has been shown in previous studies to be true for Paramecium. The organisms are at first swimming freely hither and thither. When the drop of acid is introduced, or collections are produced in other ways in certain regions, some of the individuals swim into the area in question merely through their usual movements. They do not change their course or react at all as they enter the area. But as they swim across it and reach the opposite side, where they would if unchecked pass out of the area into the surrounding water, each infusorian gives its characteristic "motor reaction." Oxytricha after moving backward turns toward its unnotched side, Loxocephalus to the aboral side, Colpidium toward its convex side, Paramecium toward the aboral side. The animal is thus prevented from leaving the area containing the chemical, but swims in another direction within this area. As it reacts in the same way every time it comes to the outer boundary of the area, it does not leave it at all. Other individuals enter in the same way, through their random movements, and remain through the same reactions, so that after a time the areas in question swarm with infusoria.

If the animals are allowed to come thoroughly to rest before introducing the chemical, usually no collection is formed within it. This shows the essential part played in the reaction by the random movements of the organisms.

It seems difficult for many minds to believe that the dense gather-

ings observed can be produced in this way. That this is the real method by which the collections occur, can be very neatly demonstrated to the eye in the following manner. A number of Paramecia or other infusoria which collect in acids are mounted on a slide. Upon the upper surface of the cover glass a small circle about the size of the drop of acid usually introduced, is made in ink with the pen. By directing the attention to the area within the ring of ink, it will be seen that many infusoria (as many as ten per second or more, in an ordinary mount of Paramecium), cross the area every instant. It is therefore evident that if all of them could be stopped within the area, a dense group would soon be produced. With the capillary pipette a drop of acid is now introduced beneath the ring; the same number of infusoria now enter the area as before, but every one remains and a dense collection soon results.[1]

In the paper already cited, Garrey maintains that the flagellate Chilomonas collects in certain acids in a manner entirely different from that above set forth. He holds that the collections (in acetic acid, for example), are produced through an orientation of the organism in the lines of the diffusing ions. The reactions to other substances, drops of which are left empty by Chilomonas (as for example a solution of sodium chloride), take place in a way entirely different, according to Garrey. Here there is no orientation; the chemicals merely cause "swift shooting movements," by which the animal is carried out of the area, or prevented from entering it. The method of reaction exhibited in collecting in acetic acid is denominated by Garrey *chemotaxis*, while that shown in keeping out of or leaving a drop of sodium chloride he calls *chemokinesis*.

In the sixth of this series of studies, reasons drawn from a study of the movements of the individual Chilomonads have been given for rejecting this distinction in kind between the reaction in collecting and that in avoiding a region containing chemicals. Certainly no such distinction can be made in Paramecium, nor in the other ciliates above mentioned. Leaving out of account the direct observations on the movements of the individuals, there are certain experiments which amount almost to a demonstration that there is no such distinction in kind, — even in Chilomonas. They demonstrate at least that collections exactly similar to those produced through the supposed "chemo-

[1] This experiment was demonstrated on the screen by means of the stereopticon before the Society of Western Naturalists at the meeting in Chicago in December, 1900.

taxis" can be produced through the operation of the admitted "chemokinesis."

Acetic acid may be taken as a type of the substances toward which, according to Garrey, Chilomonas shows orientation, or "chemotaxis;" while sodium chloride is an example of the substances which cause no orientation, but merely "chemokinesis." If a drop of acetic acid of a proper concentration is introduced into a preparation of the in-

FIGURE 4.

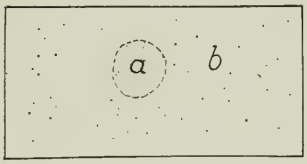

FIGURE 5.

FIGURE 6.

FIGURES 4, 5, and 6. Diagrams showing how the grouping of the organisms depends on the relations of the two fluids to each other in space. *a* represents the area occupied by the fluid into which the infusoria may pass without giving the "motor reaction;" *b* the fluid into which it cannot pass without giving the "motor reaction." When *a* is water, *b* is a salt or alkaline solution; when *b* is water, *a* is an acid solution; in either case the grouping of the organisms (Paramecium or Chilomonas), is that shown in the figures.

fusoria, the latter soon collect in the drop ("chemotaxis"), while if a drop of sodium chloride solution is introduced, it remains empty ("chemokinesis").

But suppose we mount our infusoria in a weak solution of sodium chloride, and introduce a drop of water? The salt solution is admitted to produce no orientation, but merely "chemokinesis," yet in a short time the drop of water is filled with a dense group of infusoria,—just as was the acetic acid in the former case. Apparently the collection is formed in water just as quickly as in the acid, and the present authors have been able to detect no difference in the method of formation. It is at least demonstrated that collections can as well be formed without orientation as with it, and that if these infusoria

possess the power of becoming oriented to diffusing ions, this power is a useless luxury.

According to our observations, the phenomena are identical in the two cases. The organisms swim about, in the solution in which they are mounted (water, or sodium chloride solution, respectively), and enter the drop (acetic acid, or water, respectively), without reaction. After having entered they give the usual "motor reaction" when they come to the outer boundary of the drop; hence they do not leave it, and the drop after a time swarms with the animals.

The following series of experiments is instructive and brings out clearly the facts as they appear to the present authors.

Mix a part of the infusoria (Paramecium or Chilomonas) with a weak solution of sodium chloride, not strong enough to injure them, mix others with a weak, non-injurious solution of acetic acid, and leave others in water. Now make mounts with the fluids in various relations to each other:

(1). Make a preparation (Fig. 4) in such a way that half the fluid on the slide is water (*a*) containing infusoria, while the other half is salt solution (*b*) containing infusoria. After a short time most of the infusoria will be in the half containing water alone.

(1a). Make a similar preparation (Fig. 4), save that one half (*a*) is acetic acid containing infusoria, the other half (*b*) water containing infusoria. In this case after a time most of the organisms will be found in the acid.

(2). Make a preparation (Fig. 5) in such a way that the salt solution surrounds the drop of water, the water (*a*) being introduced as a drop into the salt solution (*b*). After a time the drop (*a*) of water contains a dense swarm of the organisms.

(2a). Make a preparation as in the last, save that water (Fig. 5, *b*) surrounds the acid (*a*), which is introduced as a drop into the water. In this case there is likewise a dense aggregation formed in the drop *a* (of acid).

(3). Make a preparation (Fig. 6) such that the water (*a*) surrounds the salt solution (*b*).— the latter being introduced as a drop into the water. After a short time the drop *b* (of salt solution) is empty.

(3a). Make a similar preparation, in which the acid solution (Fig. 6, *a*) surrounds the drop of water (*b*). Soon the drop *b* (of water) is left empty.

With the same pair of substances we get, therefore, either a dense aggregation (or what has been sometimes called "positive chemotaxis"), or a certain definite area left vacant ("negative chemotaxis"), depending upon the relation in space of the two fluids to each other. And this result may be obtained whether we use as our chemical one like acetic acid, to which it has been maintained that the infusoria

show positive "chemotaxis" proper, or whether we employ a salt to which they are held to react only by "chemokinesis."

Thus with either pair of fluids, whether we do or do not get a dense aggregation of infusoria depends "on the configuration of the two fluids" — on the relation of the two fluids to each other in space. General statements embodying these relations may be made as follows. If we distinguish as *b* that fluid into which the infusorian cannot pass without causing the "motor reaction," as *a* that into which it can pass without causing the reaction, then

If *b* surrounds *a* (Fig. 5), a dense aggregation is formed in *a* ("positive chemotaxis").

If *a* surrounds *b* (Fig. 6), the small area *b* is left empty ("negative chemotaxis").

If *a* and *b* occupy equal areas (Fig. 4), after a time most of the organisms will be found in *a*. (This last case is not so strongly realized in a minute organism like Chilomonas as in a larger creature, such as Paramecium, because the distances to be passed over are so great that a weak swimmer like Chilomonas will not soon reach the area *a*, and may come to rest in large numbers in *b* without reaching *a* at all. But in any case, a considerable majority will be found in *a*.)

If *a* is water, *b* may be a solution of an alkali or of a great variety of neutral salts; in the case of Paramecium, almost any neutral salt If *b* is water, *a* may be any acid. In either case the resulting phenomena will be essentially the same.

Summary.

1. In order to test the reactions of infusoria to acids, it is necessary to remove with great care from the water containing the organisms the carbon dioxide produced by the organisms in their respiratory processes.

2. Colpidium colpoda, Cyclidium glaucoma, and Chilomonas paramecium collect in solutions of carbonic and other acids, just as Paramecium does. The spontaneous collections formed by these organisms may therefore be due to their excretion of carbon dioxide.

3. Loxocephalus granulosus and Oxytricha aeruginosa form spontaneous collections similar to those of Paramecium, but do not gather in carbonic or other acids. The spontaneous collections in these cases must therefore be due to other causes.

4. The following infusoria do not collect in carbonic or other acids,

nor were they observed to form spontaneous gatherings: Oxytricha fallax, Euplotes charon, Stylonychia pustulata, Colpoda cucullus, Spirostomum teres, Stentor cæruleus, Enchelys farcimen, Halteria grandinella, Didinium nasutum, Euglena viridis, Heteromita globosa.

5. The collections, according to our observations, take place in the manner described in previous numbers of this series of studies for Paramecium. In cases where this has been disputed, it is shown that collections essentially similar to those produced by what has been considered "chemotaxis" proper are likewise produced by what is admittedly "chemokinesis."

THE MOVEMENTS OF THE INTESTINES STUDIED BY MEANS OF THE RÖNTGEN RAYS.[1]

By W. B. CANNON.

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CONTENTS.

Introduction.

THE investigation of intestinal movements has been beset by the same difficulties that characterized the investigation of the gastric mechanism. Pathological subjects or animals subjected to the disturbing action of drugs and anæsthetics and of serious operations, have been the only sources of our knowledge. A considerable difference of opinion as to the nature of the normal movements in the intestines has resulted from observations made under these necessarily abnormal conditions. The slowly-advancing peristaltic wave and the *Pendelbewegung*, or swaying movement, described by Ludwig, have been regarded as true physiological processes. Concerning antiperistalsis and the swiftly-running vermicular contraction, observers are not so nearly in agreement. The activity of the large intestine

[1] The results of this investigation were reported to the Boston Society of Medical Sciences, November 19, 1901.

has been described as simply peristalsis of a slower rate than that seen in the small intestine.

The best known of the intestinal movements is the normal peristaltic wave. This wave is slow, having a rate of about two centimetres per minute,[1] is regular, and by most observers is said to move always in one direction. The progress of the contraction, as suggested by Nothnagel's experiments,[2] and, as clearly stated by Mall and by Bayliss and Starling, is dependent upon a local reflex. According to Mall,[3] when an object stimulates the mucosa there occurs above the point of stimulation a constriction which forces the object downward; and as it moves downward new regions immediately above the mass are by this reflex brought into constriction, and thus the wave and its stimulus advance together. "At the same time," Mall states, "a sucking force, due to active dilatation below the body, may have a tendency to drag it down." In what manner an active dilatation of the intestinal wall may occur so as to produce a "sucking force," he does not make wholly clear. Bayliss and Starling, in describing normal peristalsis in the intestine, state that the contractions above the bolus increase until there is a strong tonic constriction.[4] This passes the bolus onward, and as it advances the ring of constriction follows it. While the bolus is passing down, the gut above it is traversed by waves running as far as the constricted ring. These observers state the law of intestinal peristalsis thus: "Local stimulation of the gut produces excitation above and inhibition below the excited spot."

The pendulum movements are characterized by a gentle swaying motion of the coils, and are accompanied by rhythmical contractions of the intestinal wall. They continue with undiminished force after paralysis of the local nervous mechanism by nicotine or cocaine; they have been called, therefore, myogenic or myodromic contractions. Observers have described them variously as shortenings and narrowings of the gut, rhythmically repeated at nearly the same intestinal circumference;[5] as alternating to-and-fro movements of the long axis without

[1] Cash: Proceedings of the Royal Society, 1886, xli, p. 227.

[2] Nothnagel: Archiv für pathologische Anatomie und Physiologie, 1882, lxxxviii, p. 5.

[3] Mall: Johns Hopkins Hospital Reports, 1896, i, p. 51.

[4] Bayliss and Starling: Journal of physiology, 1899, xxiv, p. 106.

[5] Ludwig: Lehrbuch der Physiologie des Menschen. Leipzig und Heidelberg, 1861, ii, p. 615.

changes in the lumen;[1] as local or extensive periodic contractions and relaxations mainly of the circular musculature;[2] and as waves involving both muscular coats of the intestine, and travelling normally from above downward at a rapid rate (2 to 5 cm. per second).[3] They have been seen in the dog,[4] and in the rabbit and cat.[5] In the cat Bayliss and Starling noticed that when the lumen of the gut was distended by a rubber balloon, there appeared rhythmical contractions, which nearly always were most marked at about the middle of the balloon, *i. e.*, the region of greatest tension. This form of constriction, which, as my observation shows, is an indication of the manner in which the rhythmical contraction acts in the cat's intestine, Bayliss and Starling seem to have regarded with slight attention, since it did not accord with the law of peristalsis.

The swift vermicular wave may pass the whole length of the intestine in about a minute. It is often seen just after death, as well as in pathological states such as intestinal anæmia or hyperæmia, and when the bowel contains gases and organic acids from decomposing food.[6] Starling is inclined to regard this type of intestinal activity as an exaggeration of the rhythmic type; Mall, on the other hand, places it in a class by itself, and declares that its service is to rid the intestine rapidly of irritating substances. Nothnagel, who designates this form of movement with the term *Rollbewegung*, thinks it is transitional between a physiological and a pathological activity.

The existence of antiperistalsis has been so much questioned that it will be considered in a special section of this paper, where my observations may be conveniently introduced.

The common understanding of the manner in which food passes through the intestinal canal is that the chyme ejected from the stomach is pressed downward by a peristalsis, which passes slowly over a part or all of the small intestine. The peristaltic waves of the colon are supposed to constitute an independent group, similar to those of the small intestine, but weaker and slower. Movements

[1] Raiser: Beiträge zur Kenntniss der Darmbewegung. Dissertation. Giessen. 1895, p. 7. Nothnagel: Die Erkrankungen des Darms und des Peritoneum. Wien, 1898, i, Darmbewegung. p. 1.

[2] Mall: *Loc. cit.*, p. 48.

[3] Bayliss and Starling: Journal of physiology. 1899, xxiv, p. 103.

[4] *Ibid.*

[5] Bayliss and Starling: Journal of physiology, 1901, xxvi, pp. 127 and 134.

[6] Bokai: Archiv für experimentelle Pathologie and Pharmakologie, 1887, xxiii, p. 232.

of the food other than the uninterrupted advance have been mentioned by some observers. Starling [1] states that the effect of the pendulum movement is to mix the contents of the intestine and bring them into intimate contact with the mucous membrane. Grützner writes that he has been brought "by strange and peculiar observations" to believe that the fluid contents of the small intestine move irregularly forward, then forward and back, then perhaps remain quiet for some time, only to pass backward for a long distance and finally to move forward steadily to the end. In this manner the food is mixed, and brought into contact with the absorbing walls.[2] The to-and-fro shiftings of the food Grützner ascribed to advancing and retrograde contractions of the intestinal musculature, and he argued that even circular constrictions must force the liquid contents away in both directions. To support his contention Grützner introduced mercury into the intestine and observed it with the Röntgen rays. After noting a backward and forward movement of the mercury he dismissed the method, saying, "Many a flash must come from the Röntgen tube before the *normal* movement of the intestinal contents is made entirely clear by this method."

The following account is a summary of many repeated observations on different animals, and is a contribution to a clear understanding of the normal movements of the intestines and their contents.

The Method.

The method employed in this investigation is identical with that used in 1897 to observe the movements of the stomach.[3] Subnitrate of bismuth, one-tenth to one-third the weight of the food, was mixed with what the animal ate. Thus far observations have been made almost exclusively on cats. The subnitrate of bismuth was generally mixed with canned salmon, — a food which cats relish. The animal to be observed was usually not allowed to eat anything during the day previous to the observation, and moreover was commonly given from four to six teaspoonfuls of castor oil to clear the bowels.

A tranquil mood on the part of the animal was found to be quite as necessary for seeing movements of the intestine as it was for

[1] Starling: Text-book of physiology, edited by Schäfer. Edinburgh and London, 1900, ii, p. 330.

[2] Grützner: Archiv für die gesammte Physiologie, 1898, lxxi, p. 515.

[3] Cannon: This journal, 1898, i, p. 362.

securing normal activity of the stomach. For this reason female cats, which submit quietly to the confinement of the holder and the straps, proved to be much more favorable subjects than the males, which struggle violently when tied into the hammock. Curiously the crackling and rumble of the static machine, which generated the electricity for producing the X-rays, instead of frightening the animal had often a soothing effect.

The appearance of the food in the alimentary canal is shown in Fig. 1, the reproduction of a radiograph taken five and three-fourths hours after the animal finished eating. The cat lay on her back, and the photographic plate was placed over the front of the abdomen. The intestines move up and down in the body cavity with each respiration; in order to secure clear outlines, a leaden plate was slipped between the cat and the Crookes tube during inspiration and the beginning of expiration, and then removed till the beginning of inspiration. Thus the plate was exposed to the rays only during the pause recurrent at the end of each expiration, when the shadows resume approximately their former position. Records were taken, both by means of radiographs and by means of tracings made with a soft pencil on tissue paper laid over the fluorescent surface of the screen. The reliability of the latter method has been proved by comparison with radiographs taken immediately before or afterwards.

FIGURE 1. — Appearance of food in the intestines $5\frac{3}{4}$ hours after eating. This and other radiographs reduced two-thirds.

In Fig. 1 the spinal column is seen in the middle line with the pelvis below. On the right, above, is the pyloric end of the stomach, and on the left, dimly outlined as a lighter area, because of the presence of gas, is the ascending colon. In the cæcum there is a small amount of food present. Loops of small intestine containing food are to be seen in various parts of the abdomen. These loops are often distinct enough to allow movements in them to be seen without any manipulation; when this is not the case, however, and the loops overlie one another, as on the right side of Fig. 1, a slight pressure with the fingers through the abdominal wall will readily separate from neighboring loops the one to be observed.

The Movements of the Small Intestine.

When the food has been distributed through the intestine so as to present the appearance shown in Fig. 1, a noticeable feature in most or all of the loops is the total absence of movement. If the animal remains quiet, however, only a few moments elapse before peculiar motions appear in one or another of the loops, or perhaps in several, and last for some time. These motions consist in a sudden division of one of the long, narrow masses of food into many little segments of nearly equal size; then these segments are again suddenly divided and the neighboring halves unite to make new segments, and so on, in a manner to be more fully described. I have called this process the rhythmic segmentation of the intestinal contents. Further observation reveals peristalsis here and there, and under certain circumstances the typical swaying movements may be seen. All these phenomena are now to be considered in detail.

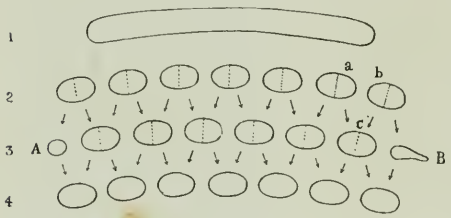


FIGURE 2. — Diagram representing the process of rhythmic segmentation. Lines 1, 2, 3, 4 indicate the sequence of appearances in the loop. The dotted lines mark the regions of division. The arrows show the relation of the particles to the segments they subsequently form.

Rhythmic segmentation of the intestinal contents. — This is by far the most common and the most interesting mechanical process to be seen in the small intestine. The nature of the process may best be understood by referring to the diagram in Fig. 2. A string-like mass of food is seen lying quietly in one of the intestinal loops, line 1, Fig. 2. Suddenly an undefined activity appears in the mass, and a moment later constrictions at regular intervals along its length cut it into little ovoid pieces. The solid string[1] is thus quickly transformed, by a simultaneous sectioning, into a series of uniform segments. A moment later each of these segments is divided into two particles, and immediately after the division neighboring particles (as *a* and *b*, line 2, Fig. 2) rush together, often with the rapidity of flying shuttles, and merge to form new segments (as *c*, line 3, Fig. 2). The next moment these new segments are divided,

[1] In lieu of any better short expression, "string" of food is used to designate the long slender mass of the contents lying in a loop of the intestine.

and neighboring particles unite to make a third series, and so on. At the time of the second segmentation (line 3, Fig. 2) the end particles are left small. Observation shows that these small pieces are not redivided. The end piece at A simply varies in size with each division; at one moment it is left small, at the next moment it is full size from the addition of a part of the nearest segment, and a moment later is the small bit left after another division. The end piece at B (probably the rear of the mass) shoots away when the end mass is divided, and is swept back at each reunion to make the large end mass again, only to be shot away and swept onward with each recurrence of the constrictions. Thus the process of repeated segmentation continues, with the little particles flitting toward each other and the larger segments shifting to and fro, commonly for more than half an hour without cessation. From the beginning to the end of a period of segmentation the food is seen to have changed its position in the abdomen to only a slight extent; whether this change is a passing of the food along the loop, or a movement of the loop itself, it is impossible to tell from the shadows on the screen. The change of position, however, is much less conspicuous than the lively division and redivision which the mass suffers so many times from the busy, shifting constrictions.

From this typical form of rhythmic segmentation there are several variations. Sometimes, and especially when the mass of food is thick, the constrictions do not make complete divisions and are so far apart that the intermediate portions are relatively large. Moreover the constrictions do not take place in the middle of each portion, but near one end; thus each portion is constricted, not into halves, but into thirds. If a little pointer is placed at the middle of a segment, when the segments are completely divided into halves, in a few seconds the pointer will be in the middle of the clear space between two segments; but in a few seconds more the first phase will return and the pointer will again be indicating a segment, — two operations intervene between similar phases. When, however, the portions are constricted into thirds, the indicator shows it, since three operations intervene between similar phases. The manner of these changes is made clearer by reference to the diagram in Fig. 3. That each portion is constricted into three pieces is proved also by watching the gradual reduction of the portion at the left end of line 1 through lines 2 and 3; and also in the gradual formation of a full-sized portion at the right end of lines 2, 3, and 4. When food undergoing this process is watched, it

appears to be affected by a series of constrictions, each of which starts at one end of the mass and marches through to the other end, leaving its impress at short intervals along the length. The progression of the dotted lines from right to left in *a*, *b*, *c*, and *d*, etc., Fig. 3, gives a notion of these advancing constrictions.

Another variation of the segmentation is shown in Fig. 4. In this type there are evidently divisions and subdivisions, *i. e.*, one more operation between the appearance and the reappearance of the same phase than is present in the simple division of the small segments in a long string of food (Fig. 2). This form of segmentation is fairly typical for the constrictions seen in food advancing through the intestine. Sometimes the divisions occur in the middle of a long string of food and leave the ends wholly unaffected.

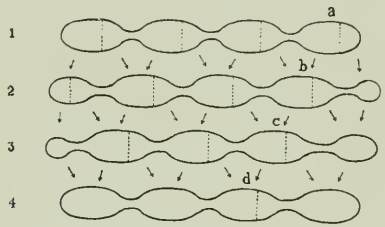


FIGURE 3.— Diagram showing the relations of the portions when they are constricted into three pieces. The dotted lines indicate regions of constriction; the arrows indicate the relationship of the pieces to the portions they subsequently form.

A remarkable feature in the segmentation of the food is the rapidity with which the changes take place. The simplest way of estimating the rate of division is to count, not the number of times the partition of the food recurs in the same place, but the number of different sets of segments observed in a given period. Thus in Fig. 4 the appearances of lines 1, 2, 3, 4, etc., would be counted, and not merely lines 1, 4, etc. Repeated observations on different animals have shown that the most common rate of division in long, thin chains of food varies between 28 and 30 times in a minute, *i. e.*, there is a change from one set of segments to another set every 2 seconds, and a return of the same phase every 4 seconds. In some cases the rate is as low as 23 times per minute. The larger masses seem to be associated with a slower segmentation; the operations indicated in Fig. 3, for example, occurred from 18 to 21 times in a minute, so that the same phase reappeared only once in 8 or 9 seconds. The segmentation frequently continues for more than half an hour; in one instance it was seen to persist with only three short periods of inactivity for two hours and twenty-two minutes. At the rate of 30 segmentations per minute it is clear that a slender string of food may commonly undergo division into small particles

more than a thousand times while scarcely changing its position in the intestine.

I have seen once, in a cat only lightly etherized, the exterior of an intestine which was dividing the food as above described. An hour and a half after a meal of salmon the anæsthetic was given, the abdomen opened, and the flaps raised so as to form walls. Warm salt solution was then poured into the abdominal cavity, and the floating coils left covered with the transparent omentum. The gastric peristaltic waves were running regularly; on the intestine there were visible at various places during the period of observation regions of constriction which had the appearance shown in Fig. 3, except that the rings were relatively nearer together. New rings of constriction took place on the same side of all the bulging parts at the margin of the constricted portion (*cf.* dotted lines, Fig. 3). As new rings occurred the old relaxed, but apparently with tardiness, for the contents gurgled as if forced through the narrowed lumen. The constrictions recurred irregularly and at much longer intervals than in the normal animal. The contracted rings were pale and bloodless.

The effect of the process of rhythmic segmentation proves it an admirable mechanism. The food over and over again is brought into closest contact with the intestinal walls by the swift kneading movement of the muscles. Thereby not only is the undigested food intimately mixed with the digestive juices, but the digested food is thoroughly exposed to the organs of absorption. Mall[1] has shown that contraction of the intestinal wall has the effect of pumping the blood from the submucous venous plexus into the radicles of the superior mesenteric vein and thus materially aids the intestinal circulation. Moreover, lacteals loaded with fat will in a few moments become empty unless the intestine is slit lengthwise so that the muscles cannot exert compression.[2] The rhythmic constrictions, therefore, both propel the blood in the portal circulation and act like a heart in promoting the flow of lymph in the lacteals. This single movement with its several results is an excellent example of bodily economy: the repeated constrictions, as already shown, thoroughly churn the food and digestive fluids together, and also plunge the absorbing mucosa into the very midst of the food masses; but not only are the processes of digestion and absorption favored by these movements, — they also, by compression of the veins and lacteals of the intestinal

[1] Mall: *Loc. cit.*, p. 68. [2] Mall: *Loc. cit.*, p. 47.

wall, serve to deport through blood and lymph channels the digested and absorbed material.

Peristalsis.[1] — The phenomena of peristalsis and segmentation are usually combined in some manner while the food passes through the small intestine. Peristalsis is observed normally in two forms; as a slow advancing of the food for a short distance in a coil, and as a rapid movement sweeping the food without pause through several turns of the gut. The latter form is frequently seen when the food is carried on from the duodenum; and it may readily be produced in other parts of the small intestine by giving an enema of soapsuds.

When a mass of food has been subjected for some time to the segmenting activity of the intestine, the separate segments, instead of being again divided, may suddenly begin to move slowly along the loop in which they lie. That this movement is not a swinging of the coil as a whole, but a peristaltic advance of separate rings of its circular musculature, is made probable by the fact that the succeeding segments follow along the same path their predecessors have taken. The advance of the little pieces may continue for seven or eight centimetres, when finally the front piece stops or meets other food. Then all the succeeding pieces are swept one by one into the accumulating mass, which at last lies stretched along the intestine, a solid string manifesting no sign of commotion.

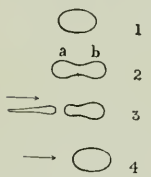


FIGURE 4.—Diagram showing combined peristalsis and segmentation.

Another form of slow peristalsis is frequently observed when the food is pushed forward, not in small divisions, but as a large lump. The relatively long string of food is first crowded into an ovoid form as the forward movement begins, and as it is collecting thus, it seems at the last to be suddenly formed into a more rounded ball, as if the mass were pulled or pushed together at the two ends. The next moment it is indented in the middle by a circular constriction (as shown in Fig. 4, line 2), which spreads it in both directions along the loop. The trailing portion (*a*) is next cut in two, and the severed part some-

[1] Without the possibility of seeing the relations of a movement to the ends of the intestine it cannot be stated absolutely whether the movement is peristaltic or antiperistaltic. Such relations can be seen on the fluorescent screen only near the stomach and near the ileocæcal valve. The evidence that advancing peristalsis is the normal movement is so overwhelming that I have assumed that when food is moving in loops not visibly related to fixed points, it is moving forward.

times flies back over its course about a centimetre. Now the whole mass is swept together again and slightly forward as shown in line 4, Fig. 4, and the segmenting process is repeated. At stage 3, Fig. 4, a constriction sometimes appears around the middle of the advanced portion (*b*). Thus with many halts and interruptions the food slowly advances.

A slight variation of the movement just described is observed when the amount of food is greater and extends farther along the intestine. Under such circumstances, as the mass moves forward, constrictions appear just in front of the rear end, which separate it from the main body, and cause it to shoot backward sometimes through the distance of a centimetre. The main body meanwhile is not disturbed. No sooner has the rear section been shot away than it is swept forward again into union with the rest of the food, and the whole mass then advances until another interfering constriction repeats the process.

Rhythmic segmentation and the pendulum movement. — There is little doubt that the segmentation of the food which I have seen is due to an activity of the intestinal musculature similar to that which causes the so-called pendulum movement. This activity, as already noted, is rhythmic, and, although accounts differ,[1] analytical methods prove that it involves both the longitudinal and the circular layers of muscle. Observations of the effect of the rhythmic contractions upon the food show that the action of the circular fibres is most prominent. It is probable, however, that the longitudinal fibres also play an important part in the process of segmentation. Examination of Fig. 2 makes clear that in line 2 the regions of constriction appear between the regions of constriction in line 3; before *c* can be formed, therefore, the constriction between *a* and *b* must relax. Contraction of the longitudinal fibres between two segments would help to enlarge the constricted lumen of the gut. It seems probable that, as the constrictions on either side of *c* occur, the longitudinal fibres between them contract; almost simultaneously the constriction between *a* and *b* relaxes, and the two particles are thus brought swiftly together. A similar process naturally would take place for each of the shifting segments. Thus the function of the longitudinal muscles would be to contract between new rings of constriction and thereby aid in relaxing the former ring between them. During my one observation of the segmenting process as seen on

[1] See page 252.

the surface of the intestine, I could not be sure that the distance between neighboring segments was shortened as the constriction relaxed; that activity of the longitudinal fibres is present, however, is indicated by observations of Raiser[1] on the intestines of the rabbit and the cat. Raiser observed the outer surface of the coils, and describes the normal movement as an alternate contraction and relaxation of single divisions of the longitudinal fibres; he notes that these short divisions shift. But whether they shift in alternation with the shifting circular constrictions, as seems probable, is an interesting point not yet determined.

Bayliss and Starling state that the swaying pendulum movements are essentially due to peristaltic waves recurring in the same place and running rapidly downward. This form of the movements I have seen only once. At this time about 90 c.c. of soapy water had been injected. This procedure has the effect of exaggerating in every particular the movements of the small intestine. In this instance a broad constriction appeared about the middle of a long string of food and persisted there while it spread down the gut. As the contraction spread, the gut swayed slowly to and fro before it. Then there was a relaxation, followed by a recurrence of the constriction in the same place, a spreading of the contraction, and a swinging of the loop just as before. This phenomenon was repeated again and again till finally the string was divided and the forward piece pushed through a tortuous course to the colon.

FIGURE 5.—Tracing showing segmentation of chyme in the duodenum. This and other tracings reduced two-thirds.

The course of the food in the small intestine. — Chyme is not forced from the stomach by every wave that passes over the antrum, but only at intervals.[2] When the pylorus relaxes, the food, moved towards the pylorus under considerable pressure, is squirted along the duodenum for two centimetres or more. Careful watching of this food shows that usually it lies for some time in the curve of the duodenum until additions have been made to it from the stomach, and a long, thin string of food is formed. While it is resting in this place it is exposed to the outpouring of the bile and pancreatic juices. All at once the string becomes segmented (see Fig. 5), and the process of rhythmic segmentation continues several minutes, thoroughly mixing the

[1] RAISER: *Loc. cit.*, p. 7. [2] CANNON: *Loc. cit.*, p. 369.

intestinal digestive juices with the chyme. In this region the alternate positions of the segments are sometimes far apart, and the to-and-fro movement of the particles may be a relatively extensive and very energetic swinging. Finally the little segments unite into a single mass, or form in groups, and begin to move forward. The peristalsis here, as already mentioned, is much more rapid than the normal peristalsis elsewhere in the small intestine. The masses once started go flying along, turning curves, whisking hither and thither in the loops, moving swiftly and continuously forward. After passing on in this rapid manner for some distance the food is collected in thicker and longer strings resembling the strings seen characteristically in the other loops. Towards the end of digestion the small masses shot out from the stomach, after a few segmentations, may move on in the rapid course without being accumulated in a larger mass until the swift movement ceases.

During the first stages of digestion in the cat's small intestine the food usually lies chiefly on the right side of the abdomen; during the last stages the loops on the left side contain the greater amount of food. In these loops the food remains sometimes for an hour or more with no sign of movement. All at once a mass begins to show irregular depressions and elevations along its length, and then suddenly it is divided, at first partially, later completely, into many little equal parts, and these repeatedly undergo division and reunion, division and reunion, over and over again, in the manner described above as rhythmic segmentation. After a varying length of time the activity wanes, and the little segments are carried forward individually and later brought together, or join and move on as a single body, or they may reunite and lie quietly for some time without further change. Thus by a combined process of kneading and peristaltic advance the food is brought to the ileocæcal valve to enter the large intestine. Records from ten different animals show that salmon does not appear in the small intestine until an hour or an hour and a half after the food is eaten. Inasmuch as five or six hours elapse after eating before this food begins to be seen in the colon, it is evident that the chyme takes four to five hours to pass the length of the small intestine. It is interesting to note that the operations are considerably shortened if the meal has consisted of bread and milk.

The Competence of the Ileocæcal Valve.

The ileocæcal valve in the cat is situated three or four centimetres from the blind end of the cæcum. Its position is usually marked in shadows of the food in the colon by a slight indentation, towards which masses about to enter the colon are ordinarily directed from a point somewhat distant in the small intestine (see Fig. 6).

Regarding the competence of the ileocæcal valve many observations have been made. Grützner has reviewed the evidence bearing on the question [1] and concludes that the valve is not competent, least of all for liquids. He declares that as soon as liquids or thin fluid masses appear in the upper part of the colon, they pass in many instances into the small intestine the moment that the pressure on the colon side rises slightly. If the colon contains a solid or a thick mushy mass, the passage towards the small intestine is scarcely possible, because every increase of pressure in the large intestine must force the two lips of the valve together and close it.

The importance of the competence of the ileocæcal valve under normal conditions cannot be appreciated until the function of the first part of the colon is considered. In order that this part of the intestinal mechanism may perform its service, the competence of the valve for the food which enters the colon from the ileum should be perfect. As a matter of fact such is the case. Not only does the activity of the colon prove this statement, but the failure of every attempt to drive the food in the colon back through the valve into the ileum confirms the proof. Again and again I have tried, by manipulation through the abdominal wall, to press the normal contents of the colon downward with sufficient force to cause them to return to the small intestine, but without success. The valve held perfectly.

The Movements of the Large Intestine.

When the large intestine is full, palpation through the abdominal wall demonstrates that the material in the lower descending colon [2] and in the sigmoid flexure is usually composed of hard, incompressible lumps, while that in the ascending and transverse colon and

[1] Grützner: Archiv für die gesammte Physiologie, 1898, lxxi, p. 495.

[2] The large intestine in the cat has no fixed ascending, transverse, and descending portions; in this paper these terms are used to designate the parts of the curve of the colon which occupy these relative positions.

the cæcum is soft, permitting the walls of the gut to be easily pushed together. The condition of the contents in these two regions seems to indicate a rough division of the large intestine into two parts, and the mechanical activities of these two parts verify the differentiation. In the descending colon the material is very slowly advanced by rings of tonic constrictions (see Fig. 7); in the ascending and transverse colon and in the cæcum by far the most common movement is an antiperistalsis.

Antiperistalsis in the colon. — The colon of cats which have been without food for a day usually contains enough gas to make the position of the gut distinguishable with the fluorescent screen (see Fig. 1). The first food to enter the colon from the small intestine is carried by antiperistaltic waves into the cæcum (Fig. 1), and all new food as it enters is also affected by these waves. Thus the contents of the colon, instead of being driven immediately toward the rectum by slow peristalsis, as is the general opinion, are first repeatedly pushed toward the cæcum by an antiperistaltic action.

These antiperistaltic waves follow one after another like the peristaltic waves of the stomach (see Figs. 5, 6, and 10). They begin either on the more advanced portion of the food in the colon (when only a small amount is present), or at the nearest tonic constriction, which is usually at the turn between the transverse and descending colon (Figs. 7 and 8). The waves rarely run continuously for a long time. When the colon is full, it is usually quiet. The first sign of activity is an irregular undulation of the walls, then very faint constrictions passing along the gut toward the cæcum. These constrictions may first appear only on the ascending colon. As they continue coursing over the intestine they become deeper and deeper until there is a marked bulging between successive constrictions. When the waves have thus become more prominent, they are seen to start near the end of the transverse colon and pass without interruption to the end of the cæcum. After these deepest waves have been running for a few minutes the indentations grow gradually less marked until at last they are so faint as to be hardly discernible. The final waves are sometimes to be observed only at the end of the transverse colon.

Such a period of antiperistalsis lasts from two to eight minutes, with an average duration of four or five minutes. The periods recur at varying lengths of time; in one instance a period began at 1.38 P. M. and was repeated at 2.06, 2.34, 2.55, 3.15, and at 3.36,

when the observation ceased; in another instance a period began at 2.43 P. M. and was repeated at 2.57 and at intervals of from ten to fifteen minutes thereafter while the animal was being watched. The waves have nearly the same rate of recurrence as those in the stomach; about five and a half waves pass a given point in a minute, *i. e.*, eleven waves in two minutes. This rate has proved fairly constant in different cats and at different stages in the process of digestion; in one case, however, the waves passed at the rate of nine in two minutes.

The stimulating effect of rectal injections on the movements of the small intestine has already been noted. Enemata have also pronounced stimulating action on the antiperistalsis of the colon. Usually the almost immediate result of a rectal injection of warm water is the appearance of deep antiperistaltic waves, which often continue running for a long period. In one case, after an injection of 50 c.c. of warm water, the waves followed one another with monotonous regularity during an observation lasting an hour and twenty minutes. The manner in which this antiperistaltic mechanism affects nutrient enemata introduced into the bowel will be discussed in the section devoted to the question of antiperistalsis.

These constrictions passing backward over the colon do not force the normal contents back through the valve into the small intestine again. I have seen hundreds of such constrictions, and only twice have there been exceptions to this rule, — once under normal conditions, when a small mass slipped back into the ileum, and at another time when a large amount of water had been introduced into the colon. The importance of the competence of the ileocæcal valve is now apparent; indeed, antiperistalsis in the colon gives new meaning and value to the location of a valve at the opening of the ileum. For, inasmuch as the valve is normally competent, the constrictions repeatedly coursing toward it force the food before them into a blind sac. The effect on the food must be the same as the effect seen in the stomach when the pylorus remains closed before the advancing waves. The food is pressed forward by the approach of each constriction; but since it cannot go onward in the blind sac, and is, moreover, subjected to increasing pressure as the constriction comes nearer, it is forced into the only way of escape, *i. e.*, away from the cæcum through the advancing constricted ring. About twenty-five waves affect every particle of food in the colon in this manner during each normal period of antiperistalsis. The result must be again a

thorough mixing of the contents and a bringing of these contents into close contact with the absorbing wall — a process which has already been variously repeated many times in the stomach and in the small intestine.

Two other movements have been observed in the ascending colon, but they are rare appearances. The first of these was a serial sectioning of the contents noticed in an animal given castor oil with the food. A constriction separated a small segment in the cæcum; another constriction then cut off a segment just above the first, and with the disappearance of the first constriction the two separated segments united. A third segmentation took place above the second, and the changes occurred again. Thus the whole mass was sectioned from one end to the other; and no sooner was that finished than the process began again and was repeated several times. A slight modification of this movement was observed in a colon containing very little food. The mass was pressed and partially segmented in the manner characteristic of the small intestine, and was thus again and again spread along the ascending colon and each time swept back into a rounded form by antiperistalsis. The second of the two movements mentioned above consisted in a gentle kneading of the contents. This was caused by broad constrictions appearing, relaxing, appearing, relaxing, over and over again in the same place. When several of these regions were active at the same time they gave the food in the colon the appearance of a restless undulatory mass. Once a constriction occurred and remained permanently in one place while the bulging parts on either side of it pulsated alternately, at the rate of about eighteen times in a minute, with the regularity of the heart-beat. Although these phenomena are somewhat striking, they are not usual, and are in no way so important as the antiperistalsis.

The changes when food enters the colon. — The passage of food through the ileocæcal valve seems to stimulate the colon to activity. As food is nearing the ileocæcal valve the large intestine is usually quiet and relaxed (Fig. 6, 4.00), though occasionally indefinite movements are to be observed; and sometimes just before the food reaches the end of the ileum the circular fibres of the colon in the region of the valve contract strongly, so that a deep indentation is present there. The indentation may persist several minutes; it disappears as the muscles relax just previous to the entrance of the food. The food is moved slowly along the ileum and is pushed through the

valve into the colon. The moment it has entered, a strong contraction takes place all along the cæcum and the beginning of the ascending colon, pressing some of the food onward, and a moment later deep antiperistaltic waves (Fig. 6, 4.03) sweep down from the transverse colon and continue running until the cæcum is again normally full, *i. e.*, for two or three minutes.

The appearance of tonic constrictions. — It has already been noted that as the food accumulates in the ascending colon it is at first confined to this region by antiperistaltic waves. With further accessions, however, the contents naturally must be pressed more and more into the transverse and descending colon. In the early stages of this accumulation, while the food lies chiefly in the ascending colon, the only activity of the muscular walls is the antiperistalsis. As the contents extend along the intestine a deep constriction appears near the advancing end and nearly separates a globular mass from the main body of the food (Fig. 6). The contents of the large intestine progress farther and farther from the cæcum ; meanwhile new tonic constrictions appear which separate the contents into a series of globular masses. And as the number of these divisions increases they take a position farther from the cæcum so that they are present chiefly in the descending colon (Fig. 7). Raiser[1] has recorded a similar appearance in the terminal portion of the rabbit's colon, in which deep circular constrictions separate the scybalous masses. He maintains that these masses are pushed onward by the constrictions. Comparing tracings made at rather long intervals (forty-five minutes), I found that the rings disappear from the transverse colon and then are present with the waste material in the descending colon. Thus in the cat also these rings which seem with short observation to be remaining in one position are in reality moving slowly away from the cæcum, pushing the hardening contents before them. The contents at this stage are no longer fluid, and consequently they must offer considerable resistance to a force pushing

FIGURE 6. — Tracings showing changes when food enters the colon and also the first tonic constriction. 4.00, the colon relaxed as food approaches in the ileum. 4.03, the colon contracted and traversed by antiperistaltic waves after the food has entered.

[1] RAISER : *Loc. cit.*, p. 12.

them through the colon. It is an advantage to have this pultaceous substance propelled in divisions rather than in a uniformly cylindrical mass, since the fibres along the length of the mass are thereby rendered effective. Such are the functions of the persistent rings, — they form the waste matter into globular masses at the end of the transverse colon and slowly push these masses onward.

FIGURE 7.— Radiograph showing the region of tonic constrictions (descending colon), and the region of antiperistalsis (transverse and ascending colon).

In the transverse colon, which is free from the slowly-moving rings, the antiperistaltic waves have full sway. In the region of the tonic rings an infrequent or even a slowly periodic relaxation and contraction are often to be observed. These changes seem to take place in all the rings at about the same time. Once I saw antiperistaltic waves running over the uppermost of four segments, but, since the rings on either side of the segment held tightly, the waves had merely the effect of churning the material of the segment and did not move it onward. Inasmuch as the material in these segments at first is soft, so that the segments are easily compressible, while the fecal masses which are the final result are relatively hard and dry, it follows that even within the confines of these persistent rings some absorption is taking place.

DEFECATION.

The process of clearing the colon is a process of repeated reduction of the amount of material present. Figure 8 (3.11) is a radiograph showing the food in the colon at 3.11 P. M. About 3.25, with a slow sweeping movement, the gut swung around so that the ascending colon was lying in the position of the last half of the transverse colon, and the transverse colon had taken the position of the descending part (Fig. 8, 3.25). At the same time the tonic constrictions disappeared and were replaced by a strong, broad contraction of the circular muscle, tapering the contents off on either side in two cones. The region of strongest contraction was apparently drawn downward with the rest of the gut by a shortening of the descending colon. As the intestine swung around, more material was forced into the rectum,

and when the swinging of the intestine stopped, the constriction which divided the lumen passed slowly downward and with the aid of the muscles surrounding the abdominal cavity pushed the separated mass out of the canal.[1] After the terminal mass had thus been pushed out, the colon with the remainder of its contents returned to nearly its former position (Fig. 8, 3.46). About two hours afterward this remnant had been spread throughout the length of the large intestine by means of the slowly-moving rings. Fig. 7 is a radiograph of the same colon pictured in Fig. 8; the radiograph was taken at 11.50 A. M., and at 12.15 P. M. the material in the lower descending colon was forced out in the manner above described. Within three hours the remaining portion had been spread into the evacuated region, as shown in Fig. 8, 3.11. The manner in which the material is spread from the region of the antiperistaltic waves into the region of the slowly advancing rings presents a problem. During normal living new food constantly arriving in the colon must force the old contents forward

FIGURE 8. — Two radiographs and a tracing showing the changes taking place in defecation. 3.11, material in the colon. 3.25, colon carried downward and terminal mass separated. 3.46, after defecation, when the colon returns to former position. Defecation occurred at 3.27.

just as the later parts of a meal force forward the earlier parts; there is no doubt, however, that most of the contents of the cæcum and the ascending colon may be passed onward even during starvation. The

[1] In this case the fæces were soft.

emptying of these regions, according to my observations, is never complete; for, after considerable time has elapsed and the large intestine is cleared and dilated with gas, some substance is still to be detected in the cæcum and clinging to the walls of the ascending colon. The only activities manifested here are the antiperistaltic waves, and the strong tonic contraction of the whole circular musculature shown in Fig. 6. It is clear that the latter activity would serve to press into the transverse colon a considerable portion of the contents of the ascending colon, and the remnant seen clinging to the walls would be the part not thus pressed forward.

Twice I have seen appearances which might account for the emptying of the first portion of the large intestine in a more thorough manner than that above described. At one time, without apparent stimulation, strong tonic contraction occurred along the entire length of the ascending colon, which forced the contents almost wholly into the transverse portion. This action seemed merely an exaggerated form of that observable after food passes the ileocæcal valve (see Fig. 6). At another time, after a mass of food had passed through the ileocæcal valve, after the ascending colon had contracted generally and the antiperistaltic waves had coursed over it in the usual manner, a deep constriction appeared at the valve and ran upward without relaxation nearly the length of the ascending colon, pushing the contents before it. For an instant the wave paused; then the constriction relaxed, and the food returned toward the cæcum. These observations indicate that either a general contraction of the wall of the large intestine or a true peristalsis may be effective in pressing waste matter from the region where antiperistalsis is the usual activity into the region where the slowly-advancing rings may carry it on to evacuation (see Fig. 7).

The Question of Antiperistalsis.

In 1894, Grützner[1] published an observation and made an assumption, about which there has since been much controversy. He maintained that when normal salt solution, holding in suspension hair, powdered charcoal, or starch grains, is injected into the rectum, it is carried upward into the small intestine and may even enter the stomach. These experiments have been repeated by several observers. Some have confirmed Grützner's results; others have failed,

[1] Grützner: Deutsche medicinische Wochenschrift, 1894, xx, p. 897.

after using most careful methods, to find any evidence of the passage of the injected material back to the stomach, and they have declared that the apparent success was due to carelessly allowing the food of the animal to become contaminated with the test materials, so that these were introduced into the stomach by way of the mouth. That antiperistalsis does not occur in the small intestine seems to be proved by Mall's experiment[1] of reversing a portion, sewing it in place, and then finding that the food does not pass the reversed region, but collects at the upper end. Sabbatani and Fasola[2] reversed stretches of small intestine of varying length, and found that the reversed portions allowed fluids to pass, but that the persistence of the physiological direction of movement caused an accumulation of undigested food in the region of the upper suture. However a portion of the intestine lay in relation to the rest, it always manifested the normal peristalsis. Many other observers[3] working directly on the intestine confirm this testimony and state that the progress of the constriction-rings is always downward and that antiperistalsis is not physiological. In 1898, however, Grützner[4] took his stand again in favor of a backward movement in the intestines, and in a somewhat metaphysical manner argued that peristalsis and antiperistalsis belong to each other just as relaxation of muscle is related to contraction. He assumed that as the contents are advanced by slow peristalsis, so are they returned by a similar movement in the opposite direction, and he mentions several pathological cases (fistula of intestine) to substantiate the assumption.

By means of the X-rays it is possible to see just what takes place when a fluid is injected into the rectum. For the purpose of determining how nutrient enemata are received and acted upon in the intestines, I have introduced thin fluid masses in large and small amounts, and thick, mushy masses in large and small amounts, in different animals. The enemata consisted of 100 c.c. of milk, one egg, ten to fifteen grams of bismuth subnitrate, and two grams of starch to hold the bismuth powder in suspension. To make the thick enema all these were stirred together and boiled to a soft mush; to make the thin enema all the parts were boiled together except

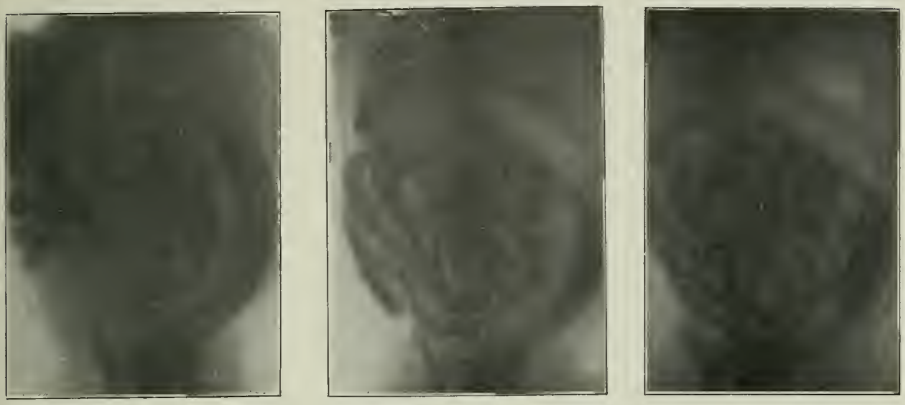
[1] Mall: Johns Hopkins Hospital Reports, 1896, i, p. 93.

[2] Sabbatani and Fasola: Archives italiennes de biologie, 1900, xxxiv, p. 195.

[3] See Fubini and Luzzati: Moleschott's Untersuchungen, 1888, xiii, p. 386, for an array of observers who declare that antiperistalsis is not a normal movement.

[4] Grützner: Archiv für die gesammte Physiologie, 1898, lxxi, p. 513.

the egg, which was added after the boiled portion was cooled. The small amount injected was 25 c.c.; the large amount almost 90 c.c., about the capacity of the large intestine when removed from the body. The animals were given first a cleansing injection, and after this was effective the nutrient material was introduced. In order to make sure of the observation, a control radiograph was first taken to show no bismuth food present, and other radiographs taken at varying intervals after the injection to record the course the food was following.



1.50 2.15 3.00

FIGURE 9. — Radiographs showing that after a large nutrient enema (about 90 c.c.) has been given, the food is forced more and more from the large into the small intestine. The enema was introduced at about 1.40 P. M. At 3.00 segmentation was occurring in many loops.

These experiments show that when small amounts of nutrient fluid are introduced they lie first in the descending colon. In every instance antiperistaltic waves are set going by the injection and the material is thereby carried to the cæcum. When large amounts are injected they stop for a moment in the region between the transverse and descending colon as if a constriction existed there. Then a considerable amount of the fluid passes the point and antiperistaltic waves carry it to the cæcum. In any case the repeated passing of the waves seems to have the effect of promoting absorption, for in the region where these waves continue running, the shadows become gradually more dim and finally the bismuth appears to be only on the intestinal walls; in other regions, *e. g.*, in the descending colon, the shadows retain their original intensity. Small injections have never in my

experience been forced even in part into the small intestine; but with the larger amounts, whether fluid or mushy, the radiographs show many coils of the small intestine containing the bismuth food.

The passage of the injected material beyond the ileocæcal valve is probably due entirely to antiperistalsis in the colon, — a factor unknown to both Grützner and his opponents. The valve, which is thoroughly competent for food coming normally from the small intestine into the large, is curiously incompetent for a substance, even of the consistency of thick cream, introduced in large amount by rectum. When the valve first permits the food to enter the ileum, the fluid pours through and appears suddenly as a winding mass occupying several loops of the intestine (Fig. 9, 1.50, about ten minutes after the injection). The mass is continuous from the valve to the other end; antiperistalsis is therefore not visible in the small intestine under the circumstances of this experiment. The antiperistaltic waves of the colon, however, continue running; the transverse and ascending colon are thus almost emptied, and the small intestine more and more filled with food (Fig. 9, 2.15 and 3.00). After a short time the typical segmenting movements can be seen in the loops, busily separating the food into small masses, and over and over again dividing and redividing them.

I have never seen food material pass back from the colon so far as the stomach; but once, about ten minutes after an injection of 100 c.c. of warm water, the cat retched and vomited a clear fluid resembling mixed water and mucus. In the fluid were two intestinal worms still alive.

The importance of the mechanism by which nutrient enemata are passed backward in the intestine is evident. In the colon the nutrient material is worked over by the antiperistaltic waves, intimately mixed with whatever digestive juices may be present, and exposed to the organs of absorption in that region. If the enemata are large, the digestive and absorptive processes are by no means confined to the colon, but may take place along extensive surfaces of the small intestine. I have repeatedly seen rhythmic segmentation active throughout many loops of the small intestine, thus exposing the injected food to the same mixing and absorbing processes as affect the nutriment which has come through the stomach in a normal manner.

The Effect of Emotions and Sleep.

Observations on the stomach of the cat showed that the peristalsis is inhibited whenever the animal manifests signs of anxiety, rage, or distress.[1] Since the extrinsic innervation of a large part of the intestinal tract is the same as that of the stomach, it is of interest to note the effect of emotional states on the movements of the intestines. Esselmont,[2] in a study of the dog's intestine, noted constantly after signs of emotion a marked increase of activity lasting for only a few moments. Fubini[3] also observed that fear occasioned more rapid peristalsis. There is no doubt that many emotional states are a strong stimulus to peristalsis, but it is equally true that other emotional states inhibit peristalsis. In the cat the same conditions which stop the movements of the stomach stop also the movements of the intestines.

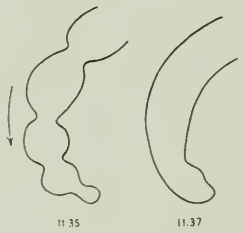


Figure 10. — Tracings showing the effect of excitement on antiperistalsis in the colon.

The female cats used in these observations ordinarily lie quietly on the holder and make no demonstration. Sometimes, however, with only a little premonitory restlessness the cat suddenly flies into a rage, lashing her tail from side to side, pulling and jerking with every limb, and biting at everything near her head. During such excitement, and for some moments after the animal becomes pacified again the movements, both of the large and small intestine, entirely cease. Such violence of excitement is not necessary to cause the movements to stop; a cat which was restless and continually whining while confined to the holder, showed no signs of intestinal movements during any period of observation (one period lasted more than an hour), although the changes in the distribution of the food observable from one period to the next proved that movements were going on during the quiet intermissions. In another cat, uneasy and fretful for fifty minutes, no activity was seen; then she became quiet for several minutes, and peristalsis of the small intestine appeared.

When the segmentation process in the small intestine is stopped by

[1] Cannon: *Loc. cit.*, p. 380.

[2] Esselmont: Report of the British Association for the Advancement of Science, 1899, p. 899.

[3] Fubini: Moleschott's Untersuchungen, 1892, xiv, p. 528.

excitement the segments unite and the series of parts returns to the form of a solid string. The change occurring in the large intestine when the antiperistalsis is inhibited by excitement is shown in Fig. 10. The tonic constrictions in the descending colon are apparently not affected by emotional states, for they do not seem to relax in the excitement which causes the movements to cease.

By holding the mouth and nostrils closed, or by pressing between the rami of the jaw, the breathing may be stopped. As soon as the cat shows distress from lack of breath every form of intestinal movement stops.

The statement is sometimes made in text-books of physiology that the gastric and intestinal mechanisms cease to act during sleep. It is worthy of note that nearly all the animals curled up and slept during the time between observations; nevertheless, the progress of the food through the intestines continued. The statement is also made that at night, even without sleep, the intestines are almost entirely at rest; that this is their normal time for repose.[1] I have seen both large and small intestines actively at work, however, from half past nine until half past ten o'clock at night.

Summary.

1. Bismuth subnitrate, 10 to 33 per cent, mixed with the food renders the movement of the intestinal contents and thereby the movements of the intestinal walls visible on the fluorescent screen.

2. The activity most commonly seen in the small intestine is the simultaneous division of the food in a coil into small segments, and a rhythmic repetition of the segmentation each time applied to the new segments formed from parts of those just divided. In the cat this rhythmic segmentation may proceed at the rate of 30 divisions per minute. The effects of the constrictions causing the segmentation are, the mixing of the food and the digestive juices, the bringing of the digested food into contact with the absorbing mechanisms, and the emptying of the venous and lymphatic radicles of their contents by compression of the intestinal wall.

3. Peristalsis is usually combined with segmentation. As the food is advancing, interfering constrictions often separate the rear end of the mass from the main body. The separation is momentary, how-

[1] Hess: Deutsches Archiv für klinische Medicin, 1887. xl, p. 104. Ludwig: *Loc. cit.*, p. 617.

ever; the rear end is swept into union with the main body again, and the whole mass is pushed onward until another constriction repeats the changes.

4. The ileocæcal valve is thoroughly competent for food entering the colon from the ileum.

5. The usual movement of the transverse and ascending colon and the cæcum is an antiperistalsis. This recurs in periods about every fifteen minutes, and each period lasts commonly about five minutes; the waves recur during a period at the rate usually of eleven waves in two minutes. This antiperistalsis gives new significance to the ileocæcal valve; for the food, now in a closed sac, is thoroughly churned and mixed by the constrictions running towards the cæcum, and again exposed to absorbing walls without any interference with the processes in the small intestine.

6. As soon as new food enters the large intestine a strong general contraction takes place along the cæcum and ascending colon, forcing some of the food onward; a moment later antiperistaltic waves begin to pass.

7. With the accumulation of material in the transverse colon, deep tonic constrictions appear one after another and carry the material into the descending colon, leaving the transverse and ascending portions free for the antiperistaltic waves.

8. In emptying the large intestine the material in the lower descending colon is first carried out by combined peristalsis and pressure of abdominal muscles; the remainder of the material is then spread into the evacuated region, and this region is again cleared; the second remainder may be similarly affected. In normal life the new food arriving in the colon must force forward the old contents of the ascending and transverse colon.

9. The observations have revealed no evidence of antiperistalsis in the small intestine, but, since the ileocæcal valve will allow nutrient material under pressure to pass backward, the antiperistalsis of the large intestine may force into the small intestine a considerable portion of a large nutrient enema. Segmentation in the small intestine affects such an enema precisely as it affects food which has passed normally through the stomach.

10. Signs of emotion, such as fear, distress, or rage, are accompanied by a total cessation of the movements of both large and small intestines. The movements continue in the cat both during sleep and at night.

THE REFLEXES CONNECTED WITH AUTOTOMY IN THE HERMIT-CRAB.

T. H. MORGAN.

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THE throwing off of the legs of crabs after injury has been long known, and the physiological processes involved in the act, have been examined in some detail by Fredericq.[1] There are, however, certain "instincts" (reflexes) connected with the autotomy that have as yet received very little attention. If a leg is quickly cut off distal to the "breaking-joint," the stump of the leg that remains may not be thrown off at once, although if held for a moment it will invariably drop off. If the stump of the leg is not held and the crab is returned to the water, the stump is generally drawn up against the side of the body or pressed down against the bottom of the aquarium, and in consequence of the resistance that it meets with it is often cast off. If the leg is not gotten rid of in any of these ways the other walking legs of the same side are brought against or across the stump and may assist in the autotomy. Very often the leg cannot be thrown off in any of these ways, and this is especially true for hermit-crabs (*Eupagurus*), in which case the stump is caught hold of by one or by both claws of the first pair of legs, or chelæ and these, pulling at the stump, furnish the resistance necessary for the autotomy to take place. It is this instinct of the hermit-crab that has especially interested me. The act appears to be one carried out as though it were a deliberate attempt on the part of the crab to get rid of the useless stump. The advantage of the result is obvious, not only because a better surface for regeneration is present at the breaking-joint, but also because relatively little blood is lost when the leg is thrown off at this place. In fact crabs often die when the leg is not thrown off at the base. The catching hold of the stump by one or by both of the claws is an act of such a kind that if we could imagine the crab endowed with intelligence, we should not hesitate to praise the action as one calculated to preserve the individual after

[1] Fredericq: Archives de zoologie expérimentale et générale, 1883.

injury. On the other hand, Fredericq has shown that the throwing off of the leg is a simple reflex act that involves the ventral nerve-cord. The leg may be thrown off even when the brain has been destroyed. Furthermore, I have found in the hermit-crab, that if the whole anterior end of the head (with its appendages) is quickly cut off, and then if one of the walking legs is cut off distal to the breaking-joint and the crab returned to water, the claws of the first pair of legs almost invariably bend over and catching hold of the stump of the leg, pull at it until it comes off. I have seen this act repeated by the same individual until a number of legs have been successively cast off.

A similar act takes place in the decapitated crab if one of the first pair of legs, or chelæ, is cut off distal to the breaking-joint. The claw of the opposite side bends over and, catching hold of the stump, supplies the resistance necessary for the throwing off of the leg. Shall we call this reflex action that leads to a beneficial result an instinct? If so, it is an instinct that involves only the nerve cord, and not the brain. I refer, of course, not simply to the reflex, described by Fredericq, by means of which autotomy takes place at the base of the leg, but especially to that part of the process involving the catching hold of the stump by the claws of another pair of legs. The following observations put the matter in even a more paradoxical light.

The fourth and the fifth pairs of legs of the hermit-crab are small in comparison to the more anterior legs. They serve to brace the animal against the shell and also to clean out the branchial chamber. They do not have a breaking-joint at the base, and cannot be thrown off after injury. If they are cut off at any level no effort is made by the crab to catch hold of them with its claws. Obviously such an act would be useless, since the leg would not come off, as can be shown by holding the legs with a pair of forceps. The fact that no attempt is made by the crab to catch hold of these legs, if they are injured, shows that the presence of a cut end does not in itself lead to catching hold of the injured part. This was further shown in another way. If the last pair of abdominal appendages are partly cut off, the piece that is left is not caught by the claws of the first pair of legs. A still more satisfactory demonstration of the same thing is given by cutting off one of the walking legs inside of the breaking-joint. Although profuse bleeding may follow, there is no effort made by the crab to catch hold of the injured region. This is an unexpected result, for if the reflex action takes place through the nerve that goes

to the ventral cord, it would seem that when the same nerve is cut off proximally to the breaking-joint, a reaction would be set up in the ventral cord that would lead to the first pair of legs being brought to the injured region. Since this is not the case we must suppose that there is another factor in the process in addition to the exposure of the cut end of the nerve. This other factor involves the presence of the stump of the leg distal to the breaking-joint. Its presence cannot be made known to the animal, however, through its cephalic sense organs, since the reaction takes place when these have been cut off.

Fredericq has shown that the throwing-off of the leg at the breaking-joint is brought about by certain muscles present in that region. If the muscles are injured the leg cannot be thrown off. This relation suggested the following experiment. With a pair of fine oculist's scissors it is easily possible to cut, either on the ventral or on the dorsal side, some of the muscles that bring about the autotomy. The leg can no longer be properly used for walking after the operation, but the crab makes no effort to catch hold of it. If the crab really acted intelligently we should expect it to attempt to get rid of the leg by catching hold of it with the claws. Or shall we suppose the crab acts even more intelligently and prefers to allow the breaking muscles to regenerate rather than regenerate a new leg? It would be gratuitous on our part to assume that either alternate has anything to do with the result. We can only infer that the autotomy reflex is not started, hence there is no action. Suppose, however, a leg that has had its muscles cut in this way is itself cut off outside of the breaking-joint. The crab will at once make violent and oft-repeated attempts to get rid of the stump by grasping it with the claws of the first pair of legs. If the muscles have been sufficiently cut apart, the stump of the leg cannot be thrown off, and may, subsequently, regenerate the distal part of the leg from the cut end.

If some of the muscles at the breaking-joint are cut, as in the last operation, and if at the same time the nerve of the leg is also cut proximally to the breaking-joint, no attempt is made by the crab to catch hold of the leg. If the leg is then cut off distal to the breaking-joint, still no attempt is made to catch hold of the stump of the leg. This latter result is what we might have anticipated from the results of the experiment in which the nerve was cut in two proximally to the breaking-joint, the leg itself being left attached. How shall we formulate these results? Shall we conclude that the entire series of reactions are purely machine-like, and that nothing takes place which

involves processes akin to intelligence on the part of the crab? There seems scarcely any room for doubt on this point, for we must suppose the crab to be entirely ignorant of the beneficial results of its action. The reactions are simple, reflex ones, and they only appear intelligent to us, since we can see that, on the whole, the result is useful to the animal.

But why so often do just those reflexes take place that we should ourselves as intelligent agents recommend because they are useful to the future welfare of the individual? Why does the crab catch hold of its leg and bring about its breaking off when the leg is cut off outside of the breaking-joint, and why does not a similar action take place when the leg is cut off inside of the same region? Why does no similar reaction take place when other appendages without breaking-joints are injured? Does the problem for the physiologist go no further than an examination of the reflex-action itself? We meet here with only a striking case of the familiar phenomenon of machine-like reflex actions taking place in the organism as though they had been specially devised as ends to means. It is at present customary to assume that the physiological problem involves only a study of the mechanism by which a process takes place, and that it is beyond the scope of physiology, and by inference beyond the range of scientific inquiry, to investigate the origin of useful reflexes, or rather to investigate how it is that so many responses are just those which are useful to the organism. In other words, is there a higher law that will account for those physiological changes being present which are useful to the organism? Pflüger, in 1877, drew attenion to this problem in his "Law of Teleology."

It is unquestionably the easiest solution of the difficulty to deny that this is a proper field for scientific investigation. Moreover, it is undoubtedly true that reactions sometimes take place that are injurious to the organism, but this does not do away with the fact that the great majority of responses are useful. Many zoölogists believe that they have found a plausible interpretation of this relation in the Darwinian theory, for if only useful responses survive, their preponderance in organisms appears to be accounted for, but I doubt if all useful responses can be explained in this way, because many of them are not of vital importance to the organism, and without a death-struggle the action of natural selection, *sensu strictu*, cannot take place. But even if it be admitted that a selective process takes place, the physiological problem remains exactly the same, for we

have to account in the first place for the appearance of the first useful variation, and in the second place find an explanation of the accumulation of variations by inbreeding. It would not be profitable to enter here into a general discussion of the origin of useful responses, but I cannot but believe that it is by no means certain that the problem is beyond the possibility of scientific investigation.

A PHYSIOLOGICAL STUDY OF THE PULMONARY CIRCULATION.

By HORATIO C. WOOD, Jr.

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THE earliest investigation concerning the vaso-motor supply of the lungs which I have been able to find is that of Brown-Séquard on the changes in the appearance of the lungs after various injuries to the pons varolii or the crura. Brown-Séquard observed, as the result of injuries to these parts, sometimes ecchymosis and effusion of blood, sometimes pallor or œdema of the lungs, which changes he attributed to vascular alterations in these organs. The untrustworthiness of direct observation of the calibre of blood-vessels, as well as the impossibility of determining whether these changes were the primary or secondary effects of the injuries to the brain, makes this evidence of little value. In my studies I have relied upon what is after all the most generally useful means of determining the calibre of the vessels, namely, the alteration of the blood-pressure.

The method which I have employed is as follows. The experiments were made upon dogs of moderate size (about 10 kilos) ; all the dogs but one were curarized and were rendered entirely insensible by morphine. In the greater number of the experiments the carotid pressure was taken simultaneously with that in the pulmonary vessels, although in one or two the pulmonic pressure only was observed. After placing a cannula in the carotid artery, and connecting the trachea with the artificial respiration apparatus, an incision was made the whole length of the chest, the chest-walls drawn apart, and after careful dissection a small branch, sometimes of the right, sometimes of the left, pulmonary artery was exposed, and a cannula placed in it. Usually I chose a branch supplying one of the superior lobes of the lung, being careful not to injure the blood-vessels or lung tissue of the neighboring lobes. Sufficient air was forced into the lungs by the artificial respiration apparatus to neutralize the diminished area in the respiratory surface. Absorbent cotton wrung out in hot water was placed lightly over the opening in the chest in order to prevent drying or chilling. The exposure of the lung is associated necessarily with a

considerable amount of shock to the animal, and I have nearly always found the carotid pressure low, and presumably the pulmonic pressure is correspondingly low.

Under the above conditions I have found that the pressure in the pulmonary artery of the dog, at the beginning of the experiment, varies between ten and twenty-five millimetres of mercury, and its relation to the carotid pressure to be usually between 1–4 and 1–10. The relations between the two arterial systems may be greatly distorted by various influences.

TABLE OF PULMONARY PRESSURE.

Author.	Mean of pressure in pulmonary.	Average ratio of pulmonic to aortic pressure.	
Velich	22.7	1 : 6	Dog
Knoll	12.2	1 : 7	Rabbit
Bradford and Dean	18	1 : 5	Dog
Wood	16	1 : 4.3	Dog

That the pressure in the pulmonary artery may be distinctly altered by appropriate measures is so generally admitted that I need only refer to the appended tables of my experiments. In the interpretation of these variations there is, however, considerable diversity of opinion, and a close analysis of these changes is necessary.

Asphyxia, when practised in animals thoroughly curarized, is a very efficient and at the same time comparatively uncomplicated vasomotor stimulant. In every case of nine experiments in which asphyxia was employed, there was a distinct elevation of the blood-pressure in the pulmonary, as in the carotid artery. Knoll, in a long series of experiments made upon rabbits, obtained an "outspoken" elevation of the pulmonary pressure in only a single case; but as the results of every other experimenter with whose work I am familiar are in accord with my own in finding that asphyxia elevates the pressure in the lesser circulatory system, as well as in the larger, it would seem that Knoll's results were due to some fault in technique, or that rabbits react very differently from dogs. It may be, however, that this investigator expected too considerable changes in the pulmonary pressure. In my experiments the rise absolutely expressed

was never so great in the pulmonary as in the aortic pressure, but in proportion to the original heights of the pressure the elevation of the pulmonic might be relatively even greater than the elevation in the carotid artery. In other words, the difference between the minimum

TABLE I.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	Pulse rate per min.	
min. sec.				
0 0	20	47	162	
0 5	..	..	..	Begin asphyxia.
0 17	20	52	162	
0 20	22	55	162	
0 25	27	71	162	
0 40	..	..	..	End asphyxia.
0 43	40	137	162	
3 0	25	68	180	
3 2 to 3 9	..	..	..	Stimulate intact right vagus.
3 5	22	60	84	
3 15	28	97	192	
3 25	..	..	..	Divide right vagus.
3 30	22	57	210	
3 45	20	50	120	Stimulation of peripheral vagus.

Weight of dog, 11.3 kilos. Right pulmonary.

and maximum pressure in the carotid artery, when expressed in millimetres, bore much the same relation to the difference between the high and low pressure in the pulmonary artery as the original pressure in the carotid bore to that in the pulmonary, although by certain influences the proportion could be absolutely destroyed.

I have found, also, that stimulation of the central end of a divided pneumogastric nerve causes simultaneous rise of the pulmonary and carotid pressures. The rise in the pulmonary pressure did not seem to be proportionately as high as that in the carotid. François-Franck

has found that stimulation of other sensory nerves, as the intercostal or crural, likewise elevates the pressure in both circulatory systems.

Three explanations of the elevation of pressure which takes place in the pulmonary arteries have been offered; first, that it is due to a damming back of the blood: second, that it results from a greater flow to the right heart: third, that it is due to direct contraction of the arteries of the pulmonary circulation. Let us consider these in order.

TABLE II.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	Pulse rate per min.	
min. sec.				
0 0	13	82	138	
0 5	..	..	..	Begin asphyxia.
0 40	13	93	144	
1 0	16	150	168	
1 15	20	139	192	End asphyxia.
15 0	16	124	168	Begin asphyxia.
16 0	21	162		
16 20	23	156	198	
16 25	24	131	198	End asphyxia.
16 30	25	157		
20 0	20	119		
20 1 to 20 11	..	..	..	Compress inferior vena cava.
20 11	18	51		
20 12	24	51		
20 19	20	105		

Weight of dog, 6.8 kilos.

According to the first theory it is supposed that the increase of resistance offered by the contracted vessels in the general circulation holds back the blood in the left ventricle, thereby increasing correspondingly the pressure in the left heart, which in turn dams back the blood in the pulmonary vein, and in this way increases the resistance

offered to the flow of blood out of the right heart. Velich has found that the rise of arterial pressure caused by the intravenous injection of the extract of the suprarenal capsules was accompanied by an elevation of the pressure in the left ventricle from 32 to 100 mm., and in the left auricle from 6 to 30 mm. These figures represent different experiments, apparently only one of each having been made. It is very difficult to interpret properly these single experiments, but they would seem somewhat to favor the stagnation theory. It has also been argued that the pressure in the aorta is elevated sooner than the pressure in the pulmonary artery, and the conclusion has been reached by a sort of "post hoc: propter hoc" logic that the lateness of the rise in

TABLE III.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Pulse rate per min.	
min. sec.			
0 0	12	240	
0 5	..	..	Begin asphyxia.
0 30	19	228	
0 45	25	228	End asphyxia.
2 20	..	..	Begin asphyxia.
2 30	20	222	
3 10	28		
3 40	24	240	End asphyxia.
3 50	18	240	

Weight of dog, 20.5 kilos.

the lesser circulation is due to the necessity of producing a sufficient resistance to dam back the blood through the heart. I have found that while it is true that the aortic pressure begins to rise before the pulmonic pressure (see Tables I and II), yet this elevation need only amount to a few millimetres (7 mm. in Table I) before the pulmonary pressure begins to go up; and it is hardly plausible that seven or eight millimetres' elevation in the pressure of the larger arteries is sufficient to overcome the reserve power of the heart.

If the rise of pressure in the pulmonary vessels is a passive one,

any condition which affects the aortic pressure must of necessity affect also the pulmonic pressure; but such is not the case. The simplest method of increasing the pressure in the aorta is by occlusion of its thoracic portion. In compressing the thoracic aorta I have found that while the carotid pressure might be more than doubled there was almost no rise at all in the pressure in the pulmonary artery (Table IV). Again, under the influence of digitalis the systemic pressure reached a point three times its previous height, while the pulmonary pressure rose one millimetre (Table IV). Bradford and Dean have obtained similar results by stimulating the peripheral end of the splanchnic nerve. Knoll suggests that the reason that these manipulations fail to elevate the pressure in the lesser circulation is that the normal heart is able to overcome the excessive resistance of high pressure and does not permit the blood to be dammed back; but that after the heart has been weakened by the accumulation of carbon dioxide in the system it permits of regurgitation. But this modification renders the theory none the more tenable. In the first place, I need only call attention again to the short interval which elapses between the beginning of the elevation of the carotid pressure and the beginning of the elevation of the pulmonic pressure; in the second place, certain influences such as the injection of suprarenal extract, irritation of a sensory nerve (see Table IV), which can hardly be accused of weakening the heart, cause a simultaneous rise in the two pressures. Moreover, as I shall show later, it is possible to produce an elevation of the pulmonary pressure without any rise whatsoever in the aortic pressure. It therefore seems proven that the rise of the pressure in the pulmonic artery cannot properly be attributed to the damming back of the blood.

The second explanation of the elevation of the pressure in the pulmonary arteries is, strangely enough, more or less the direct opposite of the foregoing. It supposes that as the blood flows more rapidly through the contracted blood-vessels of the general circulation, a greater supply of blood flows to the right heart. This supposition, I must confess, seems to me rather opposed to the laws of physics; it is hard to understand how more blood flows through a vessel of narrow calibre than would flow through a vessel of wide calibre, the pumping force being constant.

This view has been most strenuously urged by Openchowski, who has brought forward a certain amount of evidence to support his belief. His most important evidence is the following experiment.

TABLE IV.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	Pulse rate per min.	
min. sec.				
0 0	17	44	174	Right vagus has been cut.
0 5	..	..	..	Stimulate central end of vagus.
0 17	19	62	174	
0 20	19	64	180	
1 0	19	52	184	
1 5 to 1 35	..	..	..	Stimulate central end of vagus.
1 30	22	70	192	
1 35	21	69	192	
2 0	19	46	192	
2 5 to 2 16	..	..	..	Digital compression of thoracic aorta.
2 15	20	91	192	
3 0	18	48	192	
3 5 to 3 38	..	..	..	Compression of thoracic aorta.
3 20	19	87		
3 38	20	100	204	
3 38½	16	42		
3 41	26	69	204	
5 0	18	52	194	
5 5 to 5 25	..	..	..	Inject 1 gram sodium nitrite.
5 35	20	38	194	
6 15	19	30		
7 0 to 7 18	..	..	..	Inject 12 c. c. tinct. digitalis.
8 15	20	90	264	
9 25	21	112	194	
11 0	16	54		
11 15	14	31	6	

Weight of dog, 11.5 kilos. Left pulmonary.

Openchowski severed the spinal cord in the thoracic region (exact location not given), stimulated the cervical cord and obtained no elevation of the pulmonary pressure. He concluded from this that the reason the pulmonary pressure failed to rise was because the section of the spinal cord prevented the systemic vessels from receiving a constricting stimulus, and that there was consequently no increase of flow of blood to the right heart.

TABLE V.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	Pulse rate per min.	
min. sec.				
0 0	12	121	162	
0 5 to 0 20	..	..	..	Inject 0 008 gram nitroglycerin.
0 23	16	70	180	
0 50	11	67	190	
1 5	11	88	200	
1 10 to 1 20	..	..	..	Inject 0.008 gram nitroglycerin.
1 25	13	50	190	
1 40	..	..	..	Begin injection of 0.7% NaCl solution.
2 45	17	114	186	
10 0	35	149	186	Has received 950 c.c. salt solution.
11 30	38	161	180	Begin asphyxia.
13 0	41	205	126	Traube waves in the carotid but not in the pulmonary.
14 30	31	115		
16 0	18	64	81	

Weight of dog, 12 kilos.

The weight of this experiment is much lessened by the fact that the exact level of the spinal section is not given; for it is very evident that if the cord were divided above the point where the vasomotor nerves for the lungs have their exit the pulmonary circulation would be as effectually separated from the vasomotor centre as the aortic circulation. My own results, as well as those of Bradford and Dean, are indeed in direct opposition to those of Openchowski.

I have found that if the spinal cord was cut at the level of the seventh dorsal vertebra the relations between the pulmonary and aortic pressures were very greatly disturbed. In the first place, while the general blood-pressure was of course greatly lowered by the section of the cord, the pressure in the vessels of the lungs was not affected. Thus the difference between the pressures in the two circulations was much less, indicating that the pulmonary vessels retained their tone when those of the aortic system were dilated. More than this, I succeeded in some cases in obtaining a rise in the blood-pressure of the lungs while that in the rest of the body was lowered (Tables VI and VII). As the division was low down, the vasomotor supply of the anterior part of the body was not interfered with and consequently there frequently occurred some rise in the general pressure. But this rise was never proportionately so large as that which took place in the pulmonary artery. Under ordinary circumstances the elevation produced by asphyxia was not only absolutely, but also relatively, greater in the carotid than the pulmonary; in other words, the ratio between the two was larger after asphyxia than before. But, with the exception of one case, the ratio was smaller with the cord divided at the seventh dorsal vertebra. It is also interesting that in those cases where both pressures rose, the rise in the aortic system never preceded that in the lungs; in one case it very clearly followed.

If an increased rate of flow to the right heart will cause a rise of the pressure in the vessels of the lesser circulation, it follows that a diminished flow would cause an equivalent fall; but such does not seem to be the case. For example, compression of the thoracic aorta caused no fall of pressure in the pulmonary vessels, but if anything a slight rise (Table IV). To carry the proof still further, I compressed the inferior vena cava immediately below the heart (Table II), with, of course, a fall in the pressure in both sets of vessels; but while the pressure in the carotid fell from 119 mm. to 51 mm., that in the pulmonary artery fell only from 20 to 18 mm. Therefore the second explanation that the rise of pressure in the pulmonary system is due to increased flow of blood to the right heart is as untenable as the first.

From the foregoing it would seem almost proven by exclusion that the cause of the rise of pressure in the lesser circulation can be due only to contraction of the pulmonary blood-vessels. Direct confirmatory evidence of the existence of a vasomotor centre controlling the calibre of the vessels of the lungs is to be had from the behavior of

TABLE VI.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	
min. sec. 0 0	20	33	
0 5 to 1 5	..	..	Inject 0.02 gram nitroglycerin.
0 30	18	18	
3 10	18	35	
3 15 to 3 35	..	..	Inject 5 c.c. tinct. digitalis.
20 0	19	43	Have cleaned clots from pulmonary cannula. Pulse waves large and irregular.
20 5 to 20 30	..	..	Inject digitalis, amount not recorded.
20 35	19	38	
later	18	107	Close experiment.

Weight of dog, 7.5 kilos.

TABLE VII.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	Pulse rate per min.	
min. sec. 0 0	22	85	126	Cord has been cut at 7th dorsal vertebra.
0 20	..	..	..	Begin asphyxia.
1 0	25	77	120	
1 50	34	85	90	Violent movements of diaphragm. Stop asphyxia.
2 50	..	..	..	Give curare.
7 0	21	33	48	Begin asphyxia.
8 20	22	34	66	End experiment.

Weight of dog, 6.3 kilos. Not curarized at beginning of experiment.

those vessels toward certain drugs. It is well known that the reaction of the vessels of individual organs, for example the kidneys, is frequently different from that of the great vessels of the abdomen. If the calibre of the pulmonary vessels is governed by a vasomotor centre it is therefore only to be expected that it should react to various stimuli somewhat differently from the vasomotor centre governing the systemic circulation. As yet I have not had an opportunity of studying the action of many drugs upon the pulmonary vessels, but have found some differences worthy of note between the reaction of the smaller and greater circulations.

TABLE VIII.

Time in min. and sec.	Pulmonary pressure. mm. Hg.	Carotid pressure. mm. Hg.	Pulse rate per min.	
min. sec.				
0 0	22	43	192	Cord has been cut at 7th dorsal vertebra.
0 30	..	..	..	Begin asphyxia.
0 50	23	43	192	
1 10	31	68	192	End asphyxia.
3 0	26	49	192	
3 30	..	..	..	Begin asphyxia.
4 20	34	58	168	
4 50	38	54	126	
5 50	30	48		
6 50	12	26	78	Pulse not perceptible in carotid tracing. Right heart continues to beat for 1′ 50″.

Weight of dog, 9.5 kilos.

Most striking, perhaps, is the action of the nitrite group. I have experimented with nitroglycerin and sodium nitrite. As is well known, after the exhibition of these drugs there occurs a marked fall of the general blood-pressure, which Brunton, Cash and Dunstan, and others have shown to be due to a dilatation of the vessels; but the vessels of the pulmonary system are not dilated by the nitrites, indeed as a rule they seem to contract (Tables IV and V). Thus, in

one experiment (Table V), while the blood-pressure in the carotid fell from 121 mm. to 70 mm., that in the pulmonary arteries rose from 12 to 16 mm. Excessive doses of the nitrites cause a fall in the pulmonary as well as the aortic pressure, probably due to the depressant action of large doses of these drugs on the heart.

Very interesting, also, are the effects of digitalis upon the pulmonary circulation. Bradford and Dean assert that digitalin acts in the same manner upon the pulmonary pressure as it does upon the general pressure. On the other hand, Heger, experimenting with the French digitaline, found that the pulmonary pressure was never elevated, but usually fell as the carotid pressure rose. He found, also, that the right heart continued to beat after the left heart had ceased. Popper found that strophanthin elevated the pulmonary pressure relatively less than the general pressure.

The few experiments that I have made with digitalis confirm the view that the drug acts very differently on the two sets of vessels. There seemed to be no fall in the pulmonary pressure, but if anything a tendency to rise, but the elevation was always so slight as to be disregarded. Thus, in one experiment (Table IV), while the pressure in the carotid artery rose from 30 mm. to 112 mm., that in the pulmonary rose from 19 mm. to only 21 mm. This slight rise may be reasonably attributed to stimulation of the right heart.

From the foregoing experiments it seems proven that various influences, among which are asphyxia or stimulation of a sensory nerve, elevate the blood-pressure in the pulmonary artery as well as in the aortic circulation. This elevation of the blood-pressure is not a passive one but is due to a direct contraction of the blood-vessel walls; for it is possible to cause a rise of pressure in the pulmonary system without a rise in the carotid pressure; and on the other hand, an elevation of the aortic pressure does not necessitate a rise of the pulmonic pressure. Nitroglycerin and the other nitrites, while lowering greatly the general blood-pressure, cause a slight elevation in the pulmonic system. Digitalis does not affect the vasomotor system of the lesser circulation as it does that of the greater, so that the rise in the carotid pressure is not accompanied by any rise in the pulmonic pressure.

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ARTIFICIAL PARTHENOGENESIS IN STARFISH PRODUCED BY A LOWERING OF TEMPERATURE.

By ARTHUR W. GREELEY.

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IN a previous paper[1] it has been shown that the cells of Spirogyra and Stentor may be made to lose water, either by raising the concentration of the surrounding medium or reducing the temperature. The well-known experiments of Loeb[2] have established the fact that the unfertilized eggs of sea-urchins can be made to develop by raising the concentration of the sea-water, and thus causing the eggs to lose water. From these two facts, Dr. Loeb suggested to me the problem of attempting the production of larvæ from unfertilized eggs by exposing them for some time to a low temperature.

The experiment was first tried on the eggs of Arbacia. In this case, the sea-urchin was first thoroughly cleansed by a vigorous scrubbing in running tap water. The experimenter's hands and instruments were also sterilized with a weak solution of hydrochloric acid. The eggs were then shed into sea-water, and were immediately transferred to shallow dishes. The temperature was lowered to the desired point by packing the dishes in a mixture of ice and salt. In the different experiments the eggs were exposed for from one to six hours to temperatures ranging from 0° to 5° C. Two hours after removal to the room temperature, 23° C., a small proportion of the eggs began to segment irregularly, a few reaching a thirty or forty cell stage, but beyond this no results were obtained.

In 1899,[3] Morgan observed that the eggs of Arbacia may be made to segment by lowering the temperature of the sea-water. But in his experiments, as in mine, the segmentation was irregular, and took place in only a small proportion of the eggs.

[1] GREELEY: This journal, 1901, vi, p. 122.

[2] LOEB: This journal, 1900, iv, p. 423.

[3] MORGAN: Archiv für Entwickelungsmechanik der Organismen, 1900, x, p. 489.

The low temperature experiments were next tried on the eggs of the common starfish of Woods Holl, Asterias Forbesii, and the eggs were made to develop as far as the bipinnaria stage.

These experiments were performed as follows. The same precautions against contamination with sperm were taken as have already been described in the experiments on the eggs of Arbacia. But after removal from the female, the eggs of Asterias require a different treatment from those of Arbacia. Arbacia eggs are mature as soon as they are deposited in the water, and the best results with low temperatures are obtained when the eggs are subjected to the low temperature immediately after removal from the female. Asterias eggs, however, must be allowed to stand in sea-water four or five hours after they have been removed from the female. During this interval the polar bodies are extruded, the female pronucleus reforms, and the eggs finally reach a critical stage at which they may be made to develop by a reduction of the temperature. In two or three hours after this process of maturation has been completed, the eggs lose their power of response, and before maturation has taken place, no development by means of low temperatures, beyond a few irregular segmentations, could be obtained. Some lots of eggs removed from the starfish in August, when the experiments were performed, contained no eggs that would maturate in this way and reach the critical stage, and upon them a reduction of the temperature had no effect. Other lots contained a varying number of mature eggs that maturated on standing in sea-water (50 to 90% in the best lots). The process of maturation occupied four or five hours. After maturation the eggs were removed to shallow dishes, and the temperature lowered to the desired point either by packing the dishes in a mixture of ice and salt, or by placing them on ice in a refrigerator. At certain intervals the eggs were removed to the temperature of the room, 23° C., and, after standing for some time, were carefully examined to determine the proportion of eggs that had developed. The numerical results in each case are expressed by the number of swimming gastrulæ formed in five hundred eggs. Fortunately I was able to profit by the experiments of Dr. Mathews [1] on the same species, and guard against any possible development through mechanical agitation, in the eggs exposed to low temperatures, by subjecting a series of control eggs to the same treatment as regards the transference of the eggs from dish to dish. In handling

[1] Mathews: This journal, 1901, vi, p. 142.

the eggs, it is necessary to exercise the greatest care, for a very slight agitation may suffice to start development.

A few of the experiments are described as follows: —

Experiment VII, Aug. 28, 1901. — Eggs were allowed to stand in sea-water five hours after removal from female, and were then divided into two lots.

Lot 1 was exposed to a temperature of 0° C., and the eggs were removed to the room temperature at intervals of two, three and three quarters, and five and three quarters hours, but no development took place in any of the dishes.

Lot 2 was exposed to a temperature of 4° C. and the eggs removed after two and three quarters, four and a half, six, and twelve and three quarters hours. Two hours after removal to the room temperature, the eggs were found to be segmenting regularly in the dishes exposed to a temperature of 4° C. for two and three quarters, four and a half, and six hours, while none of the eggs left at 4° C. for twelve and three quarters hours segmented. Examination the next day showed that the eggs exposed to a temperature of 4° C. for two and three quarters hours had formed five, those exposed for four and a half hours had formed ten, and those exposed for six hours had formed twenty-five swimming gastrulæ in five hundred eggs. Eggs exposed to a temperature of 4° C., after standing in sea-water three hours after maturation, or eight hours after removal from the female, showed practically no development. In the control series a very few irregular blastulæ (one to a thousand eggs) were formed, none of which became motile. The results of this experiment are expressed in tabular form on the opposite page.

A further test of the comparative power of different temperatures to produce development, was made in the following experiment.

Experiment VIII, Aug. 29, 1901. — The eggs were exposed to temperatures of 1° C., 5° C., and 7° C. After exposure to 1° C. no segmentation was produced at any period of exposure from one to nine hours. After exposure to 5° C. the eggs segmented regularly, as in the preceding experiment, and formed, after three to six hours' exposure, ten to twelve gastrulæ in five hundred eggs. After an exposure to 7° C. fewer eggs segmented in each dish, and about one half as many gastrulæ were formed, for each period of exposure, as were formed after an exposure to 5° C. No development took place in the control, except a few irregular segmentations into four or eight cells.

In the next experiment an attempt was made to determine the limits of time during which the eggs must be exposed to a temperature of 5° C. and 15° C. in order to produce swimming larvæ.

Experiment IX, Aug. 30, 1901. — The lot of eggs, which had been allowed to stand in sea-water for five hours, was exposed to a temperature of 5° C. The first lot was brought back to the room temperature (23° C.) after fifteen minutes' exposure to the low temperature. No eggs developed. A second lot of eggs was brought back to the room temperature after thirty minutes. Only one blastula in three thousand eggs was formed. A third lot taken from the cold fifteen minutes later gave the same result. From now on, a marked change occurred. The eggs taken from the cold

EXPERIMENT VII.

August 28, 1901. Temperature of the room, 23° C.

Time between removal of eggs from female and experiment.	Temperature. C.	Duration of exposure to low temperature.	Number of gastrulæ formed in 500 eggs.
hours.		hours.	
5	4°	$2\frac{3}{4}$	5
5	4°	$4\frac{1}{2}$	10
5	4°	6	25
5	4°	$12\frac{3}{4}$	None
8	4°	3	1
8	4°	$9\frac{3}{4}$	None
11	4°	$6\frac{3}{4}$	"
5	0°	2	"
5	0°	$3\frac{3}{4}$	"
5	0°	$5\frac{3}{4}$	"

after one to seven hours' exposure formed one to four swimming gastrulæ in five hundred eggs. No segmentation occurred after nine hours' exposure. Another lot of eggs was exposed to a temperature of 15° C. during periods of from two to nine hours, but no development took place. These two lots of eggs contained a very small proportion of mature eggs. Only a few commenced segmentation, but all of these developed regularly, and formed perfect larvæ. The highest limit for the production of larvæ by means of low temperatures lies somewhere between 7° C. and 15° C., but I was unable to maintain a constant temperature between these two points.

EXPERIMENT VIII.

August 29, 1901. Temperature of the room, 23° C.

Time between removal of eggs from female and experiment.	Temperature. C.	Duration of exposure to low temperature.	Number of gastrulæ formed in 500 eggs.
hours.		hours.	
4½	5°	1	2–3
4½	5°	2	3–4
4½	5°	3	12
4½	5°	4	None [1]
4½	5°	6	10
4½	5°	8	5
4½	5°	10	4
6	5°	17½	None
4½	7°	2	1
4½	7°	3	6
4½	7°	4	5
4½	7°	6	6
4½	7°	8	1–2
4½	7°	10	None
5½	1°	1	"
5½	1°	2	"
5½	1°	3	"
5½	1°	5	"
5½	1°	7	"
5½	1°	9	"

[1] For some unknown reason no blastulæ were formed in this lot of eggs.

The length of the exposure to a temperature of 4° C. necessary to cause Asterias eggs to develop was further tested in the following experiment.

Experiment X, Sept. 2, 1901. — The eggs were exposed to a temperature of 4° C. during periods of from fifteen minutes to eighteen hours. After the fifteen and thirty minute periods at 4° C. no blastulæ were formed.

EXPERIMENT IX.

August 30, 1901. Temperature of the room, 23° C.

Time between removal of eggs from female and experiment.	Temperature. C.	Duration of exposure to low temperature.	Number of gastrulæ formed in 500 eggs.
hours.		hours.	
5	5°	$\frac{1}{4}$	None
5	5°	$\frac{1}{2}$	None [1]
5	5°	$\frac{3}{4}$	None [1]
5	5°	$1\frac{1}{4}$	1–2
5	5°	2	2
5	5°	3	3–4
5	5°	5	3–4
5	5°	7	3–4
5	5°	9	None
5	15°	2	"
5	15°	3	"
5	15°	5	"
5	15°	7	"
5	15°	9	"
14	5°	9	"

[1] One blastula in the entire lot of 3000 eggs.

But eggs after an exposure to 4° C. for forty-five minutes formed one, and after an exposure of one hour formed five swimming gastrulæ in five hundred eggs. After an exposure to 4° C. for two and three, four and a half, and seven and nine hours, there were formed respectively ten,

forty-two, and one hundred gastrulæ in five hundred eggs. After an eleven hours' exposure very rarely an egg reached the blastula stage, and after an exposure of eighteen hours there was no development at all. The control series contained a few irregular segmentations, a small proportion of which reached the morula stage. An exceptionally large proportion of these eggs were mature. Ninety per cent maturated.

EXPERIMENT X.

September 2, 1901. Temperature of the room, 23° C.

Time between removal of eggs from female and experiment.	Temperature. C.	Duration of exposure to low temperature.	Number of gastrulæ formed in 500 eggs.
hours.		hours.	
$4\frac{1}{2}$	4°	$\frac{1}{4}$	None
$4\frac{1}{2}$	4°	$\frac{1}{2}$	"
$4\frac{1}{2}$	4°	$\frac{3}{4}$	1
$4\frac{1}{2}$	4°	1	5
$4\frac{1}{2}$	4°	2	10
$4\frac{1}{2}$	4°	3	10
$4\frac{1}{2}$	4°	$4\frac{1}{2}$	42
$4\frac{1}{2}$	4°	7	100
$4\frac{1}{2}$	4°	9	100
$4\frac{1}{2}$	4°	11	1
5	4°-6°	$18\frac{1}{2}$	None

From the foregoing experiments it will be seen that an exposure to a temperature of from 4° C. to 7° C. during one to nine hours will cause fully maturated eggs of Asterias to develop into swimming larvæ. The optimum temperature is 4 or 5° C., and the optimum period of exposure about six or seven hours, although this varies greatly in different lots of eggs. Unfortunately in August, when the experiments were performed, great difficulty was experienced in obtaining mature eggs, viz: — those that would maturate on standing in sea-water. The proportion of mature eggs in those experimented on varied considerably, and, for this reason, there is a decided dissimilarity in the quantitative results of the different experiments, although

in no case did an experiment fail when maturated eggs were used. In some of the experiments, the control series contained a very few irregular blastulæ, which, however, did not develop into motile larvæ. Their formation was probably due to a slight agitation the eggs received in being transferred from dish to dish, although every precaution was taken to prevent it. Their number is too small to affect the result.

The development of the eggs exposed to low temperatures resembled closely that of normally fertilized eggs. Segmentation began in from one and a half to two hours after the eggs were transferred to the temperature of the room, and was preceded by the formation of a fertilization membrane. The eggs divided regularly into two, four, eight cells, and at any stage of development, the parthenogenetic eggs could hardly be distinguished from fertilized eggs. The parthenogenetic blastulæ, when removed to fresh sea-water, lived five or six days, and formed bipinnaria. A comparison of the time occupied in the development of parthenogenetic and fertilized eggs is given, the development of the parthenogenetic eggs being measured from the time of their removal to the temperature of the room.

	Fertilized eggs.	Parthenogenetic eggs.
Beginning of segmentation	$1\frac{1}{4}$ hours	$1\frac{3}{4}$ hours
Formation of swimming blastulæ . .	12 "	18–20 "
Formation of swimming gastrulæ . .	24 "	36 "
Formation of mouth invagination . .	40 "	60 "

Delage,[1] in experiments on the unfertilized eggs of Asterias, observes that immediately after the eggs have maturated, while they are in the so-called critical stage, they may be made to develop in several ways, viz: — by an increase in the concentration of the sea-water, by the chemical effect of certain ions, and by an increase in the temperature of the sea-water (to 30 or 35° C.). His conclusion that the eggs may be made to develop by warming the sea-water, is open to criticism because he was not aware of the possibility of development through mechanical agitation, and apparently used no precautions to prevent it. In several experiments, I subjected eggs of the same lot as those used in the low temperature experiments to an increase of temperature ranging from 31° to 37° C. during periods of exposure of from one to seven hours. When great care was exercised in handling the eggs, not a single segmentation was produced.

[1] Delage: Comptes rendus, 1901, cxxxiii, p. 346.

Summary.

1. After maturation has been completed, the unfertilized eggs of Asterias Forbesii can be made to develop regularly into bipinnaria by an exposure to a temperature of 4° to 7° C. for from one to nine hours.

2. Segmentation of the Asterias egg cannot be produced by raising the temperature of the sea-water.

ON THE PROLONGATION OF THE LIFE OF THE UNFERTILIZED EGGS OF SEA-URCHINS BY POTASSIUM CYANIDE.

By JACQUES LOEB and WARREN H. LEWIS.

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Introduction.

IN a former paper Loeb pointed out that the experiments on artificial parthenogenesis have a bearing upon the problem of the prolongation of life.[1] The unfertilized mature eggs of a sea-urchin die comparatively soon when deposited in sea-water. The same eggs, however, live a longer time when caused to develop either artificially, by extracting a certain quantity of water from them or naturally, by allowing a spermatozoon to enter. Loeb concluded from this that there are two kinds of processes going on in the egg: one which leads to the death and disintegration of the egg — a mortal process: and a second which leads to cell divisions and further development. The latter process inhibits or modifies the mortal process.

With this assumption the problem of the prolongation of the life of a cell was given a concrete form. According to this idea death and disintegration are due to specific processes which take place in the egg, and possibly in other or all living matter. These processes must be checked in order to render life possible. If this theory was of any value it was certain to lead to the discovery of artificial means by which the life of unfertilized eggs might be prolonged.

The specific life phenomena are, as far as their chemical side is concerned, chiefly, if not altogether, catalytic phenomena. Hence it was to be expected that a checking of the specific mortal processes should be brought about by agencies which inhibit catalytic phenomena without permanently altering the constitution of living matter.

Among all the agencies which act in this way, potassium cyanide seemed to meet this condition most perfectly. It weakens or inhibits a number of enzymatic processes in living matter without necessarily

[1] Loeb: This journal, iv, 1901, p. 455.

altering the constitution of the latter. When the potassium cyanide is permitted to evaporate, the original condition of the system may be restored.

A series of experiments on the effects of KCN on the unfertilized eggs of sea-urchins confirmed our expectations and proved that by adding a small quantity of KCN to sea-water the unfertilized eggs of the sea-urchin can be kept alive a comparatively long time at a temperature of 20° C. or above.

Method.

The experiments were made at summer temperature, which probably never went below 20° C. during our experiments, but was considerably higher at times. As a rule the ovaries of several females were used for each experiment, and the precautions described in former papers were applied to guard against contamination of the eggs by spermatozoa. A large quantity of eggs were kept in normal sea-water to serve as control material. The rest were distributed into various finger-bowls or flasks which contained sea-water to which various quantities of KCN had been added. After certain intervals some of the eggs were taken out of these solutions and put into normal sea-water. Half an hour after the transfer had been made sperm was added to the eggs to determine whether they could be fertilized. The development of these eggs was carefully watched. In a number of experiments tests were also made to see whether or not the eggs were still able to develop parthenogenetically by extracting water from them.

Eggs kept in Normal Sea-water.

It was found that unfertilized eggs, when kept from 1 to 23 hours in normal sea-water, could not only be fertilized but also reach the pluteus stage (at a temperature of about 20° C.). After that time they began to weaken. They could either not be fertilized at all, or their development stopped at an early stage. Eggs that had been in sea-water for from 24 to 32½ hours reached at the best only the gastrula stage, and only a small percentage of eggs developed. As a rule the eggs could no longer be fertilized after 32 hours. In the few cases where they were fertilized after that time they showed only the beginning of a segmentation. The latest date at which eggs that had been kept in normal sea-water could be fertilized was 48 hours. In this case, however, the effect of the fertilization consisted

in a few eggs showing the first segmentation: no membrane was formed. We were not able to repeat this observation. As a rule the eggs after from 24 to 32 hours became a sticky mass and assumed a dirty brownish color and this was the beginning of the complete disintegration and putrid decay.

Eggs kept in KCN Solution.

Our stock solution of KCN was $\frac{n}{10}$. Of this solution we added small amounts to sea-water. A solution of 99 c. c. sea-water and 1 c. c. of the $\frac{n}{10}$ KCN will be called an $\frac{n}{1000}$ KCN solution. When we say that the eggs were kept in an $\frac{n}{500}$ KCN solution, it means that they were kept in 98 c. c. of sea-water to which had been added 2 c. c. of the $\frac{n}{10}$ KCN solution. All the KCN solutions in which the eggs were kept were made of sea-water to which a small amount of $\frac{n}{10}$ KCN solution had been added. This way of proceeding was necessary, as the eggs of sea-urchins are very sensitive to changes of osmotic pressure as well as to KCN solutions that are stronger than $\frac{n}{500}$.

In the first series of experiments the solutions, in which the eggs were kept, were put into finger-bowls which were covered by glass plates. These glass plates did not fit air-tight, and hence a slight but continuous evaporation of KCN occurred. This evaporation was increased once or twice a day when we removed the cover in order to get out some of the eggs. It is thus evident that in these experiments the concentration of the KCN was a maximum at the beginning and decreased continuously during the experiment. We shall see later that this fact is not without influence upon the result.

We found that the duration of life was longest when the eggs were kept in KCN solutions that varied between $\frac{n}{500}$ and $\frac{n}{1250}$. Most experiments were made with $\frac{n}{1000}$ KCN solutions (99 sea-water + 1 $\frac{n}{10}$ KCN). While the control eggs (that had been left in normal sea-water) could no longer reach the pluteus stage when fertilized 24 hours after they had been put into the sea-water, the eggs that had been kept in a $\frac{n}{1000}$ KCN solution *in every case* reached the pluteus stage when fertilized 72 hours after they had been put into the solution. When taken out of the KCN solution and put back into normal sea-water they developed into normal plutei, when sperm was added. In a few experiments we even got plutei from eggs that had been in the poisoned sea-water from 90 to 100 hours.

These eggs not only reached the pluteus stage, but kept on devel-

oping, and remained alive as long as eggs that had been fertilized immediately after being taken out of the ovary. Some of the plutei which developed from eggs that had been fertilized after a stay of 72 hours in poisoned sea-water lived five or six days and longer, which is a considerable time if we remember that the temperature of the water was never lower and often higher than 20° C.

The longer the eggs remained in the poisoned sea-water the smaller the percentage became of those that yielded to fertilization and the earlier their development stopped. Eggs that had been in a loosely covered $\frac{n}{1000}$ KCN solution for five days never developed beyond the blastula stage.

Eggs that had been in the poisoned sea-water over 72 or 80 hours as a rule no longer formed a membrane upon impregnation. Moreover, the first segmentations were liable to be irregular.

The Optimal Concentration of the KCN Solution.

We have said that we used in most experiments a $\frac{n}{1000}$ KCN solution. The weaker as well as the stronger solutions were less effective.

Concentration of KCN.	Result of fertilization after a 75 hours' stay in the solution.
Pure sea-water	No egg segments.
$\frac{n}{64000}$ KCN	No egg segments.
$\frac{n}{32000}$ KCN	No egg segments.
$\frac{n}{16000}$ KCN	No egg segments.
$\frac{n}{8000}$ KCN	Very few eggs show a beginning of segmentation.
$\frac{n}{4000}$ KCN	Very few eggs show a beginning of segmentation.
$\frac{n}{2000}$ KCN	Few eggs go through the early stages of segmentation.
$\frac{n}{1000}$ KCN	Many eggs segment and develop into swimming larvæ.
$\frac{n}{750}$ KCN	Many eggs segment and develop into swimming larvæ.
$\frac{n}{400}$ KCN	A few eggs develop into swimming larvæ.
$\frac{n}{300}$ KCN	No egg segments.
$\frac{n}{250}$ KCN	No egg segments.
$\frac{n}{200}$ KCN	No egg segments.
$\frac{n}{100}$ KCN	No egg segments.

When the sea-water contained too little KCN it had little or no effect upon the prolongation of life. When we added too much it evidently altered the constitution of the egg and the latter could no longer be fertilized when taken out of the solution. The results of experiments with various concentrations of KCN can be seen from the preceding table.

The first vertical column at the left indicates the concentration of the KCN in the sea-water. Eggs of the same females were distributed into these mixtures, and after 75 hours eggs were taken out, put into pure sea-water (which was changed repeatedly); half an hour later sperm was added. The second column indicates the development of these various groups of eggs.

It should be added that the last two solutions were more harmful than pure sea-water, inasmuch as they completely annihilated the power of development of the eggs in less than 24 hours.

The Rôle of the Evaporation of KCN.

In all the experiments mentioned thus far the KCN solutions which contained the eggs were kept in finger-bowls covered loosely with glass plates. It goes without saying that the KCN evaporated slowly but steadily from these solutions and that therefore the experiments were actually experiments with KCN solutions whose concentration diminished steadily. This could be ascertained without titration by the fact that the odor of the solutions grew steadily weaker. It was necessary to determine whether or not this diminution in the concentration of the KCN solution had any effect upon the result of these experiments.

We put the KCN solution and the unfertilized eggs into the same loosely covered finger-bowls, but renewed the solution every 24 hours. When we used $\frac{n}{1000}$ KCN solutions the prolongation of life which we had noticed before was diminished. Eggs that were put into a $\frac{n}{1000}$ KCN solution which was renewed every 24 hours lost their power of development almost completely in less than 75 hours. After that time only a small percentage began to develop when fertilized, and those that segmented never reached the swimming stage. Of those, however, that were kept in $\frac{n}{1000}$ KCN solutions which were not renewed, not only practically all segmented but all reached the pluteus stage. We then tried the effects of $\frac{n}{1000}$ KCN solutions in small corked flasks, in which the evaporation of the KCN

was prevented. As was to be expected, the results were decidedly poorer than with $\frac{n}{1000}$ KCN solutions, which evaporated slowly. After 66 hours a few of the eggs were able to segment when fertilized, but they did not reach the swimming stage. But even in these experiments the unfertilized eggs that were put into the $\frac{n}{1000}$ KCN solution lived and preserved their capability of being fertilized considerably longer than the eggs kept in normal sea-water. These experiments were repeated many times with the same striking result.

Maximal Prolongation of Life.

From the previous experiments it appeared as if there were two conditions to be considered in the attempt to prolong the life of the unfertilized eggs of the sea-urchins. First, a comparatively high initial concentration of the KCN solution (about $\frac{n}{1000}$) seemed necessary (perhaps to stop suddenly certain injurious processes in the egg). Second, if this high initial concentration was maintained it injured the constitution of the egg. We thought that by decreasing the concentration of the KCN solution more carefully, still better results might be obtained. This was indeed the case. We will describe the most striking experiment of this kind. The unfertilized eggs were put into a $\frac{n}{750}$ KCN solution. After 24 hours they were transferred into a $\frac{n}{1400}$ KCN solution, after 48 hours into a $\frac{n}{2000}$, after 72 hours into a $\frac{n}{2500}$, after 96 hours into a $\frac{n}{3000}$, and after 120 hours into a $\frac{n}{3000}$, in which they remained for the rest of the time. The solutions were kept in closed flasks. After certain intervals a portion of the eggs was transferred into normal sea-water and brought together with fresh sperm to test their power of development. The first portion of eggs was fertilized after having been in the solution 66 hours. About 80 per cent of the eggs segmented regularly, formed membranes and reached the pluteus stage. The second portion was taken out of the solution and fertilized after 90 hours. About 30 per cent of the eggs segmented regularly, but formed no membranes. They also reached the pluteus stage. The third lot was taken out and fertilized after $99\frac{1}{4}$ hours. About 20 per cent of the eggs segmented, some regularly, some irregularly. None had formed a membrane. They also reached the pluteus stage. The same occurred with the next lot of eggs, which were taken out after 112 hours, with the difference only that fewer eggs segmented. The eggs taken out after having been 120 hours in

the poisoned sea-water, upon fertilization, yielded a number of swimming gastrulæ but no plutei. After 139 hours a new lot of eggs was taken out and fertilized. But few eggs segmented, some regularly, and none formed a membrane. They reached the blastula stage and swam about. After 144 hours the results were still similar, with the exception that the blastulæ did not swim. The eggs that were taken out after 161 and 168 hours were still alive, and upon the addition of sperm reached the eight-cell stage, but did not develop further. They formed no membranes and few segmented. We discontinued the experiment at this point.

We performed, with the same material, several other series of experiments. One series of experiments was performed with a $\frac{n}{750}$ KCN solution in finger-bowls covered with glass plates, which allowed some evaporation to occur. The solutions were changed every 24 hours simultaneously and in exactly the same way as in the chief experiment mentioned above. The slight evaporation seemed to be less advantageous, as the eggs ceased earlier to reach the pluteus stage, and after 144 hours were no longer even able to segment. It might be said that on the whole their duration of life was a little over 24 hours shorter than that of the eggs of the first series of experiments. Two further control experiments accompanied this series. One lot of eggs was left in the $\frac{n}{750}$ KCN solution into which the eggs had originally been put. They were kept in finger-bowls covered with glass plates. These eggs lived but a little over five days ; that means, when fertilized, after having been in the poisoned sea-water 120 hours only a few eggs began to segment and these did not develop beyond the four-cell stage. The eggs that had been 139 hours in this solution did not segment when put back into normal sea-water and exposed to sperm. Eggs taken from this solution after 66 hours reached the pluteus stage when fertilized, but those taken out after 90 hours reached the gastrula stage only when fertilized.

Finally one lot of these eggs was left in normal sea-water. It need hardly be said that after 66 hours not a single egg could be fertilized or was alive. Everything was disintegrated. We have varied these experiments in several ways, but it would be tedious to enumerate all these detailed experiments. It seems possible that by a further improvement of the methods the unfertilized eggs of the sea-urchin may be kept alive even longer than seven days.

Artificial Parthenogenesis.

In all the experiments mentioned thus far the power of development of the unfertilized eggs had been tested by adding sperm to the eggs. We tried whether the KCN preserved also the power of the egg to develop parthenogenetically. It was also our intention to find out whether or not the power of the eggs to develop parthenogenetically disappeared sooner than their power of developing sexually. In a number of experiments we removed unfertilized eggs at various intervals from the KCN solution, put them into 100 c.c. of sea-water, to which was added about 15 c.c. of a $2\frac{1}{2}$ *n* KCl solution. After from $1\frac{1}{2}$ to 2 hours they were put back into normal sea-water. Loeb has shown that the unfertilized eggs of Arbacia reach the pluteus stage when treated in this way. In order to be able to express ourselves briefly we shall call this method osmotic fertilization. In one experiment we took eggs that had been in a $\frac{n}{1000}$ KCN solution (in loosely covered dishes) for 42 hours, and fertilized them osmotically. About 75 per cent of the eggs developed and many reached the pluteus stage. A second lot of unfertilized eggs were exposed to osmotic fertilization after they had been in the $\frac{n}{1000}$ KCN solution for 66 hours. About 12 per cent of the eggs developed and some of them reached the pluteus stage. At the same time eggs of the control material of the same culture which had been kept in normal sea-water were also fertilized osmotically. From the eggs that had been in the normal sea-water for 23 hours we were able to produce plutei by osmotic fertilization. But after 42 or 66 hours not an egg even segmented when fertilized osmotically. In another series of experiments we got almost identical results. In this series we obtained swimming blastulæ from the control material by osmotic fertilization after the eggs had been in normal sea-water for 31 hours. In a third series we obtained parthenogenetic gastrulæ from eggs that had been in a (loosely covered) $\frac{n}{500}$ KCN solution for 74 hours. In a fourth series we obtained a beginning of a parthenogenetic development up to the 32-cell stage from eggs that had been in a $\frac{n}{750}$ KCN solution for 113 hours. Finally we tried how long the eggs would yield to osmotic fertilization when kept in a KCN solution whose strength was diminished a certain amount every 24 hours. We began with a $\frac{n}{750}$ KCN solution and dropped gradually down to a $\frac{n}{2000}$ solution. In this case we obtained parthenogenetic gastrulæ from eggs that

had been in the poisoned sea-water for 99 hours and blastulæ from eggs that had been kept in the KCN solution 113 hours. Even after 140 hours some eggs reached the 4 to 16-cell stage when fertilized osmotically. In each of these experiments the power of developing upon osmotic fertilization lasted almost, but not quite, as long as the power of developing upon natural fertilization. On the whole we might say that the power of parthenogenetic development ceased from about 12 to 24 hours earlier. This difference is probably due to the fact that the exposure of the eggs to sea-water of a higher concentration in the act of osmotic fertilization injures the eggs slightly.

Effects of Lack of Oxygen.

The poisonous effects of KCN upon higher animals are chiefly if not wholly due to the inhibition of the oxidative process in the tissues. The observations of Geppert, Spitzer, and Bredig point in this direction. We had, therefore, to consider the possibility that the processes that lead to the death of the unfertilized egg, and which are stopped by the KCN, are oxidations. We tried whether or not the life of the unfertilized eggs of the sea-urchin can be prolonged by depriving them of oxygen. We used two different methods of depriving the eggs of oxygen. First, we kept them in gas chambers in which the air was driven out by a powerful current of carefully cleaned hydrogen. The second method consisted in putting the eggs with a few c.c. of sea-water into minute open flasks which were put into test-tubes containing a fresh mixture of 180 gms. KOH in 120 c.c. of water to which a solution of 5 gm. pyrogallol in 15 c.c. of H_2O was added. The test-tubes were then sealed. At various intervals a tube was broken and the egg exposed to fresh sperm in normal sea-water.

In the latter series of experiments, the eggs died a little earlier than the control eggs kept in normal sea-water. After 29 hours the former were dead. Those taken out and fertilized after 22 hours, reached only the blastula stage. It is possible that the slight rise in temperature while the pyrogallol was put in and the tubes sealed, accounts for this difference. The eggs that were kept in gas chambers through which a constant current of hydrogen was sent, lived longer. At 4, 14, 27, and 38 hours after the beginning of the experiment, the unfertilized eggs could not only be fertilized, but developed into swimming larvæ. Even after 64 hours, a few of the eggs were

still able to segment into two cells when taken out and brought into contact with sperm. But this was the ultimate limit. While this result is slightly better than those obtained with eggs kept in normal sea-water, it is so far inferior to the results obtained with KCN solutions, that we may say that the prolongation of life of the unfertilized egg by KCN is, if at all, only to a slight degree due to the prevention of the oxidative processes by KCN.

The Effects of a Low Temperature.

A priori one should expect that the best way to prolong the life of the unfertilized eggs of the sea-urchin would be to keep them at a low temperature. The lowering of the temperature ought to stop the action of the enzymes. O. Schultze has shown that the fertilized eggs of frogs can be kept alive on ice for two weeks. We do not know whether any experiments of that kind have been made on the unfertilized eggs of frogs. It would not be correct to conclude that fertilized and unfertilized eggs behave necessarily alike in this respect. At the suggestion of one of us, Dr. Lyon investigated the effects of a KCN solution upon the fertilized eggs of the sea-urchin, and his results show that there is a characteristic difference between the two cases. As Dr. Lyon's paper will appear shortly, we do not need to discuss this difference here. We put one lot of a culture of unfertilized eggs on ice in normal sea-water (Lot I), a second lot (Lot II) on ice, in an $\frac{n}{1000}$ KCN solution. A third lot of the same eggs was put into an $\frac{n}{1000}$ KCN solution, and kept at room temperature (20° C. or above) (Lot III), and a fourth lot was kept in normal sea-water at room temperature.

After 50 hours 75 per cent of the eggs of Lot I, when they were taken from the ice, and sperm was added, developed, and many of them reached the gastrula stage, while 90 per cent of those of Lot II developed. Lot III were equally good, but all of Lot IV were dead. After 63 hours, another portion of the eggs were put back into normal water at 20° C., and exposed to fresh sperm. Only 35 per cent of the eggs of Lot I developed, while 90 per cent of Lot II developed. After 76½ hours, again a portion of eggs was taken out of these solutions and fertilized. Those of Lot I and II only reached the early segmentation stages, while those of Lot III developed into swimming larvæ.

This experiment shows clearly that the lowering of temperature (provided it does not go below the freezing point) produces changes

in the egg which weaken its chances for development, although one series of experiments should not carry too much weight. But while it seems as if in certain forms (*i. e.*, the frog) the lowering of the temperature had only the effect of diminishing the velocity of chemical changes, and of getting the animal into that condition which Claude Bernard calls latent life, in other forms it is decidedly different. A lowering of the temperature causes, for example, the growth of wings in plant lice. Hence a decided process of cell multiplication and growth is called forth by a lowering of temperature.

In several former papers Loeb has pointed out the similarity of the effects of a lowering of the temperature and of a loss of water in cells. In certain Copepods and larvæ of Polygordius, he found that loss of water on the part of the animal, as well as lowering of temperature, transformed them from negatively to positively heliotropic forms. At his suggestion, Mr. A. W. Greeley studied the effect of a reduction of temperature, and showed that by a lowering of the temperature (above the freezing point of water), Stentor cœruleus undergoes a definite series of morphological changes, which can also be produced by an abstraction of water from the animal. He made it probable that a reduction of temperature causes the cells of certain organisms to lose water. In unfertilized starfish eggs Mr. Greeley succeeded in producing the development of larvæ, by keeping the unfertilized eggs on ice at a certain stage and for a certain length of time. The unfertilized eggs of sea-urchins could be caused in the same way to at last reach the 32-cell stage. This, then, shows that the eggs undergo a change when their temperature is kept for some time at a little above 0° C. Hence it appears intelligible that keeping the eggs of sea-urchins on ice does not prolong their life as much as if we keep them in an $\frac{n}{1000}$ KCN solution.

Experiments on the Eggs of Starfish.

The eggs of Arbacia are not transparent enough to permit us to convince ourselves whether or not the internal processes in the unfertilized egg are brought to a standstill through KCN. If this were not the case, we should have to consider the possibility that KCN preserves the unfertilized eggs longer on account of a bactericidal effect. But observations on the more transparent eggs of the starfish (Asterias Forbesii) left no doubt that the preservation or lack of disintegration of the unfertilized egg is due to the interrup-

tion of certain progressive changes which in normal sea-water are going on in the egg. When the egg of the starfish is laid it is still immature. Its nucleus is large, and during the first two hours the reduction of the nucleus and the throwing out of the polar bodies occurs. If the eggs are not shaken, the nuclei become invisible, and the eggs, after 24 hours, look dirty, indicating the beginning of disintegration. We put the eggs of starfish, immediately after they were taken out of the ovary, into an $\frac{n}{1000}$ KCN solution. None of the changes characteristic of the process of ripening occurred in these eggs, and after 48 hours even, the nucleus was as large and distinct as at the time they were put into the poisoned sea-water. But the eggs which had been put into normal sea-water had undergone the above-mentioned steady series of internal changes to complete disintegration. Nothing could be more striking than to compare the progressive series of changes in the eggs kept in normal sea-water with the unaltered appearance of the eggs put into the poisoned sea-water.

Conclusions.

1. We have shown that the unfertilized eggs of the sea-urchin, kept in normal sea-water at a temperature of about 20° C., gradually lose their power of development. Their power to reach the pluteus state disappears, as a rule, after they have been in sea-water for about 23 hours. In the majority of cases the power of segmentation is lost when the eggs have been in sea-water 48 hours or even less. At that time, as a rule, the eggs form a sticky and discolored mass.

2. We have shown that the life of the unfertilized eggs of the sea-urchin can be prolonged materially by adding KCN to the sea-water. The best concentration of the KCN for this purpose is a mixture of about 100 parts of sea-water and one part of an $\frac{n}{10}$ KCN solution. But while this concentration is necessary at the beginning, life lasts longer when the concentration of KCN is gradually diminished during the experiment. By allowing part of the KCN to evaporate gradually, or by transferring the eggs into weaker and weaker solutions of KCN in sea-water, we could obtain plutei from eggs that had been in the KCN solutions for 112 hours, and we could get the beginning of a development of eggs that had been 168 hours in such poisoned sea-water. The eggs had, during all that time, been kept at a temperature of 20° C., or above.

3. Not only the power of sexual, but also that of parthenogenetic development is prolonged through the potassium cyanide.

4. These experiments are another proof of the fact that, while weak solutions of KCN are able to stop certain processes in the cell, the old conditions of the system may be re-established when the solution of KCN is allowed to evaporate. If the KCN solution is of a higher concentration than $\frac{n}{500}$ or $\frac{n}{300}$, the eggs may be injured permanently.

4. Lack of oxygen does not prolong, or prolongs but little, the life of the unfertilized eggs.

5. The lowering of the temperature seems to be far less effective for the prolongation of life in the unfertilized eggs of sea-urchins than the addition of KCN to sea-water.

6. As long as we consider death as something merely negative (namely, the cessation of certain processes), it must appear extremely paradoxical that the life of the unfertilized egg of the sea-urchin should be prolonged by applying one of the most deadly poisons. But the paradoxical element disappears, when we start from the assumption which led us to these experiments, namely, that in the unfertilized eggs specific mortal processes are going on, which are checked or modified by the process of sexual or osmotic fertilization. These specific mortal processes are also checked by potassium cyanide, which substitutes for the destructive action of these processes a condition of suspension of life ("vie latente" of Bernard).

7. We may next consider the question, What is the nature of the mortal processes in the unfertilized egg, and how can fertilization check them? No definite answer is possible at present. The mortal processes may consist in self-digestion, or in other enzymatic processes, or they may not be catalytic at all. How the act of fertilization can modify or check such processes, is not beyond analogy. We know that a full supply of oxygen decreases the fermentative action of zymase. Spitzer has made it probable that the cell nucleus contains an oxydizing agent, namely, the nucleoproteids. The process of fertilization results, in the egg of the sea-urchin, in a rapid series of successive cell divisions, in each of which the contents of the nucleus are scattered throughout the cell. It is easily conceivable that this periodic spreading or mixing of the contents of the nucleus and the cells may modify the chemical processes in the egg and check the mortal processes.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE CALIFORNIA HAGFISH, *POLISTOTREMA STOUTI*. — II. THE ABSENCE OF REGULATIVE NERVES FOR THE SYSTEMIC HEART.

By CHARLES WILSON GREENE.

[*From the Physiological Laboratory of the University of Missouri.*][1]

THE Cyclostomes, or hagfishes, have become classical in morphological literature. Their primitive vertebrate characters and their position in the vertebrate series make them in much demand for embryological and morphological research. Very little physiological work has been done with this group, yet their physiological interest promises to be great.

The hagfishes are relatively large and tenacious of life, two qualities very favorable to physiological research. The representative of the group on our western coast, especially abundant in Monterey Bay, reaches a length of forty to fifty centimetres. It is readily taken by means of the trawl or in traps, and can be kept alive in the aquarium with ease. Its tissues live under experimental conditions for hours or even days.

In this brief paper I shall present the results of a series of experiments made to establish the relation of the nervous system to the activity of the heart in Polistotrema stouti. The slight cartilaginous skeleton of the animal permits the preparation of organs with great facility. On the other hand, the slightest muscular movement of any part of the body is sufficient to displace the heart and to render the recording of its movements a matter of peculiar difficulty. The successful records finally made were secured by pinning the body muscles firmly to a holder and independently supporting the heart, and by curarizing the animal.

Stimulation of the vagus nerve. — Johannes Müller described the

[1] The experiments upon which this paper is based were performed at the Hopkins Seaside Laboratory, California. I take this opportunity to express my obligations to the Directors, Dr. C. H. Gilbert and Dr. O. P. Jenkins, for the facilities of the Laboratory.

vagus nerve of the hagfish as sending branches to the gill sacs, to the heart, and to the stomach-intestine. A small filament, doubtless Müller's cardiac branch, runs toward the heart in Polistotrema stouti but by macroscopic methods I have never been able to trace it nearer than the tissue just dorsal to the pericardial wall.

The motor fibres to the gill sacs furnish a ready means of determining whether or not a given stimulus is effective. The muscles of the gill sacs are always thrown into contraction by even a weak stimulus applied to the vagus, so also are the well developed constrictor cardiæ at the entrance of the stomach. The strength of current

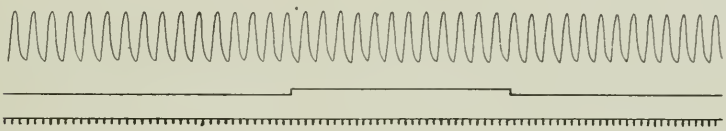

FIGURE 1. — *Experiment, December 28, 1899.* Record of contractions of the ventricle upon simultaneous stimulation of the vagus nerves in the hagfish, *Polistotrema stouti*. Two thirds the original size. Strength of the interrupted current, 500 units. Du Bois Reymond coil (Petzold manufacture) fed by one Edison-Lalande cell. Time in seconds.

necessary to produce contraction of these gill sacs is from twenty to thirty units by the proportionate scale of the Petzold inductorium used when fed by one Edison-Lalande cell.

The vagus was stimulated at different points along its course from the point of origin within the cranium to a point just anterior to the heart itself. It was stimulated with the nerve intact and with the nerve cut. Each nerve was stimulated by itself and also both together. The strength of the current was varied from one to one thousand units and the rate of interruption was also varied. The results of a series of such tests are presented in the table on page 320.

The heart rates given in this table are computed from counts made for equal periods of time immediately preceding, during, and following vagus stimulation. The error of measurement reaches a maximum of four to five tenths of a beat per minute. Slight variations due to causes which produce a general increase or decrease of rate may, of course, fall within the period under consideration. Of these outside factors among the most important are those influences affecting the return of blood to the heart. Taking into consideration these factors, it seems to me that in no case has a change of heart rate of sufficient magnitude occurred to justify the assumption of a direct

vagus influence. The accompanying figure gives one of a series of experiments in which the strength of current was varied from ten to one thousand units.

The failure to discover inhibitory nerves in the vagus of the hagfish was a great surprise to me, hence I immediately began experiments to determine by what other path the heart received its supply of regulative nerves. The spinal nerves are too delicate to isolate, hence experiments were necessarily confined to an exploration of the brain and spinal cord.

TABLE SHOWING HEART RATE WITH VAGUS STIMULATION.

Date 1899.	Nerve stimulated.	Strength of stimulus.	Duration of stimulation.	Rate before stimulation.	Rate during stimulation.	Rate after stimulation.
		units.	sec.			
	Vagus	200	18	26.6	26.4	26.5
	Vagus	400	18.4	25.2	25.2	25.2
Dec. 28	Right vagus	500	10	25.2	24.8	24.8
" "	Right and left vagus	500	30	24.5	24.6	24.5
" "	Right and left vagus	10	13	24.0	23.8	24.0
" "	Right and left vagus	100	17.6	24.0	24.1	24.0
" "	Right and left vagus	1000	18	23.3	23.3	23.3

Cranial stimulation. — The brain was stimulated with platinum electrodes, the bipolar and unipolar methods both being used. The stimulation of different parts of the brain, especially of the medulla and of the roots of the cranial nerves, gave entirely negative results in so far as any influence affecting the heart contractions is concerned.

Stimulation of the spinal cord. — It was hoped that by stimulating different sections of the spinal cord any regulative nerves that might reach the heart by this path would be discovered. My results here also were wholly negative, although numerous experiments were performed. It is true that in this series of experiments the heart rate was sometimes slightly increased. The stimulation of the cord was always followed by vigorous contractions of the great lateral muscles, and any one who has ever handled a live hagfish will realize what a pronounced effect the muscular action will have on the tension of

the heart. These effects cannot be absolutely eliminated except in the curarized animal and the changes in tension and pressure are great enough to produce a change of one or two contractions per minute. I have reached the conclusion, therefore, that regulative nerves for the heart do not pass out through the spinal nerves in the hagfish.

At the suggestion of Dr. Howell, to whom I am greatly indebted for inspiration and advice, I have repeated the experiments along two lines, namely, stimulation of the vagus with the heart not exposed to the air, as it was in the experiments quoted above, and stimulation by applying the electrode directly to the venous sinus. These experiments were kindly performed for me by Mr. W. F. Allen during the summer of 1900, and I have since repeated the experiments myself. Stimulation of the vagus in the neck had no effect on the undisturbed heart. Upon stimulating the venous sinus there was an increase of two or three beats per minute following the first stimulation, an increase that remained permanent for that series. In the experiment quoted the rates were counted in the order given.

HAGFISH NO. X.

Natural heart rate per minute 28, 27, 28, 28, 28.
Vagus stimulated, heart not exposed to air 30, 30, 31, 30, 30.
Sinus venosus stimulated 30, 31, 30, 31, 31.

If the experiments occurred in the order: 1 normal rate, 2 vagus stimulation, 3 sinus stimulation, 4 normal, 5 vagus stimulation, etc., it was found that an increase in rate of one or two beats per minute followed the first stimulation but that the rate did not return afterward to the former normal. My interpretation of this slight change is that it is a direct effect of the general muscular contractions of the animal. When the heart is left in the pericardium it is covered by a sheet of ventral muscle and the pericardial epithelium. Any body movements occurring greatly facilitate the flow of the blood and at the same time produce a decided change in the pressure on the heart as suggested above. These two factors act to increase the heart rate.

Curara acts in the usual way when injected into the hagfish. However, it takes a much larger dose to produce paralysis, and it acts more slowly than on a frog or a mammal. Curara eliminates the influence of the motor nerves of the cord on the body muscles, and also the vagus influence on the muscles of the gill sacs. Stimulation

of the vagus in the curarized animal does not produce the usual contractions of the gill muscles nor of the constrictor cardiæ, yet direct stimulation of these muscles is followed by contractions. In the curarized hagfish, stimulation of the vagus produces no visible cardiac effects. Records taken under these conditions continue with uninterrupted rhythm, force, and sequence.

The experiments outlined in this paper were performed first during the summer of 1899, but the results were so exceptional that no report was published until further verification could be had. The experiments were repeated at intervals during the late summer, the early autumn, the following Christmas holidays; in March and September, 1900; and in July, 1901.

The animals were taken directly from the aquarium, and were in good condition. They gave unquestioned evidence of the physiological activity of the muscular and of the nervous tissues for hours after being experimented upon. The experiments were performed during the four seasons of the year. The results are uniform throughout the entire series of experiments, and lead to the conclusion, which may now be announced with confidence, that *the California hagfish, Polistotrema stouti, does not possess regulative nerves for the heart.*

Since the demonstration of the inhibitory nerves for the heart by the Weber brothers, this class of nerves has been shown to be present in numerous species throughout the vertebrate series. I can find no exception whatever in the literature available. It never occurred to me that an exception was possible until I attempted to use the hagfish for demonstrating the vagus influence upon the heart to my class in physiology at the seashore.

Harrington[1] found a certain amount of cardiac resistance to vagus inhibition in guinea pigs during October and January. At this season, stimulation of the vagus nerves produced only a certain amount of cardiac slowing with fall of blood-pressure, but never heart standstill, no matter how strong the stimulus. From February to April, Harrington secured the usual complete standstill of the heart with a sudden and pronounced fall of blood-pressure. He suggested that the difference noted in his experiments was due to a variation in vitality associated with the season, possibly due to a diminution of fresh air and light during the winter months. But in Harrington's

[1] Harrington: This journal, 1898, i, pp. 383–394.

experiments there was not a total absence of vagus influence on the heart.

A large number of bony fishes and several sharks and rays have been shown to possess depressor nerves for the heart. Fishes are mentioned in the original list of animals given by the Weber brothers in which cardiac inhibition was produced by vagus stimulation. I have myself demonstrated the presence of such nerves in eleven different species of bony fishes and one shark, common in Monterey Bay.

Among the invertebrates investigated, some have been shown to possess complex cardiac regulative nervous mechanisms. Perhaps the best known of the invertebrate cardiac nervous systems, is that of the cephalopod molluscs. In the octopus and in the squid, both inhibitory and accelerator nerves are distributed to the heart.[1] The cardiac fibres pass to the heart by way of the visceral nerve. The fibres to the branchial heart are exclusively motor, *i. e.*, accelerator.

Conant and Clark,[2] in an excellent research, demonstrated that the American edible crab, Calinectes hastatus, possesses a cardiac nervous mechanism. From the anterior part of the thoracic ganglion, are given off two pairs of accelerator, and one pair of inhibitory nerves, which run to a pericardial plexus. Illustrative tracings are presented, showing cardiac acceleration and inhibition following the stimulation of these nerves.

The land snail, Helix, possesses cardiac inhibitory nerves, as proven by the papers of Young, Foster, Biedermann, and Ransom.[3] On the other hand, the sea snail, Aplysia, possesses a cardiac accelerator nerve, but no inhibitory nerve. Ransom stimulated the "visceral nerve" and always obtained cardiac acceleration. More recently, Bottazzi and Enrique have demonstrated accelerator nerves for Aplysia depilans and A. limacina. They also failed to find inhibitory nerves.

The presence of cardiac regulative nerves in so many invertebrates has tended to strengthen the assumption that such nerves were present in all of the lower vertebrates, an assumption which has not before been questioned.

[1] Ransom: Journal of physiology, 1884. v, pp. 261–347. Bottazzi et Enrique: Archives italiennes de biologie, 1901, xxxiv, pp. 111–143.

[2] Conant and Clark: On the accelerator and inhibitory nerves to the crab's heart, Journal of experimental medicine, 1896. i, pp. 1–7.

[3] Ransom: *Loc. cit.*, p. 261. Other literature is cited in Ransom's article.

The present experiments show that in the hagfish, the lowest of the craniata, there are no cardiac regulative nerves. In this fact we have a striking illustration of the automaticity of cardiac muscular tissue. The hagfish heart is comparable to the heart of an embryo before nerves have entered. Any regulation of the heart's action must depend upon the conditions which affect the muscle directly, *i. e.*, tension, nutrition, etc. The volume and pressure of the blood coming to the heart and the changes in the pressure upon the internal organs produced by the ever-varying movements of the plastic body of the animal are the factors that must have a decided influence on the hagfish heart. Questions as to the influence of nutrition I wish to discuss at another time.

Among zoölogists there is some discussion as to whether or not the apparent primitive structure of the hagfish may be, in reality, a retrograde or degenerate condition. This question may be raised concerning the physiology of the heart. It is, indeed, a question that cannot be arbitrarily settled, and one that must be taken into consideration in any discussion of the acquirement of a cardiac-regulative nervous mechanism in the vertebrate series.

THE PHYSIOLOGICAL ACTION OF FORMALDEHYDE.

By WALDEMAR KOCH.

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THE disinfectant action of formaldehyde was first pointed out by Loew.[1] A number of years later F. Blum[2] introduced formol or formalin (a concentrated solution of formaldehyde) into histological technique as a hardening and preserving agent. Since then, with cheapened methods of manufacture, formaldehyde has found more general application in the arts and trades.

Its use as a preservative for food materials, naturally led to the investigation of its physiological properties. F. Blum[3] had already studied its action on proteids, and Benedicenti,[4] extending this investigation, called attention to the relation of these proteid compounds to aldehyde ammonia combinations, on account of the ease with which formaldehyde could be again split off by means of steam distillation. He was also the first one to point out the action of formaldehyde on the blood, changing hæmoglobin to hæmatin. The more recent experiments of M. Fischer[5] on animals leave no doubt that formaldehyde is an intense protoplasmic poison. The observations of F. W. Tunnicliffe and O. Rosenheim[6] on children directly contradict Fischer's results, but can be explained by considering the extremely dilute solutions used. The results obtained by various investigators with reference to the action on enzymes indicate that proteolytic enzymes are more or less hindered in their action, while starch-, sugar-, or fat-splitting enzymes are very little affected. The first attempt to offer an explanation of the intense bactericidal action of formaldehyde was made by Van't Hoff. In his "Vorlesungen über theoretische und physikalische Chemie" Vol. III, p. 118, he states: "Oxide of

[1] Loew: Münchener medicinische Wochenschrift, 1888, p. 412.

[2] F. Blum: Anatomischer Anzeiger, 1895, xi, p. 718.

[3] F. Blum: Zeitschrift für physiologische Chemie, 1896, xx, p. 127.

[4] Benedicenti: Archiv für Physiologie, 1897, p. 210.

[5] M. Fischer: Journal of the Boston Society of Medical Sciences, 1900, v, p. 18.

[6] F. W. Tunnicliffe and O. Rosenheim: Journal of hygiene, 1901, i, p. 319.

methylen (formaldehyde or formalin) oxydizes itself even on exposure to the air. The active oxygen formed as a result of this reaction may be made to account for the antiseptic properties of formaldehyde." [1]

The present investigation was begun with a view to testing the above suggestion. If formaldehyde must form active oxygen in order to develop its antiseptic action, it should not be poisonous to anærobic forms of life. As yeast can easily be made to grow anærobically, it was used in this research.

Two tubes half filled with a fermenting solution were inoculated with a little compressed yeast, and into one was placed a small thin glass bulb, filled with a one per cent formaldehyde solution, freed from oxygen by boiling and sealing the bulb while steam was escaping. The air was then expelled from the tubes by a stream of hydrogen and after fermentation was well under way the formaldehyde bulb was broken and both tubes were closed. After an hour the tube containing the formaldehyde had developed little or no gas, while the control tube gave considerable pressure. A microscopic examination of the yeast cells exposed to the formalin showed them to be irregular in outline, with the protoplasm of a granular appearance, reminding one of starved or drying yeast cells in which katabolic reactions have come to a stop. In order to study more carefully the immediate effects of formaldehyde, fermentation tubes filled with a fermenting solution were sealed by filling the bend between upright tube and bulb with mercury and inoculated with about ten milligrams (dry weight) of compressed yeast. The amount of gas collected in such a tube can be considered a measure of the growth taking place. The fermenting solution was made up as follows:

Glucose	100 grams.
Sodium potassium tartrate	4 grams.
Ammonium nitrate	2 grams.
Sodium carbonate	2 grams.
Water	1000 c.c.
Sodium indigo sulphonate sufficient to give a blue color.	

The indigo in this solution soon decolorizes, forming with glucose an unstable compound, which breaks up in the presence of air or oxygen, thus forming a valuable indicator for small quantities of

[1] "Methylenoxyd (Formaldehyde oder Formalin) oxydiert sich schon an der Luft, womit wohl Sauerstoffactivierung und dadurch antiseptische Wirkung zusammenhängt."

oxygen. The mixture has no power of absorbing oxygen like pyrogallol, but serves merely as an indicator. Formaldehyde does not give such a colorless compound with indigo.

	Concentration of formaldehyde added.	Before adding formaldehyde.		After adding formaldehyde.	
		Time in hours.	Gas collected. c.c.	Time in hours.	Gas collected. c.c.
I.	Control [1]	2.5	3.75	9.0	9.0
II.	0.1 per cent	2.5	2.8	21.0	12.0
III.	1.0 " "	2.0	4.7	21.0	3.6
IV.	1.0 " "	0.0	0.0	20.0	0 0
V.	5.0 " "	2.5	3.0	14.0	0.6
VI.	10.0 " "	2.5	5.1	18.5	1.5

Capacity of tubes 12–15 c.c.

[1] Added one-half c.c. of water instead of formaldehyde after yeast had grown two and one half hours.

The amount of formaldehyde necessary to prevent the growth of yeast will be seen from the above table. In each case one-half cubic centimetre of formaldehyde of the strength indicated was added, after the yeast had grown for several hours, except in IV, where it was added immediately. As usually about ten cubic centimetres of fermenting solution remained in the tube, the concentration of the formalin added must be divided by twenty to give the strength of solution to which the yeast was exposed. We see thus that 0.05 per cent (1 : 2000) kills growing yeast, while 0.005 per cent (1 : 20,000) does not. Another interesting fact is obvious on comparing III and IV, namely, that the immediate addition of the formalin prevents all fermentation, while adding the formalin to fermenting yeast permits varying and always considerable amounts of gas to collect. Two factors must be considered in explaining this result. First the retention of carbon dioxide in the meshes of the growing yeast from which the gas is gradually liberated, and secondly fermentation due to zymase after the cell is dead. The fact that zymase is not sensitive

to formaldehyde was first indicated by the work of A. Macfayden, G. Morris, and S. Rowland,[1] and is also very strikingly shown in the following table. For a number of hours after the addition of the

Time in hours.	Control.		Potassium arsenite. 15 per cent solution.		Formaldehyde. 1 per cent.	
	Gas collected. c.c.	Rate.	Gas collected. c.c.	Rate.	Gas collected. c.c.	Rate.
1.0	1.2	0.4	0.9	0.4	0.9	0.4
1.5	1.6	0.7	1.3	0.5	1.3	0.7
2.0	2.3	0.8	1.8	0.6	2.0	0.7
2.5	3.1	0.8	2.4	0.6	2.7	0.7
3.0	3.9	0.7	3.0	0.6	3.4	0.5
3.5	4.6	0.5	3.6	0.6	3.9	0.9
4.0	5 1	0.9	4.2	0.7	4 8	0.9
4.5	6.0	0.9	4.5[1]	0.3	5.7[2]	0.4
5.0	6.9	1.2	4.8	0.3	6.1	0.3
5.5	8.1	1.0	5.1	0.1	6 4	0.2
6.0	9.1	1 0	5.2	0.2	6.6	0 1
6.5	10.1	0 9	5.4	0.3	6.7	0.2
7.0	11.0	1.0	5.7	0.3	6 9	0.3
8.0	12.0	1.4	6.0	0.1	7.2	0.2
9.0	13.4	0.9	6.1	0.2	7.4	0 4
10.0	14.3	0 7	6.3	0.3	7.8	0.3
11.0	15.0		6.6	0.3	8 1	0.6
23.0	Tube empty		6.9		8 7	

[1] Added one half c.c. K_2HAsO_3 sol.
[2] Added formaldehyde one half c.c.

arsenite as well as the formalin a slow but steady evolution of gas continues, gradually coming to a stop. The complete parallelism of

[1] A. Macfayden, G. Morris, and S. Rowland: Berichte der deutschen chemischen Gesellschaft, 1900, xxxiii, p. 2764.

the arsenite and the formalin is apparent from the above table, and seems to point pretty clearly to the fact that the zymase continues its action in the presence of formaldehyde as well as in the presence of potassium arsenite, though a much stronger solution of arsenite is required.

We have seen then, that the action of formaldehyde does not depend on active oxygen, neither does it affect zymase. Another possibility to be considered is its interference with the proteolytic enzyme, which has been found to be present in yeast by Kutscher[1] and others. The fact that formalin interferes with tryptic digestion has been observed by a number of investigators. Whether or not the trypsin is itself affected by formalin has never been clearly shown. The following experiments were therefore performed to settle this point.

Experiment I. — To 10 c.c. trypsin solution (covered with toluol) added 2 c.c. 10 per cent peroxide of hydrogen. *Fibrin digested.*

Experiment II. — To 10 c.c. trypsin solution (covered with toluol) added 1 c.c. 1 per cent formaldehyde and *immediately* afterwards 2 c.c. 10 per cent peroxide of hydrogen to destroy formalin. *Fibrin digested.*

Experiment III. — To 10 c.c. trypsin solution (covered with toluol) added 1 c.c. 1 per cent formaldehyde. *Fibrin not digested.* After 12 hours added 2 c.c. 10 per cent peroxide of hydrogen to destroy formalin. *A fresh piece of fibrin was digested.*

Experiment IV. — To 10 c.c. of a fresh trypsin solution (covered with toluol) added the fibrin which had not been digested in Exp. III. *It was slowly digested.* A fresh piece of fibrin treated with a stronger solution of formaldehyde *was not so easily digested.*

These experiments show that the enzyme, like a starch or fat splitting enzyme, is not directly affected by formaldehyde. The fibrin, on the other hand, is rendered more or less indigestible, depending on the strength of the formaldehyde and the time of exposure.

We may conclude from this that formaldehyde does not act by forming active oxygen, nor by destroying the zymase, but brings about the death of the cell indirectly by rendering its proteid food supply useless and by preventing the digestion of proteids always going on within the protoplasm of the cell, a reaction intimately connected with the life of the organism.

[1] KUTSCHER: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 476.

ON THE RELATION OF LIPASE TO FAT METABOLISM — LIPOGENESIS.

By A. S. LOEVENHART.

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SINCE it has been shown that lipase is reversible in its action [1] the author has felt that this fact should throw light on the history of fat in the organism, its absorption, its storing up and its utilisation.

Inasmuch as all that follows depends on the reversible action of lipase, it may be well to give a brief account of the experiments which demonstrate this fact. It was found that lipase hydrolyses ethyl butyrate with great readiness and in the work referred to this ester was used because it offers many experimental advantages over a true fat. The evidence that lipase is reversible in its action may be briefly stated as follows: — first, the hydrolysis of ethyl butyrate by lipase is incomplete; second, the hydrolysis is inhibited by the products of the reaction, and third, we were able to synthesise ethyl butyrate by the action of lipase on butyric acid and ethyl alcohol. When a fresh aqueous extract of the pancreas is treated with a mixture of dilute butyric acid ($\frac{n}{10}$ to $\frac{n}{20}$) and ethyl alcohol (sufficient in quantity to bring the whole to $1\frac{1}{2}$ per cent) the very characteristic odor of ethyl butyrate soon develops even at ordinary temperature and in the presence of antiseptics, whereas if the pancreatic extract is first boiled the mixture never develops the odor of ethyl butyrate. When this experiment is performed on a large scale a light oil can be distilled from the mixture which possesses the odor and general properties of ethyl butyrate. Further, it can be hydrolysed by lipase, and butyric acid can easily be proven to be one of the products of hydrolysis. The experiment is readily performed and with an active pancreas the odor of butyric ether can be detected in an hour.

These experiments leave no doubt that lipase is reversible in

[1] KASTLE and LOEVENHART: American chemical journal, 1900, xxiv, p. 491.

its action. Since the above work appeared, Hanriot,[1] unaware of our work has found that lipase can synthesise monobutyrin from butyric acid and glycerine.

In the physiological application of the reversible action of lipase the inference is made that lipase is also reversible in its action on the higher fats. The chemical analogy between the fats and other ethereal salts puts this inference almost beyond question. Both are hydrolysed by the same agencies and all the methods of synthesising the one are applicable to the other. Thus hydrochloric acid will accelerate the hydrolysis of both fats and ethyl butyrate. Under proper conditions it can also effect their synthesis. Similarly we know lipase can hydrolyse both the fats and ethyl butyrate, and since we have proven that it is capable of inducing the synthesis of ethyl butyrate it appears highly probable that it will also induce the synthesis of fats. The light which these experiments throw on the physiological transformation of fat will be considered under two heads, viz. 1. The absorption of fat. 2. The storing up and utilisation of fat, — Lipogenesis.

The Application of the Reversible Action of Lipase to the Theory of Fat Absorption.

Serious objections have been urged against the various theories which have been advanced from time to time to explain the manner in which fat passes from the lumen of the intestine into the central lacteal.

Without going too deeply into a discussion of the previous work on fat absorption, it may be said that the old view first advanced by Brücke, that the fat is taken up by the intestinal mucosa in particulate form has been gradually abandoned in the light of more recent work.

Recently, however, Hofbauer[2] has attempted to show that fat is absorbed without being previously hydrolysed. He fed fat stained with alcanna red and other pigments and showed that although these pigments are insoluble in water the fat appears in the thoracic duct colored red. Pflüger[3] has shown, however, that the coloring matter is soluble in bile, soaps, etc., thus explaining its absorption. Hence

[1] Hanriot: Comptes rendus de la société de biologie, 1901, p. 70.

[2] Hofbauer: Archiv für die gesammte Physiologie, 1900, lxxxi, p. 263.

[3] Pflüger: *Ibid.*, p. 375.

Hofbauer's work seems to throw no light on the subject. As Rachford[1] has shown that the pancreatic juice is capable of hydrolysing all the fat of a fatty meal in the period of pancreatic digestion there is no reason to believe that much of it escapes hydrolysis.

As to the absorption of the products of cleavage there is a divergence of opinion, Munk maintaining that it is absorbed as free fatty acid, while others believe that it is absorbed as soap. However the fatty acid is absorbed, there is certainly a synthesis of fat in the epithelium, as these cells can be seen to contain fat granules during absorption.

Ewald[2] found indication that the dry powdered mucous membrane of the small intestine is capable of synthesising fat from a proper mixture of glycerine and soap. Hamburger[3] has recently repeated Ewald's experiments with some modification using the mucosa of large intestine and has reached the same results.

In both cases the only test for fat formation was an increase in material soluble in ether.

The question as to how the fat which is synthesised in the epithelial cells reaches the central lacteal, has also been a matter of controversy. These granules cannot be egested as such from the attached border of these cells. The view that the fat granules leave the cells as such from the attached border is perhaps less acceptable than that they are taken up in this form from the lumen of the intestine.

It seems even more certain that the fat leaves the cells in solution than that it enters them in this condition. It is believed that the demonstration of the reversibility of the action of lipase throws new light on the question of fat absorption and its passage to the central lacteal.

In considering fat absorption in the light of this view two facts must be borne in mind, first, the hydrolysis of fats by lipase is incomplete unless the products of cleavage are removed from the field of activity, and second, lipase is capable of synthesising fat from fatty acid and glycerine, and here too the action of the lipase is incomplete. A mixture of fatty acid and glycerine reaches equilibrium in the presence of lipase only when a certain amount has been synthesised into fat, and it may be said that lipase merely has

[1] RACHFORD: Journal of physiology, 1891, xii, p. 72.
[2] EWALD: Archiv für Physiologie, supplement, 1883, p. 302.
[3] HAMBURGER: *Ibid.*, 1900, p. 433.

the power of hastening the production of equilbrium in such a mixture. The absorption of the products of digestion makes the splitting of fats in the intestine more or less complete. In the epithelial cells there is undoubtedly a synthesis of fat from the absorbed fatty acid and glycerine, and it is believed that this synthesis is occasioned by an enzyme contained in these cells, which is capable of effecting fat synthesis or fat decomposition according to conditions.

The occurrence of a lipolytic enzyme in these cells is proven by the following experiments:

Intestinal mucosa. — The small intestine of the pig was removed very soon after death and thoroughly washed with running water, this being continued for some time after the washings became perfectly clear. The intestine was then opened and the mucosa scraped from the upper part. Five grams of this were ground with white sand and extracted with 50 c.c. water. To test the activity of the extract, tubes were prepared containing 4 c.c. water, 1 c.c. of the extract, 0.26 c.c. ethyl butyrate and 0.1 c.c. toluene.

After acting at 40° for fifteen minutes the tubes required 0.2 c.c. KOH to neutralise the butyric acid formed, this corresponding to a hydrolysis of 0.5 per cent. On standing ten hours at room temperature (21°) they required 0.95 c.c. $\frac{n}{20}$ KOH, corresponding to 2.38 per cent hydrolysis.

Blanks in which the boiled extract was used remained neutral. The lipolytic activity of the intestinal mucosa shows great individual variation. Occasionally much greater activity than that given above has been found and some specimens show very slight activity.

The part played by the lipase in these cells during fat absorption would seem to be as follows: when the fatty acid and glycerine are taken up by the cells, the lipase in them begins to establish equilibrium between these products, which requires that a certain amount combine to form fat. In the epithelial cell at this stage, fat, fatty acids, and glycerine are simultaneously present. Inasmuch as fatty acid and glycerine are constantly diffusing out of the cell through its attached border, the fat contained in it would soon disappear if these products were not being simultaneously taken up by the cell from the lumen of the intestine.

But while the cell is absorbing the products of fat digestion, fat will remain in it and the quantity found, together with the length of time it remains there, will depend on the relative rates

of diffusion of the fatty acid and glycerine through the free and attached borders of the cell, viz., the relative rates of entrance and exit. Hence during rapid absorption the epithelial cells are seen to contain more fat than when absorption is slower. According to the view I have just advanced, the free fatty acid and glycerine pass into the central lacteal and the occurrence of fat in the intestinal mucosa is not an important factor in fat absorption, since it must again be hydrolysed. In the thoracic duct, however, we find that the split products have been largely but not entirely recombined to form fat.

Munk[1] found that the lymph from the thoracic duct contains from 5 to 10 per cent of its fatty acid in the free state, thus showing that the synthesis is not complete. Some have supposed that this synthesis takes place in the epithelial cells, but this leaves no explanation as to how the fat leaves these cells. Munk thought that the synthesis occurs in the lymphatic glands. With a view of throwing some light on this question a study of the lipolytic activity of lymph and lymphatic glands was undertaken.

Lymphatic glands. — The lymphatic glands of a large bitch were removed immediately after death and 20 grams were extracted with with 100 c.c. water.

Tubes containing 4 c.c. water, 1 c.c. extract, 0.26 c.c. ethyl butyrate, and 0.1 c.c. toluene and kept at 40° showed a hydrolysis of 0.5 per cent in fifteen minutes. After forty hours at room temperature (21°) they showed 2.76 per cent hydrolysis.

Lymph. — The lymph was collected from the thoracic duct of dogs. The following may be taken as representing the average activity of the lymph. To 2.5 c.c. of lymph serum was added 0.2 c.c. litmus and 1.3 c.c. $\frac{n}{10}$ butyric acid, which brought the litmus to the neutral tint; then 1 c.c. water was added, making the total volume 5 c.c. To this 0.26 c.c. ethyl butyrate and 0.1 c.c. toluene were added. After thirty minutes at 40° the active tubes had become bright red. After acting for two and a half hours 0.5 c.c. $\frac{n}{20}$ KOH was required to neutralise the butyric acid, therefore 1.25 per cent of the butyrate had been hydrolysed. Blanks using the boiled serum similarly treated remained perfectly neutral. When the lymph was not neutralised previous to adding ethyl butyrate it was found to become speedily acid from the hydrolysis. The activity of lymph was

[1] Munk: Virchow's Archiv für pathologische Anatomie, 1884, xcv, p. 407.

tested in still another way. To 11 c.c. of lymph serum, 0.3 c.c. litmus and 2.4 c.c. $\frac{n}{20}$ butyric acid were added to bring it to a neutral tint. 5 c.c. of this mixture was placed in each of two test-tubes and to No. 1 there was added 0.26 c.c. ethyl butyrate, while No. 2 was kept as a check. After acting seven hours at 40° and sixteen hours at room temperature, No. 1 required 1.7 c.c. $\frac{n}{20}$ KOH to bring it to the color of No 2, this corresponding to a hydrolysis of 4.27 per cent. It will be thus seen that both lymph and lymphatic glands possess marked lipolytic activity, which, however, is not great compared with the liver and pancreas.

It must be borne in mind in this connection that the results reached with ethyl butyrate may be applied qualitatively but not quantitively to fats and other ethereal salts. The equilibrium in every case will depend on the nature and strength of the combining acid and alcohol. There is reason to believe, however, apart from physiological considerations that in the case of the higher fats equilibrium would be established when most of the fatty acid and glycerine are combined as fat. Euler,[1] working on the equilibrium between ethereal salts, their constituent acid and alcohol, and water, finds that the hydrolysis of the ester is greater, the stronger its constituent acid. He also finds that the state of equilibrium varies greatly for different alcohols, and in an entirely irregular way for alcohols of the same series, hence the equilibrium in the case of each ethereal salt will have to be investigated separately. The theory of fat absorption advanced above and supported by the experimental evidence appears to me to be in accordance with all the facts in the case and offers a clear and satisfactory explanation of the process.

The Storing up and Utilisation of Fat. — Lipogenesis.

In connection with the proof of the reversibility of the action of lipase it occurred to the author to ascertain to what extent the fat synthesis known to occur in the body could be induced by the lipolytic enzyme.

In spite of the advance made in the study of syntheses occurring in the organism we have as yet but little or no conception as to the vital agencies which induce these syntheses.

In speaking of the great progress in synthetic organic chemistry

[1] Euler: Zeitschrift für physikalische Chemie, 1901, xxxvi, p. 405.

Bunge[1] remarks: "Nevertheless the processes employed in no way represent the synthetic processes in the living cell, for all artificial syntheses can only be achieved by the application of forces and agents which can never play a part in vital processes, such as extreme pressure, high temperature, concentrated mineral acids, and free chlorine, agents which are immediately fatal to any living cell." It would certainly seem strange if there should be operating in the living organism special synthetic agencies, and yet that no investigator had found the slightest evidence of their existence.

It was with a hope of throwing some light on these vital synthetic agents that a study of the reversible action of lipase on ethereal salts was undertaken by Kastle and the author.[2] If lipase be the agent which occasions both fat synthesis and fat splitting, it will follow that the enzyme occurs wherever these processes are taking place in the body, and as fat is found in all tissues to a greater or less extent we may expect to find lipase in varying quantities in all the tissues. This has been borne out by experiment. In searching for the greatest occurrence of lipase, Kastle and I tested the lipolytic activity of several organs and tissues.

In our work extracts of 10 grams of the tissue in 100 c.c. of water were used. 1 c.c. of this stained extract was diluted with 4 c.c. of water, and after heating five minutes at 40°, 0.26 c.c. ethyl butyrate and 0.1 c.c. toluene were added. After forty minutes they were titrated with $\frac{n}{20}$ KOH, using litmus as the indicator. The initial acidity of the extracts, though quite small, was always taken into account. The following results are quoted:

	c.c. $\frac{n}{20}$ KOH required.	Per cent of Hydrolysis.
Pancreas	1.4	3.52
Kidney	0.7	1.76
Liver	4.1	10.29
Submaxillary gland . .	0.5	1.26

We proved also that intestinal and gastric mucosa possess lipolytic activity. In a similar manner I have studied the lipolytic activity of the following tissues: liver, pancreas, mammary gland (active and resting), blood and lymph, intestinal mucosa, lymphatic glands, brain, spleen, somatic muscle, and heart muscle. In most cases these

[1] Bunge: Lehrbuch, 1894, p. 288; Schäfer's Text-book of physiology, 1898, i, p. 893.

[2] Kastle and Loevenhart: American chemical journal, 1900, xxiv, p. 491.

tissues were taken from the pig and dog. Usually 10 per cent extracts were employed and these were allowed to act on the ethyl butyrate for fifteen minutes, but for tissues of weak activity 20 per cent extracts were employed and allowed to act for a longer time.[1]

Mammary gland. — The enormous fat synthesis which occurs in this gland when active suggested the occurrence of a large amount of lipase in it and the facts agree perfectly with the theory. A 10 per cent extract of the active mammary of a bitch was tested as described above. After thirty minutes at 40° it required 1.55 c.c. $\frac{n}{20}$ KOH, corresponding to 3.79 per cent hydrolysis. On standing at room temperature for forty hours the tubes required 9.1 c.c. $\frac{n}{20}$ KOH, corresponding to 23.3 per cent hydrolysis.

This is even slightly greater than the activity which has been found for the pancreas of the dog. The resting gland was found to possess less than one-tenth the lipolytic power of the active gland. The above is the only active mammary gland that has been tested. In this connection it is interesting to note that Bartlet,[2] Henriques and Hansen,[3] and others have found that increasing the fat in the diet of cows increases the fat in milk; moreover, small quantities of the foreign fat taken with the food were found in the milk. On continuing the fatty diet for some time, however, the fat content of the milk fell to normal.

It should be stated that in the following account of the lipolytic activity of the tissues the figures given are the averages of the several concordant determinations. In every case blanks in which the boiled extract was used were carried through and in no case did they show the slightest hydrolysis of the ester. In many cases blanks in which the unboiled extracts without the ethyl butyrate were used were carried through in order to detect any increase in the acidity of the extract itself. The results were always negligible.

Liver. — In the dog, pig, and man this has been found to have the greatest lipolytic activity of any tissue tested. When 10 per cent ex-

[1] In speaking of the occurrence of lipase in the tissues, HANRIOT: Comptes rendus de la Société de biologie, 1896, p. 925, states that the liver, blood, and pancreas possess large quantities of it, while the thyroid gland, spleen, testicle, adrenal, urine and lymph contain only small amounts. He gives no data from which the relative activities of these tissues can be estimated.

[2] BARTLET: 14th Annual Report. Maine Agricultural Experiment Station, 1898, p. 114.

[3] HENRIQUES and HANSEN: Extract in the Journal of the Chemical Society, 1900, p. 668.

tracts of these livers were used, the results, on testing in the usual way were as follows:

Duration of experiment 15 minutes; temperature, 40°.

	c.c. $\frac{n}{20}$ KOH required.	Per cent of Hydrolysis.
Man[1] . .	0.9	2.26
Dog . .	1.5	3.76
Pig . . .	3.4	8.54
Pancreas. —		
Dog . .	1.0	2.51
Pig . . .	1.4	3.51
Kidney. — Conditions same.		
Dog . .	0.82	2.06
Pig . . .	0.70	1·76
Lung. — Conditions same.		
Dog . .	0.6	1.51
Brain. — Conditions same.		
Dog . .	0.15	0.38
Adrenal. — Conditions same. Duration of experiment 60 min.		
Dog . .	0.12	0.30

Spleen. — This organ possesses slight activity. A 20 per cent extract when tested as usual showed no change in one hour. After forty hours at room temperature it required 1.4 c.c. $\frac{n}{20}$ KOH for neutralisation, hence, 3.51 per cent hydrolysis.

Heart muscle. — A 20 per cent extract of the human heart from the same subject as the liver tested was used. The tube became bright red in one hour and in two hours it required 0.3 c.c. $\frac{n}{20}$ KOH for neutralisation, hence 0.75 per cent of the ester was hydrolysed. Dog and pig hearts show the same activity as the human heart. In these tests the myocardium was carefully cleared of any fatty tissue.

Somatic muscle. — This has about the same lipolytic activity as heart muscle. A 20 per cent extract acting for fifteen minutes at 40° and then for sixteen hours at ordinary temperature required 0.5 c.c. $\frac{n}{20}$ KOH 1.25 per cent hydrolysis.

Blood. — Hanriot,[2] in trying to determine the mechanism by which

[1] The individual died of intestinal obstruction. The autopsy was held eight hours after death and all of the organs were found normal. The tissue was tested immediately.

[2] Hanriot: Comptes rendus de la Société de biologie, 1896, p. 753. Archives de physiologie, 1898, p. 797.

reserve fat is utilised by the organism, in 1896 discovered that the blood possesses great lipolytic power. He made an interesting comparison of the lipolytic activity of the blood of a series of animals, using monobutyrin as the reagent in testing the activity. In my work serum was obtained by centrifugalising cat's blood. It was tested as follows: 1 c.c. serum, 4 c.c. water, 0.1 c.c. toluene and 0.26 c.c. ethyl butyrate were kept at 40° for thirty minutes when the mixture required 0.25 c.c. $\frac{n}{20}$ KOH for neutralisation, corresponding to 0.63 per cent hydrolysis. On returning to the bath for one hour and a half it contained a coagulum and required 0.7 c.c. $\frac{n}{20}$ KOH for neutralisation, the hydrolysis therefore being 1.76 per cent.

From this we see that blood has great lipolytic activity. In this calculation the amount of acid used in overcoming the initial alkalinity of the blood was not taken into consideration. Hence, to form a more accurate idea of the activity of blood, the following experiment was tried: 5 c.c. serum was neutralised with 1.7 c.c. $\frac{n}{20}$ butyric acid, using litmus as the indicator.

This was divided into two equal parts, and to each was added 0.1 c.c. toluene. They were heated five minutes at 40°, and to A was added 0.26 c.c. ethyl butyrate, none of the ether being added to B. After one hour, A required 0.9 c.c. $\frac{n}{20}$ KOH to return it to the color of B, hence 2.26 per cent of the ether was hydrolysed.

Bile. — Notwithstanding the great activity of the pig's liver the bile possesses but the merest trace of lipolytic activity, as is shown by the following experiments: Tube No. 1, 1 c.c. fresh pig's bile, 4 c.c. water, 0.1 c.c. toluene, and 0.26 c.c. ethyl butyrate. After acting for five hours at 40° and nineteen hours at ordinary temperature, it required 0.1 c.c. $\frac{n}{20}$ KOH 0.25 per cent hydrolysis. Blanks with the boiled bile, toluene, litmus, and ethyl butyrate, and with fresh bile to which ethyl butyrate had not been added, remained unchanged, and only required one drop $\frac{n}{20}$ KOH to turn them from a dirty yellow to a bluish green. Bruno[1] stated that the bile contains a lipolytic enzyme, and assists the pancreatic juice in its fat-splitting function.

The results obtained above are in accordance with the old view, that the bile aids in fat absorption only through its solvent action and not by any power to split fats. The lipolytic activity of intestinal mucosa, lymph, and lymphatic glands, was discussed in connection with fat absorption.

[1] Bruno: Archives des sciences biologiques, St. Petersburg, 1899, vii, p. 87; Chemisches Centralblatt, 1900, [ii] p. 916.

Adipose tissue. — The theory of the part played by lipase in fat synthesis indicated the occurrence of lipase in adipose tissue, and the findings are entirely in accordance with the theory. The following experiment illustrates the degree of activity found. Approximately a 40 per cent extract of the subcutaneous fat of the pig was prepared and tested in the usual way. After standing for thirty minutes at 40°, the tubes required 0.5 c.c. $\frac{n}{20}$ KOH for neutralisation, corresponding to 1.25 per cent hydrolysis. After forty hours at room temperature, the tubes required 2.55 c.c. $\frac{n}{20}$ KOH, corresponding to 6.40 per cent hydrolysis. Inasmuch as it is impossible to extract this tissue with any degree of thoroughness, its lipolytic activity, in all likelihood, exceeds that found in the above-quoted experiments. In this connection it was decided to test the power of the extract of subcutaneous fat to synthesise ethyl butyrate in a way similar to that used to test the synthetic action of pancreatic extract.

When a 25 per cent extract of subcutaneous fat is mixed with butyric acid and ethyl alcohol in the proportions and under the conditions described in connection with the pancreas experiments and in the presence of thymol the odor of ethyl butyrate was very apparent after twenty-four hours, whereas the blank in which the boiled extract was used remained free from the odor of ethyl butyrate. This proves the synthetic action of the lipase of this locality. The lipolytic power of fatty tissue from other parts of the pig was also tested. Pericardial and perinephric fat were both found to be active, but markedly less so than subcutaneous fat. This is in accordance with the fact that, during inanition, the fat in these localities is the last to disappear. The lipase which is accountable for the formation of the fat here seems to have subsequently largely disappeared, and hence the difficulty of absorbing it during inanition.

It is a well known fact that during inanition, or a state of malnutrition, an animal is capable of absorbing its own fat as food. What is the mechanism by which this is brought about? In answer to this question we may suppose that blood and lymph, as the result of continual oxidation, become poor in fatty acid and glycerine. When the lymph bathing the fat cell becomes poor in these products, however, the lipase restores equilibrium by effecting an hydrolysis of the fat.

If the passage of the fat from the lumen of the intestine into the lymph and blood and thence to the subcutaneous tissue, be accom-

plished by a series of hydrolyses and hydrosyntheses, it may be asked why an animal does not ordinarily lay on the fat taken as food. This would seem to be very well accounted for by the old explanation, that since the fat laid on differs only quantitatively from the fat of the food, it may be due to the greater destruction of one constituent of the fat than the others, whereby the whole is brought to the normal fat of the particular animal. That the fat of the food may be laid on directly has been conclusively proven by Lebedeff,[1] Munk,[2] and others. The same result has recently been reached by Rosenfeld[3] also, who found that by feeding one dog with cocoa butter and another with mutton fat, the fats deposited in each case corresponded with the fat of the food. Further, he was able to produce a deposit of mutton tallow in goldfish and carp by feeding it to them. He concludes, therefore, that the peculiarities in the fat of different animals is accounted for by the fat in the food.

As food the fats and carbohydrates occupy co-ordinate positions, both serving for the production of body heat, and the ultimate fate of both being oxidation. Starting with Bernard's discovery of glycogen and the sugar-producing power of the liver, much has been said both for and against his theory of "glycogenesis;" but it stands to-day as the best explanation of the facts regarding the storing and transportation of carbohydrates in the body. The liver is the primary store for glycogen, secondary deposits being found in all the tissues of the body. In the case of fats we have somewhat analogous conditions, and hence we may speak of the storing and utilisation of fats as "lipogenesis." In the case of fats the areolar tissue is the great primary store, secondary deposits being found in all the tissues. In some animals even this difference in the storing of fats and carbohydrates is not to be noted. In many fish, notably the cod, the liver, at certain seasons of the year, becomes the great depository for fat. The liver we have found to possess powerful lipolytic activity, and hence, under proper conditions, it should be capable of storing fat. Moreover, this is in accordance with the experiments of Noël Paton,[4] who

[1] Lebedeff: Centralblatt für die medicinische Wissenschaften, 1882, p. 129. Zeitschrift für physiologische Chemie, 1882, vi, p. 149. Archiv für die gesammte Physiologie, 1883, xxxi, p. 11.

[2] Munk: Archiv für Physiologie, 1883, p. 273. Virchow's Archiv für pathologische Anatomie, 1884, xcv, p. 407.

[3] Rosenfeld: Verhandlungen des XVII. Congresses für innere Medicin, 1899, p. 503.

[4] Paton: Journal of physiology, 1896, xix, p. 167.

showed that the fat contained in the liver of frogs is increased after a fatty meal. It is believed that both phases of lipogenesis are induced by lipase, a fat-splitting and fat-forming enzyme. Against this view it may be urged, however, that the amount of lipase found in the tissue is no index of the amount of fat they contain. Thus the liver contains more lipase than any other tissue, and yet it contains normally comparatively little fat. It seems that besides the presence of lipase, there must exist in the tissues certain conditions which favor the storing up of fat. For instance, in the liver the great lymph formation and the enormous and varied activities of this organ may not be favorable for fat accumulation. Indeed, it seems that the conditions for this are more favorable in the more sluggish connective tissue. Under pathological conditions, however, where the function of the liver is interfered with, we note the ease and rapidity with which fat may be stored up. Further, it will be seen below, that the hydrolysis by hepatic lipase is much more complete than with pancreatic lipase. Whether, on the other hand, its synthetic power is equally great or correspondingly less, has not yet been determined. The observation of Hanriot that fœtal blood does not contain lipase up to the sixth month, is not opposed to the theory of the part played by lipase in lipogenesis. The absence of lipase from the blood should offer no obstacle to the laying on of fat if the tissues contain lipase.

Fate of Lipase in the Body.

In this connection it has been found that the urine possesses but a trace of lipolytic activity, and that at times none at all can be noted. Extracts of fæces, on the other hand, show some activity. A 20 per cent extract of fresh fæces was prepared and tested in the presence of toluene to prevent the action of bacteria. After two and a half hours, it required 0.2 c.c. $\frac{n}{20}$ KOH, corresponding to 0.5 per cent hydrolysis. The blank containing the boiled extract remained neutral. Whether this activity is derived from the pancreatic juice or from lipase contained in the bacteria I am unable to say.

We have found that pancreas kept until putrefaction was well advanced, still showed lipolytic activity in the presence of toluene, though it was much diminished.

Distribution of Lipase in the Tissues in Phosphorus Poisoning.

In the light of the recent work on phosphorus poisoning[1] it was decided to poison dogs slowly by the subcutaneous injection of sub-lethal doses of a one per cent solution of phosphorus in olive oil. Three dogs were experimented with, and during the poisoning they were fed largely on fatty food. They were killed after two weeks. The organs showed fatty degeneration, and on testing the lipolytic activity of the heart, liver, and kidney it was found that in no case did it vary far enough from the normal to indicate that the fatty changes were due to changes in the distribution or amount of lipase in the tissue.

On the Non-Occurrence of Soaps in the Body.

After having synthesised ethyl butyrate from butyric acid and ethyl alcohol, the question presented itself as to whether it would be possible to synthesise the ether from sodium butyrate and ethyl alcohol by means of lipase. This was soon found to be impossible. This fact indicates that perhaps soaps do not exist in the blood and lymph, and that instead, the free fatty acid occurs there in simple solution.[2]

On looking into the matter, it was found that there had already been accumulated a number of facts which would lead us to believe that no soaps exist in the blood. The toxicity of the soaps and the non-toxic nature of the constituent free fatty acid, offer us strong evidence against the existence of soaps in the blood. Rassmann[3] first showed the poisonous action of sodium oleate on injection into the blood current. Ten years later, Munk,[4] unacquainted with Rassmann's work, studied very thoroughly the poisonous action of soaps on rabbits by intravenous injection. He found that 0.13 gm. of sodium oleate per kilo caused death in spite of artificial respiration. On injection he found that the pupils became widely dilated, a fall of about $\frac{2}{3}$ in blood pressure occurred, the gas

[1] Taylor: Journal experimental medicine, 1899, iv, p. 399. Athanasiu: Archiv für die gesammte Physiologie, 1899, lxxiv, p. 511.

[2] The solubility of free fatty acid in the blood and lymph has not as yet been determined.

[3] Rassmann: Ueber Fettharn, Inaugural Dissertation, Dorpat, 1880.

[4] Munk: Archiv für Physiologie, Supplement, 1890, p. 117.

exchange decreased $\frac{1}{2}$ to $\frac{1}{4}$, and the heart stopped in wide diastole. The blood also lost its coagulability.

On injecting soaps into animals which had been given morphine, they fell into a coma resembling that caused by albumoses and peptones. Injection of smaller quantities than 0.13 gm. per kilo, caused more or less grave symptoms in proportion to the dose. The sodium soaps of palmitic and stearic acids are even more toxic than sodium oleate.

Munk found that injection of free oleic acid has no effect. This alone, it seems to me, strongly indicates that the blood can only form soaps from the free fatty acids with extreme slowness if at all, or otherwise he would have gotten some symptoms of intoxication upon injecting the free oleic acid. Rachford,[1] in working on the properties of the pancreatic juice, performed an experiment which well illustrates the inability of the body juices to neutralise the higher fatty acids. He found that if pancreatic juice was well shaken with neutral olive oil, and then allowed to stand twenty-four hours, the oil separated at the top and was strongly acid, while the juice at the bottom was strongly alkaline, and still in twenty-four hours no soap had been formed and no emulsion produced. It seems that there might be some union between the alkali of the blood and the proteids which prevent this neutralisation. Pflüger[2] has recently shown, however, the slowness and incompleteness with which one per cent sodium carbonate saponifies the higher fatty acids at 37°. Hoppe-Seyler[3] prepared soaps from the blood and lymph, and from this he maintained that they normally exist there. In his method of extracting the soaps, however, heat, and large amounts of alcohol were employed, and this method would surely break up such combinations between the alkali and the proteid, if they existed, and would be most favorable for the formation of soap from the free fatty acid. No conclusions can be drawn from the work of Hoppe-Seyler. Friedenthal[4] believes that the poisonous action of the soaps is due to their power to precipitate calcium. Whatever may be the manner of their action, our inability to synthesise the ethereal salts from their soaps by means of lipase, is in harmony with the facts above mentioned, all of which point to their non-existence.

[1] Rachford: Journal of physiology, 1891, xii, p. 72.

[2] Pflüger: Archiv für die gesammte Physiologie, 1901, lxxxvi, p. 1.

[3] Hoppe-Seyler: Zeitschrift für physiologische Chemie, 1883–4, viii, p. 503.

[4] Friedenthal: Archiv für Physiologie, 1901, p. 145.

On the Limit of the Action of Lipase on Ethyl Butyrate.

With reference to the mechanics of the action of lipase it is important to determine the limit of its action in order to see if lipase of different origins reaches the same limit, and also to determine to what extent this limit is affected by the amount of ethyl butyrate and enzyme present.

In order to see whether lipase from the liver and pancreas reaches the same limit, extracts of these organs were prepared and diluted until they were about equal in their power to hydrolyse ethyl butyrate; the pancreatic extract was about 20 per cent, while the hepatic was approximately a 7 per cent extract. Tubes containing 1 c.c. of the extract, 4 c.c. of water, 0.1 c.c. toluene, and 0.1 c.c. litmus were heated at 40° for five minutes and then 0.26 c.c. ethyl butyrate was added. After acting for fifteen minutes they were titrated.

	c.c. $\frac{n}{20}$ KOH required.	Per cent of Hydrolysis.
Pancreatic . .	2.2	5.52
Hepatic . . .	1.95	4.89

It was intentional here to have the pancreas extract slightly stronger than that of the liver. Mixtures were now made up as follows:

Pancreas.	c.c.	Liver.	c.c.
Water	37.2	Water	38.5
Litmus	1.0	Litmus	1.0
Pancreas extract . .	10.0	Liver extract . .	10.0
$\frac{n}{20}$ KOH[1]	1.8	$\frac{n}{20}$ KOH[1] . . .	0.5

the total volume being in each case 50 c.c. To each was added 0.65 c.c. ethyl butyrate and 0.5 c.c. toluene. These were placed in bottles and kept at 40°. They were frequently vigorously shaken. At certain intervals 5 c.c. of the mixture were drawn off and titrated, with the results shown in Table, page 347.

From the series in the accompanying Table we see that solutions of pancreatic and hepatic lipase of the same strength reach very different limits. Although the pancreatic and hepatic extracts used in these series were of equal strength when acting for fifteen minutes, yet when each was allowed to act until the limit was reached the liver extract had decomposed one and three-fourths times as much ethyl butyrate as the pancreas extract. This would naturally lead us to

[1] To neutralise the initial acidity.

doubt the identity of the lipase of the liver and pancreas. Hanriot[1] states that the lipase of the blood and the pancreas are not identical.

Time in hours.	Pancreas.		Liver.	
	$\frac{n}{20}$ KOH required. c.c	Per cent of Hydrolysis.	$\frac{n}{20}$ KOH required. c.c.	Per cent of Hydrolysis.
27	4.05	40.5	7.37	73.7
46	4.45	44.5	7.62	76.2
72	4.50	45.0	7.85	78.5
144	4.90	49.0	8.5	85.0

He bases this statement on two facts: First, although extracts of the pancreas and serum have the same activity in the presence of sodium carbonate, the serum shows almost twice the activity of the pancreas extract when the carbonate is neutralised. Second, if serum and extracts of the pancreas be prepared having at 15° the same activity, at 42° the serum becomes twice as active as the pancreas extract. Kastle and I, in a comparison of pancreatic and hepatic lipase, reached a result quite similar to Hanriot's second experiment. Hence it would seem that the lipase of the blood and liver may be identical. As further pointing to the non-identity of liver and pancreatic lipase we found that strychnine sulphate and also phenol acting at a dilution of one part in 5000 lessen the activity of pancreatic extract 30 per cent while they are without effect on hepatic extract. On the other hand osmic and salicylic acids are much more harmful in their effects on liver than on pancreas extract.

Hence we see that pancreatic and hepatic lipase seem to differ in three ways:

1. The velocity of their action is affected differently by changes of temperature.

2. They are affected differently by certain substances. These points were previously brought out by Kastle and myself.

3. When solutions possessing the power of hydrolysing equal amounts of ethyl butyrate in fifteen minutes are allowed to act until the limit is reached in each case, the liver extract hydrolyses

[1] Hanriot: Comptes rendus de la société de biologie, 1897, p. 778.

nearly twice as much of the ether as does the pancreas. It seems that the lipase of the blood, liver, and kidney presents the same characteristics, while that occurring in other localities resembles the pancreatic. This has not yet been definitely proven. These facts would lead one to believe that these enzymes are chemically different. Yet they both manifest their presence in the same way, viz., by the hydrolysis of ethereal salts.

There are other cases where substances of totally different chemical natures show the same catalytic activity. The catalysis of hydrogen peroxide is effected alike by colloidal metal solutions (Bredig), by many metallic peroxides and by all plant and animal extracts (Schönbein, Loew).

Among these substances there is no apparent chemical relation. Other similar examples are to be found among the proteolytic and diastatic enzymes.

The effect of the amount of lipase on the limit of its action on ethyl butyrate is brought out in the following series in which a 10 per cent liver extract was used. Into tightly stoppered tubes the following mixtures were placed.

	A	B	C	D	E	F	G
Extract, c.c. . . .	5.0	4.0	3.0	2.0	1.0	0.5	0.1
Water used, c.c. . .	0.0	1.0	2.0	3.0	4.0	4.5	4.9

To each of these tubes were added 0.1 c.c. toluene, 0.2 c.c. litmus, and 0.26 c.c. ethyl butyrate. They were placed in the thermostat at 38°.

After seventy-two hours they were titrated:

	A	B	C	D	E	F	G
$\frac{n}{20}$ KOH required, c.c. . .	23.52	19.20	14.85	10.80	7.00	4.25	1.90
Hydrolysis, per cent . .	59.04	48.19	37.27	27.11	17.57	10.67	4.77

After one hundred and seventeen hours tubes similar to the first and carried through simultaneously were titrated:

	B	C	D	E	F	G
$\frac{n}{20}$ KOH required, c.c.	18.70	14.75	10.90	6.90	4.80	2.10
Hydrolysis, per cent	46.93	37.02	27.35	17.32	12.05	5.29

Thus the limit had been reached after seventy-two hours. From this series it follows that when a large amount of enzyme is acting the quantity of ether hydrolysed when the limit is reached is proportional to the amount of enzyme present. For small quantities of the enzyme, however, the hydrolysis is somewhat greater proportionally than when a larger amount is used.

In interpreting the above results, it must be remembered that lipase is readily destroyed by acid and that the butyric acid produced by its action in the above experiment undoubtedly had a very detrimental action on it. Kastle and I found that the velocity of the hydrolysis is proportional to the amount of enzyme acting and hence in the limit experiments in which large amounts of enzyme were used there was acting a greater amount of acid for its destruction. On the other hand, in these cases the mixture contained much more proteid and this may possibly have exercised a protective influence over the enzyme.

In order to determine the relation of the quantity of ethyl butyrate to the limit of the action, mixtures were prepared containing liver, or pancreas extracts, and varying quantities of ethyl butyrate, as follows:

Pancreas. — No. 1. 18.75 c.c. water.
1.25 c.c. $\frac{n}{20}$ KOH[1].
0.30 c.c. toluene.
5.00 c.c. 20 per cent pancreatic extract.
1.30 c.c. ethyl butyrate.

No. 2. Mixture was made up in the same way, except that it contained 0.65 c.c. ethyl butyrate.

The mixtures were kept in stoppered bottles at 38°.

After two hundred and fifty-nine hours they had reached the limit, and titration showed the following:

	Quantity titrated. c.c.	c.c. $\frac{n}{20}$ KOH required.	Ester hydrolysed. mgr.
No. 1	5.0	10.5	68
No. 2	5.0	9.32	61

Liver. — No. 1 19.25 c.c. water.
0.75 c.c. $\frac{n}{20}$ KOH[1].
0.30 c.c. toluene.
5.00 c.c. 10 per cent liver extract.
1.30 c.c. ethyl butyrate.

No. 2. Mixture was made up in the same way, except with 0.65 c.c. ethyl butyrate. Temperature, 38°. After two hundred and fifty-nine hours equilibrium had been reached.

	Quantity titrated. c.c.	c.c. $\frac{n}{20}$ KOH required.	Quantity ether hydrolysed. mgr.
No. 1	5.0	13.6	87
No. 2	5.0	12.3	80

[1] To neutralise initial acidity.

From these series it follows that the amount of ethyl butyrate hydrolysed by lipase when the reaction is allowed to proceed to the limit, is largely independent of the excess of ethyl butyrate present.

Summary of Results.

The conclusions reached in this paper may be briefly summed up as follows:

1. The reversible action of lipase furnishes us with a clear explanation of fat absorption.

2. Lipase has been found to occur in all the tissues of the body that have been tested, most notably in the liver, active mammary gland, blood, lymph, and intestinal mucosa. As pointed out in the above, special interest attaches to the fact that lipase has been found in considerable quantities wherever fat synthesis is known to take place as in active mammary gland and subcutaneous fat.

3. The close analogy between the storing up of fat and carbohydrates in the body is pointed out, and as we conveniently call the storing and translocation of carbohydrate "glycogenesis," similarly we may call the corresponding process in the case of fats "lipogenesis." It seems, further, that both phases of lipogenesis may be brought about by the enzyme lipase, which is either fat-forming or fat-splitting, according to conditions.

4. The inability of lipase to synthesise ethyl butyrate from sodium butyrate and alcohol, together with much collateral evidence, has led to the belief that the free fatty acids rather than the soaps exist in the blood and lymph.

5. The fatty changes occurring in phosphorus poisoning are not due to changes in the distribution or amount of lipase in the tissues, as no disturbances of this character were noted.

6. Lastly a study of the limit of the action of lipase on ethyl butyrate has revealed the following:

1. The limit is nearly proportional to the amount of enzyme acting.

2. It is nearly independent of an excess of ethyl butyrate.

In conclusion I desire to express my thanks to Professors Abel and Howell, in whose laboratories this work was done.

THE PHYSIOLOGICAL ZERO AND THE INDEX OF DEVELOPMENT FOR THE EGG OF THE DOMESTIC FOWL, *GALLUS DOMESTICUS*.

A CONTRIBUTION TO THE SUBJECT OF THE INFLUENCE OF TEMPERATURE ON GROWTH.

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CONTENTS.

I. Introductory.

SINCE the time of the Egyptians it has been recorded that warmth is the chief factor in producing development in the eggs of birds. Those workers who have incubated eggs of the domestic fowl, from Von Baer and Harvey down to the present have established the optimum temperature at 38° C., with a range from 35° C. to 39° C. within which the development is usually normal. It has been shown also that cold below 35° C. when not too intense or too prolonged to destroy the embryo retards, or even suspends, the processes of growth during its application.

Prévost and Dumas, Dareste, and others give 28° to 30 ° C. as the physiological zero for the egg of the domestic fowl. Rauber places this minimum at 25° C. Below this temperature it was presumed

that no development takes place. The disagreement as to the exact degree of the physiological zero as shown by the above opinions, founded as they must have been largely upon guesswork, together with the lack of precise data, led me to undertake the following investigation of the subject.

Besides establishing the physiological zero at 20.00°–21.00° C., I have determined more exactly the normal size and variation of the blastoderm and of the area pellucida, the index of development from the physiological zero to 30.75°, the normal volume of the egg, its variation and relation to the diameter of the blastoderm, the growth of the blastoderm independently of the appearance of the primitive streak, and the dependence of ontogenetic differentiation upon rise in temperature.[1] The description of the variations (monsters) produced during these experiments is reserved for later publication.

II. Apparatus and Methods.

In Series A, a Cyphers incubator, provided with its own meta-thermostat, a normal thermometer, calibrated and divided to one-fifths of a degree, and an oil lamp were used.

In Series B, the same outfit was employed with the substitution of gas, flowing from a gas-pressure regulator, and a calibrated thermometer divided to one-tenths of a degree. For this series it became possible to place the apparatus in a basement room in the Hall of Natural History, Trinity College. Having four brick walls, the range of temperature in this room for the period of incubation was generally within one degree.

In Series C, the basement room itself became the incubator. The room was cooled by a ton of ice buried in sawdust in order to reduce the summer temperature to that required. The clutch of eggs was kept at the desired temperature by regulating the distance of the eggs from the ice. In this and Series D, thermometers calibrated and certificated by the Groszherzogliche Sächsische Prüfungsanstalt für Glasinstrumente, were used.

In Series D, through the courtesy of Mr. W. C. Wade, one of the large rooms of his cold storage building was used for incubations **16**, **17**, **18**, and **19**.

Here the air is kept constantly at about 7.5° C. by means of circu-

[1] The results embodied in Series A were read June 28, 1900, before Section F, American Association for the Advancement of Science, at the New York meeting. Edwards, C. L.: Science, 1900, xii, pp. 310–311.

lating brine cooled by ammonia. Incubations **20**, **21**, **22**, and **23** were carried on in the room used for Series B and C.

A Bausch and Lomb copper incubator, 45 cm. × 35 cm. × 70 cm., provided with Roux's bimetallic thermostat and a gas pressure regulator, was used. Besides the main gas flow in which the thermostat was placed, a separate tube was connected with the pressure tank for a minimum safety flame. For both tubes micro-burners were employed.

All temperature readings are from Centigrade scales. The readings were recorded a number of times each day during the period of incubation, and their average taken for the index of development. The extreme range of temperature fluctuation was generally within one degree and sometimes within about half of one degree.

For Series A, eggs were procured from a countryman. For all other incubations and the data concerning fresh eggs I had my own poultry yard. In order to eliminate any question of special races a mixed flock of brown leghorns, barred plymouth rocks and white wyandottes was used.

The eggs were gathered every half hour and during summer weather were placed in a refrigerator at 16° C.

In order to test the possibility of injuring the embryos by subjecting the eggs to a temperature of 16° in a refrigerator the following data were secured.

Date when freshly laid egg was placed in the refrigerator at 16°. 1901.	Date when egg was transferred from refrigerator to incubator at 38°.	Length of time in refrigerator at 16°.	Date of taking out embryo.	Age of embryo.	Condition of embryo.
July 13, 3.30 P. M.	July 14, 7 P. M.	27.5 hrs.	July 18, 10 A. M.	3 days, 15 hrs.	Normal
July 13, 2.30 P. M.	July 14, 7 P. M.	28.5 hrs.	July 18, 10 A. M.	3 days, 15 hrs.	Normal
July 15, 9 A. M.	July 17, 9 A. M.	48.0 hrs.	July 19, 9.40 P. M.	2 days, $12\frac{2}{3}$ hrs.	Normal
July 15, 9 A. M.	July 17, 9 A. M.	48.0 hrs.	July 22, 9 A. M.	5 days.	Normal

It was shown by Colasanti,[1] in eggs kept at a temperature of −7° to −10° C. for from one to two hours that the germ was absolutely

[1] COLASANTI, G. : Reichert's und Dubois-Raymond's Archiv für Anatomie, Physiologie, und wissenschaftliche Medicin, 1875.

uninjured, Pictet[1] demonstrated that after a longer period at from −2° to −3° the embryo is killed, but from −1° the egg would survive, and Rabaud[2] kept eggs at −18° for half an hour without fatal results.

In Series A, B, and C, the eggs were given an average incubation of six and one-half days in order that the effect of each temperature should be complete. It is apparent, however, that the exact length of the period is not important, provided it extends over a number of days. In Series D, each incubation lasted five days. The blastoderms and embryos were fixed in 10 per cent nitric acid, placed in Kleinenberg's picro-sulphuric acid for about twenty-four hours, gradually dehydrated, and then stained in Mayer's cochineal. Such unfertilized eggs as occasionally appeared were thrown out of the series.

In general it was found best to take the measurements from the blastoderm as it rested upon the yolk after fixation, as sometimes there is a slight change in the dimensions, due to the reagents, or some distortion caused by separating the early blastoderms from the vitelline membrane, when the blastoderm is removed.

In order to get some standard for the age of embryos included within the first forty-eight hours, the following table was made based upon the data given by Duval.[3] This author has drawn the 5, 10, 15, and 16 hour primitive streaks. Considering the magnification as given I find from Duval's figures that the actual length of the primitive streak at 5 hours is 0.625 mm.; at 10 hours, 1.25 mm.; at 15 hours, 1.6 mm.; at 16 hours 1.93 mm. The total growth in length between the 5 and 10 hour stages is 0.625 mm., or 0.125 mm. per hour. It is possible that during the first hour or two there is no detectable trace of the primitive streak but in the absence of data and in order to include those cases in the following incubations where the primitive streak measures 0.5 mm., 0.4 mm., 0.3 mm., and 0.2 mm., I have extended the table over the first four hours by employing the above hourly increment of 0.125 mm. The hourly increment of growth between the 10 and 15 hour stages is 0.07 mm., and between the 15 and 16 hours, 0.33 mm. Féré[4] did not find his chicks developing to the Duval ages.

After 48 hours Féré embryos equal 28–33 hours of Duval.
After 72 hours Féré embryos equal 46–52 hours of Duval.

[1] Pictet, Raoul: Extrait des Archives des sciences physiques et naturelles. October 1893, Genève, 1895.

[2] Rabaud: Comptes Rendus, t. 128, 1899, pp. 1183–5.

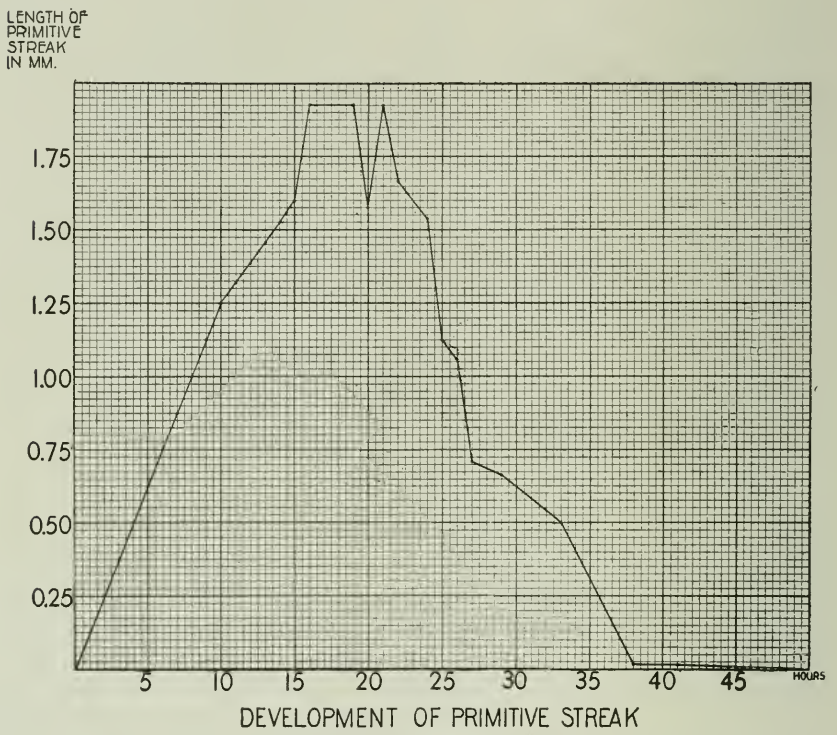
[3] Duval, Mathias: Atlas d'embryologie, Paris, 1889.

[4] Féré, Ch.: Journal de l'anatomie et de la physiologie, 1894, xxx, No. 4.

DUVAL.

Fig.	Age in hours.	Primitive streak. Length in mm.	Number of mesodermic somites.	Notochord. Length in mm.	Neural folds. Length in mm.	Neural groove. Length in mm.	Primary optic vesicles.	Secondary optic vesicles.	Auditory pit.
	1	0.125							
	2	0.250							
	3	0.375							
	4	0.500							
36	5	0.625							
	6	0.750							
	7	0.875							
	8	1.000							
	9	1.125							
47	10	1.250							
	11	1.320							
	12	1.390							
	13	1.460							
	14	1.530							
64	15	1.600							
65	16	1.930							
67	19	1.930		0.463					
68	20	1.580			Low 0.790				
70	21	1.930	1		1.501	Present.			
71	22	1.666	1		Approach ant.	"			
72	23	1.600	3		Lie together ant.	Post. $\frac{2}{3}$ open			
76	24	1.540	5–6		Lie together ant.	Post. 1.540 open			
77	25	1.150	6		Lie together ant.	Post. $\frac{2}{3}$ open			
81	26	1.070	8		Lie together ant.	Post. $\frac{1}{2}$ open			
86	27	0.710	8–9		Partly united ant.	Only last open	Beginning		
89	29	0.666	10–11		3 cerebral ves.	Mostly closed	Present		
92	32						Present		Rudiment
93	33	0.500	15			Mostly closed	Present		Deep, wide open
98	38	trace	17			Mostly closed	Present		Deep, wide open
100	41	"	18						
102	43	"	19					Rudiment.	Begin to close
107	46	"	27					Present	Begin to close
109	48	"	27					Nearly closed	Nearly closed

The development of the primitive streak is shown in the following curve plotted from the above table.



When considered in direct relation to the length of the incubation period there is a considerable individual variation in the growth of the embryo. This well known fact can be shown in an interesting manner by some instances taken from the data given by Keibel and Abraham,[1] but arranged by hours rather than in accord with the serial order of normal stages as given by these authors.

Before the 20 hour stage, where the Normentafel begins, four figures are drawn. The two for 9 hours of incubation have primitive streaks, 1.2 mm. and 1.6 mm. long, thus being of the 9.5 and 15 hour stages according to the scale I have constructed from Duval. The two for 12 hours have primitive streaks 1.5 mm. and 1.6 mm. long

[1] Keibel, F.: Normentafeln zur Entwicklungsgeschichte der Wirbelthiere. Zweites Heft. Keibel, F., and Abraham, K.: Normentafel zur Entwicklungsgeschichte des Huhnes (Gallus Domesticus), Jena, 1900.

and so represent the Duval 13.5 and 15 hour stages. An embryo incubated for 20 hours has 4 mesodermic somites, making it equal to the 23–24 hour stage of Duval. Of the seven 24 hour chicks one has 1–(2) mesodermic somites, a second 3, and a third, 7–8, while the length of the primitive streak in the four remaining is 1.20, 1.30, 1.35, and 1.40 mm. For this period then there is presented a range of stages from 9.5 to 26 hours of Duval. One 32 hour embryo has 6 mesodermic somites, while a second has 9. A 31 hour chick has 7 somites, while one incubated 40.5 hours has 5(–6), and one 48 hours only 2! Having 18–20 somites are chicks of 42, 43, 43.5, and 48 hours' incubation. The eleven chicks of 48 hours' incubation have respectively, 2, 16, 17–18, 19–20, 20, 22–23, 23, 23, 23–24, 25–26, and 28 mesodermic somites. The average is 20.04 somites, instead of 27 found in the figure by Duval of the 48 hour stage.

In spite of this variation, I believe that the scale of development should be measured by hours. It is desirable that ultimately this scale should be established upon averages found by a statistical study of a very large number of embryos. However, for the present necessity, we are not, in all probability, very far wrong in following the Atlas of Duval as a standard.

In the following series I have given the age according to the above table founded upon Duval, and, wherever possible, the serial number of Keibel and Abraham.

III.

SERIES A.[1] — INCUBATION 1.

Twelve eggs, incubated at 30.75° from April 2, 2 P. M. to April 10, 11 A. M., 7 days, 19 hours.

No. of egg.	Age in hours.	Serial number. Keibel and Abraham.	Age in hours. Keibel and Abraham.	Hydropic vesicles.[2]	General remarks.
1.1	108	73	104	Present	
1.2	108	73	104	"	
1.3	96	58	78		
1.4	96	58	78	"	
1.5	96	58	78	"	
1.6	96	58	78		
1.7	96	58	78	"	
1.8	72	52	67	"	
1.9	72	52	67	"	
1.10	72	52	67	"	
1.11	72	52	67		
1.12	48	39 c	51	"	Two embryos on one blastoderm.[3]

Temperature range from 30.25° to 31.25°, — 1.00°. Average age, 85.92 hours or 54.83 per cent of normal development.

[1] Including incubations of the spring of 1900 at the University of Cincinnati, O.

[2] In a large proportion of the following cases hydropic vesicles occur. They are of various sizes and sometimes so numerous that they form a foamwork. Sections show that these vesicles are formed by the expansion of spaces in the primitive lower layer or later in the mesoderm. Sometimes the vesicles result from the folding in opposite directions of ectoderm and lower layer cells.

[3] For cases showing from two to seven distinct embryos on the same blastoderm cf. BANCHI, A.: Monitore Zoologico Italiano, 1895, vi; also cases by G. F. WOLF, VON BAER, ALLEN THOMSON, REICHERT, DÖNITZ, AHLFELD, RAUBER, DARESTE, GERLACH, MORIGGIA, BOURCHARDT, E. HOFFMANN, P. MITROPHANOW, A. PTIZIN, KLAUSSNER, and HANCOCK, given by BANCHI.

SERIES A. — INCUBATION 2.

Six eggs, incubated at 29.25° from April 10, 5 P.M. to April 16, 11 A.M., 5 days, 18 hours.

No. of egg.	Length of primitive streak in mm.	Number of mesodermic somites.	Age in hours.	Serial number. Keibel and Abraham.	Age in hours. Keibel and Abraham.	General remarks.
2.1	..	9	27	18	32.0	Embryo abnormal.
2.2	..	8	26	16	24.0	Neural folds contorted.
2.3	..	6	25	14a	32.0	Neural folds contorted.
2.4	..	5	24	12	33.0	Cephalic end of neural groove trifid. Three additional primitive streak-like branches posteriorly.
2.5	..	3	22	8a	24.5	Neural folds enlarged; contorted. Primitive streak bifid.
2.6	2.00	..	16	3	24.0	

Temperature range from 29.00° to 29.50°, — 0.50°. Average age, 23.33 hours or 14.91 per cent of normal development. Considerable variation is shown in this clutch, being especially marked in 2.4 and 2.5 with characters as given above.

Mitrophanow,[1] describes a number of anomalies of the primitive streak similar to that occurring in egg No. 2.4, which were produced by lowering the temperature to 32°–34°. In one case of a posteriorly bifid primitive streak the structure assumes a crescentic shape, and Mitrophanow suggests that here is an instance of atavism to the phylogenetic blastopore, the trace of which, as Duval believes, is a part of normal development. Mitrophanow believes that irregularities in the form of the area pellucida are found with these lateral branchings of the primitive groove, one part directly following the other because of the interdependence during the early stages of all parts of the formative area of the blastoderm. As Dareste[2] demonstrates, by varying the particular point of application of the heat, the growth of the vascular area may be changed from the ordinary circular form to an ellipse, the direction of whose long axis directly depends upon the source of the incubating heat.

[1] MITROPHANOW, P.: Archiv für Entwickelungsmechanik der Organismen, 1895, i, pp. 370 to 376.

[2] *Loc. cit.*, pp. 288 to 293.

Kaestner,[1] following in lines known since Panum,[2] secured many interesting deviations from the normal development by suddenly lowering the temperature a number of degrees, and thus causing a suspension of development for a greater or less time. This cooling must come within the first two days of incubation, must last for a considerable length of time, and during the interruption, the egg must be placed horizontally. The duration of the interruption period is inversely proportional to the ontogenetic stage. If, for instance, the egg is cooled to about 7° below 28° (assumed as the physiological zero), the development can be suspended for three weeks, if at the beginning of the first day of incubation; if at the end of the first day, for six days; at the sixth day for seventy-two hours; at the ninth day for forty-eight hours, and finally, in the second half of incubation up to the time of hatching, for twenty-four hours.

As Warynski has observed, the yolk when cooled rises and presses the blastoderm against the vitelline membrane, to which it sticks. If this happens during the first two days, while the embryo is unprotected by the amnion, the pressure causes an arrest of development and consequent malformations. The exact character of these, and the region of the embryo in which they occur, cannot be predicted, since it is a matter of chance as to the part of the blastoderm which will adhere to the vitelline membrane. As Kaestner pointed out, the rising of the blastoderm probably depends upon a change in specific gravity consequent upon a change in volume due to cooling.

Harvey[3] found that in eggs opened after three days' incubation, the heart of the embryo beat slower and slower until it ceased, when, after a period of suspended animation, the warmth of tepid water, or even of the finger, caused the pulsations to return. Dareste[4] opened the shell, leaving the vitelline membrane intact, examined the embryo with a lens, and thus was sure of the cessation of the heart-beat. Upon the application of warm water the contraction recommenced, and normal chicks were hatched after cooling at 20°, 15°, 8°–10°, and even 1°–2°. The development was delayed, so that the eggs cooled for two days hatched in 23 instead of 21 days.

Oxygen also has a direct influence upon the growth of the various

[1] Kaestner: Anatomischer Anzeiger, 1896, pp. 136–45.

[2] Panum: Virchow's Archiv für pathologische Anatomie, 1878, lxxii, pp. 69–91, 165–197, 289–324.

[3] Harvey, William: Exercitationes de generatione animalium. London, 1651.

[4] *Loc. cit.*, p. 131.

parts of the blastoderm and of the embryo. And so other environmental conditions. It is not yet possible to give an exact analysis of the part taken by these conditions in stimulating the cells to produce local malformations, which, of course, are the direct manifestations of increased or suppressed growth.

SERIES A. — INCUBATION 3.

Twelve eggs, incubated at 28.25° from April 16, 11 A. M. to April 23, 11 A. M., 7 days.

No. of egg.	Length of primitive streak in mm.	No. of mesodermic somites.	Age in hours.	Serial number. Keibel and Abraham.	Age in hours. Keibel and Abraham.	Hydropic vesicles.	General remarks.
3.1	..	9	27	18	32		One posterior lateral branch of primitive groove.
3.2	..	7	25	15	31		
3.3	..	5	23	11	33		
3.4	..	4	22	9	20		Neural folds only developed anteriorly and posteriorly.
3.5	..	4	22	9	20		
3.6	..	3	22	7	24	Few present	
3.7	..	3	22	7	24		
3.8	..	3	22	7	24		
3.9	..	3	22	7	24		
3.10	3.0[1]	..	16	Not given	..		Lateral branches of the primitive streak.
3.11	1.5	..	13.6	Fig. 3	12		
3.12	..	..	00.0				

Temperature range from 28.00° to 28.50°, — 0.50°. Average age,[2] 19.72 hours or 12.60 per cent of normal development. Variations are shown in 3.1 and 3.10.

[1] The Duval table gives 1.93 mm. as the longest primitive streak which before the development of notochord and mesodermic somites indicates the 16 hour stage. Embryo 3.10 shows continued growth, then, at the 16 hour stage.

[2] This is taken by dividing by the full number of eggs even though in some cases no trace of the embryo is present.

SERIES A. — INCUBATION **4.**

Ten eggs, incubated at 27.00° from April 24, 11 A M. to April 30, 11 A. M., 6 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Figure, Keibel and Abraham.	Age in hours. Keibel and Abraham.	Hydropic vesicles.	General remarks.
4.1	..	..	7.0	2.0	Not given	Not given	Present	Primitive groove in form of a central blastopore-like pit.
4.2	1.8	15.5	8.0	3.0	"	"	None	
4.3	1.8	15.5	7.0	2.8	"	"	Present	
4.4	1.5	13.6	8.0	2.5 × 3.0 (2.75)	3	12	"	
4.5	1.5	13.6	8.0	3.0	3	12	"	
4.6	1.5	13.6	8.0	1.9	3	12	"	
4.7	1.0	8.0	7.5	2.0	Not given	Not given	"	Primitive groove widened.
4.8	1.0	8.0	5.5	2.0	"	"	"	
4.9	1.0	8.0	4.5	1.8	"	"	"	
4.10	0.8	6.4	0.8	2.5	"	"	"	

Temperature range from 26.75° to 27.25°, — 0.50°. Average length of primitive streak,[1] 1.32 mm. Average age, 10.22 hours, or 6.53 per cent of normal development. Average diameter of blastoderm, 7.15 mm. Average diameter of area pellucida, 2.37 mm.

[1] This and the average diameter of blastoderm and area pellucida, are taken from the number of eggs presenting these features clearly defined.

SERIES A. — INCUBATION 5.

Nine eggs, incubated at 26.00° from April 30, 4.45 P. M., to May 8, 11.45 A. M., 7 days, 19 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Hydropic vesicles.	General remarks.
5.1	1.3	11	4.5	1.8	Present	
5.2	1.3	11	7.0	2.0	"	Primitive groove slightly widened.
5.3	1.2	9	6.0	2.0	"	Several lateral branches of the primitive groove.
5.4	1.2	9	7.0	2.0	"	
5.5	1.0	8	6.5	2.0	"	
5.6	1.0	8	6.0	3.0	"	
5.7	1.0	8	6.0	3.0	None	
5.8	..	..	4.0	1.0	"	No trace of primitive streak. Degenerated.
5.9	..	..	5.0	2.0	Present	No trace of primitive streak.

Temperature range from 25.80° to 26.20°, — 0.40°. Average length of primitive streak, 1.14 mm. Average age, 7.11 hours, or 4.54 per cent of normal development. Average diameter of blastoderm, 5.78 mm. Average diameter of area pellucida, 1.98 mm.

SERIES A. — INCUBATION 6.

Nine eggs, incubated at 25.5° from May 15, 11 A. M. to May 22, 1 P. M., 7 days, 2 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Hydropic vesicles.	General remarks.
6.1	2.0	16.3	11	3.0 × 4.5 (3.75)	Present	
6.2	2.0	16.3	6.0	3.0	"	
6.3	1.5	13.6	8.0	3.0 × 2.5 (2.75)	"	Primitive groove widely open 7 mm. long.
6.4	..	..	9.0	2.0 × 3.0 (2.5)	"	Mere trace of primitive streak.
6.5	..	..	9.0	2.0 × 4.0 (3.0)	"	Central embryonic shield.[1]
6.6	..	..	4.5	2.0	"	Same as 6.5.
6.7	..	..	4.0	2.0 × 1.5 (1.75)	"	" " " , degenerated.
6.8	..	..	6.0	2.0	"	Embryonic shield. Several primitive streak-like structures of indefinite form.
6.9	..	..	7.5	3.0	"	Same as 6.8.

Temperature range from 24.80° to 26.20°, — 1.40°. Average length of primitive streak, 1.83 mm. Average age, 5.13 hours, or 3.28 per cent of normal development. Average diameter of blastoderm, 7.2 mm. Average diameter of area pellucida, 2.31 mm.

In this clutch were found one 3-day chick and one with 22 mesodermic somites (40 hours), which probably show misplaced confidence in the farmer who gathered the eggs, and so they have not been tabulated.

[1] In this and the following groups of blastoderms where the primitive streak has not yet appeared, the first stage of ontogeny known as the " embryonic shield " is often present. This stage is due to the rather indefinite multiplication of cells of the primitive lower layer. The cell mass is quite as frequently central and circular as posterior and shield-shaped but I shall speak of it by the conventional name.

SERIES A. — INCUBATION 7.

Nine eggs, incubated at 25.0° from May 23, 3.30 P. M., to May 29, 5 P. M., 6 days, 1.5 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Hydropic vesicles.	General remarks.
7.1	1.6	15	4.0	2.0	None	Primitive streak and groove bifid almost the whole length.
7.2	1.5	13.6	6.0	2.0 × 2.3 (2.15)	Present	Primitive streak sickle-shaped.
7.3	1.3	11.0	7.0	3.0 × 2.5 (2.75)	None	
7.4	0.5	4.0	5.0	2.0 × 2.5 (2.25)	Present	
7.5	..	..	6.0	2.0	"	Central embryonic shield.
7.6	..	..	5.5	2.5	"	Same as 7.5.
7.7	..	..	10.0	3.0 × 2.0 (2.5)	"	" " "
7.8	..	..	7.0	3.0 × 2.5 (2.75)	"	" " "
7.9	..	..	6.0	2.0	"	" " "

Temperature range from 24.75° to 25.25°, — 0.50°. Average length of primitive streak, 1.23 mm. Average age, 4.84 hours, or 3.09 per cent of normal development. Average diameter of blastoderm, 6.28 mm. Average diameter of area pellucida, 2.31 mm.

IV.

SERIES B.[1] — INCUBATION 8.

Eleven eggs, incubated at 23.13° from April 18, 3.30 P. M., to April 25, 4 P. M., 7 days, 5 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.
8.1	2.4	17.5	10.0	4.0 × 3.2 (3.6)	Oval	Present	
8.2	1.8	15.5	12.4 × 14.0 (13.2)	3.5 × 2.5 (3.0)	"	"	
8.3	1.7	15.3	8.0	2.35 × 2.0 (2.18)	"	"	Wings of mesoderm prominent posteriorly.
8.4	1.5	13.6	10.0 × 8.6 (9.3)	3.2 × 2.7 (3.05)	"	"	
8.5	1.25	10.0	7.0	2.6	Round	"	
8.6	1.2	9.6	8.0	2.7 × 2.5 (2.6)	Oval	"	Primitive groove indistinct.
8.7	1.15	9.2	7.5 × 6.0 (6.75)	2.55 × 2.3 (2.43)	"	"	Wings of mesoderm prominent anteriorly.
8.8	1.1	8.8	8.0 × 8.75 (8.38)	3.0 × 2.9 (2.95)	"	"	
8.9	1.05	8.4	10.0	2.85	Round	"	
8.10	0.95	7.6	9.0 × 10.0 (9.5)	3.1 × 2.6 (2.85)	Oval	Very few present	
8.11	0.9	7.2	10.0	3.2 × 2.7 (2.95)	"	Present	

Temperature range from 22.39° to 23.79°, — 1.40°. Average length of primitive streak, 1.36 mm. Average age 11.15 hours, or 7.12 per cent of normal development. Average diameter of blastoderm, 9.10 mm. Average diameter of area pellucida, 2.82 mm.

[1] This and the following Series of incubations were carried on during 1901, at Trinity College.

SERIES B. — INCUBATION 9.

Ten eggs, incubated at 22.44° from April 27, 4.30 P. M. to May 4, 4 P. M., 6 days, 23 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General Remarks.
9.1	1.7	15.3	5.2 × 5.6 (5.4)	2.7 × 2.9 (2.8)	Nearly round	None	Merest trace of primitive groove.
9.2	1.5	13.6	7.8	2.8 × 2.9 (2.85)	Oval	Present	
9.3	1.5	13.6	6.6	2.8 × 3.6 (3.2)	Oblong	None	
9.4	1.4	12.0	7.6	2.65 × 2.85 (2.75)	Slightly oval	Present	
9.5	1.2	9.6	7.6	2.6 × 3.1 (2.85)	Oval	None	
9.6	1.2	9.6	6.8	2.5 × 2.8 (2.65)	Nearly round	Present	
9.7	1.1	8.8	8.2	2.8 × 3.0 (2.3)	Round	"	
9.8	1.1	8.8	7.8	3	Round	"	
9.9	1.1	8.8	7.0 × 7.8 (7.4)	2.3 × 2.85 (2.58)	Oval	"	
9.10	0.4	3.2	6.4	2.2	Nearly round	"	Only a trace of primitive streak.

Temperature range from 22.17° to 22.67°, —0.50°. Average length of primitive streak, 1.22 mm. Average age, 10.33 hours, or 6.60 per cent of normal development. Average diameter of blastoderm, 7.16 mm. Average diameter of area pellucida, 2.78 mm.

SERIES B. — INCUBATION **10**.

Eleven eggs, incubated at 21.87° from May 6, 7 P. M. to May 13, 11 A. M., 6 days, 16 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.
10.1	1.2	9.6	5.4	3.2 × 3.5 (3.35)	Slightly oblong	Present	Primitive groove only very slightly developed.
10.2	1.1	8.8	5.8 × 6.0 (5.9)	2.5	Nearly round	"	No primitive groove.
10.3	0.9	7.2	5.6	2.6 × 2.8 (2.7)	"	Very few present	Primitive streak 0.7 mm. broad. No primitive groove. Transverse diameter 12 mm. the longer.
10.4	0.8	6.4	4.8	2.3 × 3.0 (2.65)	Oval	Present	No primitive groove. Primitive streak only faintly marked off from rest of blastoderm.
10.5	0.7	5.6	5.2	2.5 × 3.0 (2.75)	Oblong	"	Same as **10.4**.
10.6	0.3	2.4	4.2	2.65 × 2.85 (2.75)	Round	"	Same as **10.4**.
10.7	..	..	6.0	2.8	"	"	Indefinite central embryonic shield.
10.8	..	..	5.8	2.7 × 3.5 (3.1)	Oblong	"	
10.9	..	..	4.6	2.4	Round	None	
10.10	..	..	4.2	1.8 × 1.9 (1.85)	Nearly round	Present	Same as **10.7**.
10.11	..	..	3.6 × 3.8 (3.7)	..	Not well defined	None	Same as **10.7**.

Temperature range from 20.77° to 22.12°, — 1.35°. Average length of primitive streak, 0.83 mm. Average age, 3.64 hours, or 2.33 per cent of normal development. Average diameter of blastoderm, 5.03 mm. Average diameter of area pellucida, 2.44 mm. Five blastoderms show no trace of the embryo.

SERIES B. — INCUBATION 11.

Eleven eggs, incubated at 21.38° from May 14, 7 P. M. to May 20, 2 P. M., 5 days, 19 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.
11.1	1.0	8.0	6.4	2.0 × 2.3 (2.15)	Oval	Few present	No primitive groove.
11.2	0.9	7.2	6.0	3.15 × 3.5 (3.33)	Nearly round	Present	No primitive groove. Primitive streak not clearly defined.
11.3	0.8	6.4	7.0	3.4 × 3.7 (3.55)	"	"	No primitive groove.
11.4	..	..	5.8 × 6.4 (6.1)	..	Not defined	Few present	Embryo undeveloped.
11.5	..	..	5.6	..	"	Present	" "
11.6	..	..	5.6	..	"	"	" "
11.7	..	..	4.6 × 5.6 (5.1)	2.9	Nearly round	"	" "
11.8	..	..	4.8	..	(Like 11.4)	"	" "
11.9	..	..	4.4 × 4.7 (4.55)	..	"	"	" "
11.10	..	..	3.9	..	"	None	" "
11.11	..	..	3.4 × 3.7 (3.55)	..	"	"	" "

Temperature range from 21.17° to 21.62°, — 0.45°. Average length of primitive streak, 0.9 mm. Average age, 1.96 hours, or 1.25 per cent of normal development. Average diameter of blastoderm, 5.33 mm. Average diameter of area pellucida, 2.98 mm. Only 3 blastoderms show primitive streaks.

SERIES B. — INCUBATION **12.**

Twelve eggs, incubated at 21.11° from May 28, 10 A. M., to June 3, 4 P. M., 6 days, 6 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.
12.1	1.0	8.0	5.3 × 6.4 (5.83)	3.35	Nearly round	Present	Primitive streak triangular, apex forward. No groove.
12.2	1.0	8.0	5.0 × 5.8 (5.4)	2.8 × 3.0 (2.9)	"	"	Transverse diameter of the area pellucida the longer. Primitive streak thin; no groove.
12.3	0.5	4.0	6.0 × 6.4 (6.2)	2.9 × 3.1 (3.0)	"	"	
12.4	..	..	5.5	2.8	"	"	Embryonic shield.
12.5	..	..	4.6 × 5.4 (5.0)	2.6 × 2.8 (2.7)	"	"	Transverse diameter of the area pellucida the longer.
12.6	..	..	4.9 × 5.1 (5.0)	..	Not defined	None	Emb. undeveloped.
12.7	..	..	4.1 × 5.2 (4.65)	2.5 × 3.0 (2.75)	Oval	Present	Like **12.4.**
12.8	..	..	4.6	..	Not defined	"	" "
12.9	..	..	4.4 × 4.6 (4.5)	..	"	"	" "
12.10	..	..	4.2 × 4.4 (4.3)	2.3 × 2.5 (2.4)	Slightly oblong	"	Emb. undeveloped.
12.11	..	..	3.9 × 4.2 (4.05)	..	Not defined	None	" "
12.12	..	..	3.1 × 3.6 (3.35)	..	"	Present forming foam-work	" "

Temperature range from 20.72° to 21.57°, — 0.85°. Average length of primitive streak, 0.83 mm. Average age, 1.67 hours, or 1.07 per cent of normal development. Average diameter of blastoderm, 4.87 mm. Average diameter of area pellucida, 2.84 mm.

No trace of primitive groove, and the 3 primitive streaks are midway between undefined masses of cells and clearly defined primitive streaks.

SERIES B. — INCUBATION 13.

Twelve eggs, incubated at 20.92° from May 21, 3 P. M. to May 27, 2 P. M., 5 days, 23 hours.

No. of egg.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.
13.1	5.7	..	Not defined	Present	Embryo undeveloped.
13.2	5.2 × 6.0 (5.6)	2.5 × 3.0 (2.75)	Oblong	"	" "
13.3	5.2	..	Not defined	"	Embryo undeveloped. Blastoderm slightly degenerated.
13.4	5.0 × 5.3 (5.15)	..	"	"	Embryo undeveloped.
13.5	5.0	2.5 × 3.0 (2.75)	Oblong	"	" "
13.6	4.9	1.9 × 2.2 (2.05)	Oval	"	" "
13.7	4.8	2.0	Nearly round	"	" "
13.8	4.5	2.4	"	"	" "
13.9	4.2	..	Not defined	"	Embryo undeveloped. Blastoderm slightly degenerated.
13.10	3.8 × 4.0 (3.9)	..	"	"	Embryo undeveloped.
13.11	3.6 × 4.0 (3.8)	..	"	"	" "
13.12	3.5	3.0	Round	Forming a foam work.	" "

Temperature range from 20.67° to 21.47°, — 0.80°. Average diameter of blastoderm, 4.89 mm. Average diameter of area pellucida, 2.49 mm. No trace of an embryo in any of the blastoderms of this clutch.

SERIES B. — INCUBATION 14.

Thirteen eggs, incubated at 20.72° from June 7, 2 P. M. to June 13, 9.30 A.M., 5 days 20 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General Remarks.
14.1	0.9	7.2	5.4 × 5.8 (5.6)	2.4 × 2.9 (2.65)	Oblong	Present	No primitive groove. Primitive streak not well defined.
14.2	0.5	4.0	5.7	2.35 × 2.8 (2.57)	"	"	Same as 14.1. Primitive streak, 1 mm. wide.
14.3	..	..	5.8	2.8	Round, not well defined	"	Embryonic shield.
14.4	..	..	5.8	2.6 × 3.0 (2.8)	Oblong	"	Same as 14.3.
14.5	..	..	5.3	2.9	Round	"	" " "
14.6	..	..	5.0	2.6	"	"	" " "
14.7	..	..	4.7	2.7	"	"	" " "
14.8	..	..	4.7	2.5 × 2.8 (2.65)	Oblong	"	" " "
14.9	..	..	4.3 × 4.6 (4.45)	2.7	Round	"	" " "
14.10	..	..	4.4	..	Not defined	"	" " "
14.11	..	..	3.9 × 4.8 (4.35)	2.5 × 2.9 (2.7)	Oblong	"	" " "
14.12	..	..	3.2 × 3.4 (3.3)	..	Not defined	None	" " "
14.13	..	..	3.0	..	"	"	" " "

Temperature range from 18.07° to 21 57°, — 3.50°. Average length of primitive streak, 0.7 mm. Average age, 0.86 hours, or 0 54 per cent of normal development. Average diameter of blastoderm, 4.77 mm. Average diameter of area pellucida, 2.71 mm.

In most of the blastoderms of this group the area pellucida was not well defined. The primitive streaks, also, were ill-defined masses of cells.

V.

SERIES C. — INCUBATION **15**.

Eight eggs, incubated at 20.05° from July 14, 5 P. M., to July 21, 8 A. M., 6 days, 15 hours.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.
15.1	1.0	8.0	5.1 × 5.2 (5.15)	2.6	Round	Present	No primitive groove; primitive streak an undefined posterior cell-mass.
15.2	..	..	5.2	2.8	"	"	Emb. undeveloped.
15.3	..	..	4.6 × 5.0 (4.8)	..	Not defined	"	" "
15.4	..	..	4.5 × 4.8 (4.5)	2.4 × 2.8 (2.6)	Oblong	"	" "
15.5	..	..	4.4 × 4.6 (4.5)	2.6 × 2.7 (2.65)	Nearly round	"	" "
15.6	..	..	4.4	2 7 × 3.0 (2.85)	Round	"	" "
15.7	..	..	3.8 × 4.2 (4.0)	2.6 × 2.8 (2.7)	Nearly round	"	" "

Temperature range from 19.14° to 20.94°, — 1.80°. Average length of the primitive streak, 1.0 mm. Average age, 1.14 hours, or 0.07 per cent of normal development. Average diameter of blastoderm, 4.67 mm. Average diameter of area pellucida, 2.73 mm. The trace of the embryo in the one case, **15**.1, is a patch of lower layer cells, so ill defined as to make a dubious primitive streak.

VI.

SERIES D. — INCUBATION **16**.

Eleven eggs, incubated at 20.13° from August 13, 10.30 A.M. to August 18, 10.30 A.M., 5 days.

No. of egg.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	Volume of egg in c.c.
16.1	5.0	2.8	Round	None	50.0
16.2	4.7 × 5.2 (4.95)	2.4 × 2.6 (2.5)	Oblong	"	55.0
16.3	4.6 × 5.2 (4.9)	3.0	Round	"	62.5
16.4	4.6 × 5.1 (4.85)	2.1 × 2.4 (2.25)	Oblong	"	47.5
16.5	4 7	2.8	Round	"	56.5
16.6	4.4	2.4	"	"	48.0
16.7	4.2 × 4.5 (4.35)	2.5 × 2.9 (2.7)	Oblong	"	56 0
16.8	4.0 × 4.6 (4.3)	2.4 × 3.2 (2.8)	"	Very small present	56.0
16.9	3.9 × 4.5 (4.2)	2.2 × 2.6 (2.4)	"	None	45.0
16.10	3.7 × 4.3 (4.0)	1.8 × 2.2 (2.0)	"	"	55.0
16.11	3.7	2 1	Round	"	43.0

Temperature range from 19.7° to 20.6°, — 0.9°. Average diameter of blastoderm, 4.49 mm. Average diameter of area pellucida, 2.52 mm.

SERIES D. — INCUBATION **17**.

Twelve eggs, incubated at 21.08° from August 19, 9 A. M. to August 24, 9 A. M., 5 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.	Volume of egg in c.c.
17.1	1.00	8	5.0 × 5.4 (5 2)	2.6	Round	None	Primitive streak an indefinite patch of cells.	50.0
17.2	..	..	5.8	3.1	"	Very small	Embryonic shield	55.0
17.3	..	..	5.4	3.0	"	None	Same as **17.2**.	47.5
17.4	..	..	5.0 × 5.6 (5 3)	2.2	"	"	" " "	55.0
17.5	..	..	5.3	2.2	"	"	..	55.0
17.6	..	..	5.0	2.2	"	"	Same as **17.2**.	60.0
17.7	..	..	4.8	2.2	"	"	" " "	52.5
17.8	..	..	4.3 × 4.8 (4.55)	2.4 × 2.6 (2.5)	Nearly round	"	" " "	47.5
17.9	..	..	4.5	2.4	Round	Very small	" " "	50.8
17.10	..	..	4.3	2.0	"	None		55.0
17.11	..	..	4.2	2.4	"	"	" " "	55.0
17.12	..	..	3.7 × 4.5 (4.1)	2.2 × 2.5 (2.35)	Oblong	"	" " "	46.0

Temperature range from 20.6° to 21.5°, — 0.9°. Average age, 0.67 hours, or 0.56 per cent of normal development. Average diameter of blastoderm, 4.87 mm. Average diameter of area pellucida, 2.43 mm.

SERIES D. — INCUBATION 18.

Twelve eggs, incubated at 22.51° from September 6, 2 P. M., to September 11, 2 P. M., 5 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.	Vol. of egg in c.c.
18.1	0.7	5.6	5.2	2.6	Round	None	..	47.5
18.2	0.2	1.6	5.4 × 5.9 (5.65)	2.7 × 2.8 (2.75)	"	"	Of crescentic form	52.0
18.3	..	..	5.0 × 5.2 (5.1)	2.4	"	Few present	Embryonic shield.	51.0
18.4	..	..	4.4 × 4.9 (4.65)	2.2 × 2.6 (2.4)	"	None	..	48.0
18.5	..	..	4.6	2.4	"	"	..	50.0
18.6	..	..	4.6	2.2	"	"	..	52.5
18.7	..	..	4.5	2.2	"	"	Embryonic shield.	48.0
18.8	..	..	4.4	2.0	"	Present	Degenerated.	60.0
18.9	..	..	4.2	2.0 × 2.4 (2.2)	Oblong	"	Embryonic shield.	50.0
18.10	..	..	4.2	2.2	Round	None	..	47.0
18.11	..	..	4.1	2.2	"	Few present	..	45.0
18.12	..	..	4.1	2.1	"	None	Embryonic shield.	55.0

Temperature range from 22.30° to 22.90°, — 0.60°. Average length of primitive streak, 0.45 mm. Average age, 0.6 hours, 0.5 per cent of normal development. Average diameter of blastoderm, 4.61 mm. Average diameter of area pellucida, 2.30 mm. All show more or less degeneration.

SERIES D. — INCUBATION **19**.

Nine eggs, incubated at 22.89° from August 26, 5 P. M., to August 31, 5 P. M., 5 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.	Volume of egg in c.c.
19.1	1.0	8	5.2	2.6	Round	None	Primitive streak not well defined.	47.5
19.2	0.9	7.2	4.4	2.1	"	"	Like **19**.1.	50.0
19.3	..	..	4.7 × 5.1 (4.9)	2.2	"	"	Embryonic shield.	62.5
19.4	..	..	4.8 × 5.0 (4.9)	2.2	"	"	..	52.5
19.5	..	..	4.6 × 5.0 (4.8)	2.4	"	Present	Embryonic shield.	45.0
19.6	..	..	4.8	2.8	"	Few present	Small indefinite patches of lower layer cells.	45.0
19.7	..	..	4.6 × 4.8 (4.7)	2.2	"	None	Embryonic shield.	53.0
19.8	..	..	4.5	2.4	"	"	" "	51.0
19.9	..	..	4.15 × 4.5 (4.33)	2.1 × 2.3 (2.2)	Oblong	"	..	52.5

Temperature range from 22.5° to 23.2°, — 0.7°. Average length of primitive streak, 0.95 mm. Average age, 1.67 hours, or 1.39 per cent of normal development. Average diameter of blastoderm, 4.73 mm. Average diameter of area pellucida, 2.34 mm.

SERIES D. — INCUBATION **20.**

Ten eggs, incubated at 23.40° from September 17, 9.30 A. M., to September 22, 9.30 A. M., 5 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.	Volume of egg in c.c.
20.1	0.7	5.6	4.5	2.4	Round	Present	..	54.0
20.2	0.4	3.2	5.7	2.6	"	"	..	47.0
20.3	..	..	5.0	2.4	"	None	..	50 0
20.4	..	..	4.8	2.2	"	Present	Degenerated.	45.0
20.5	..	..	4.7	2.7	"	None	Embryonic shield.	49.0
20.6	..	..	4.2 × 5.2 (4.7)	2.0	"	"	" "	51.0
20.7	..	..	4.5	2.5	"	Present	" "	49.0
20.8	..	..	4.5	2.15	"	"	" "	53.0
20.9	..	..	4.2 × 4.7 (4.45)	2.1 × 2.4 (2 25)	Oblong	"	" "	50.0
20.10	..	..	4.4	2.0	Round	"	" "	56 0

Temperature range from 23.2° to 24.7°, — 1.5°. Average length of primitive streak, 0.55 mm. Average age, 0.88 hours, or 0.73 per cent of normal development. Average diameter of blastoderm, 4.73 mm. Average diameter of area pellucida, 2.32 mm.

SERIES D. — INCUBATION 21.

Twelve eggs, incubated at 24.24° from September 12, 2 P. M., to September 16, 2 P. M., 4 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.	Vol. of egg in c.c.
21.1	1.40	12.0	5.8 × 6.0 (5.9)	2.7	Round	Present	..	62.0
21.2	1.20	9.5	4.9 × 5.1 (5.0)	2.6	"	"	..	52.5
21.3	1.20	9.5	4.8	1.9	"	"	..	50.0
21.4	0.80	6.5	5.6 × 5.4 (5.5)	3.1	"	..	..	61.0
21.5	0.35	2.5	4.9	2.8	"	None	..	48.0
21.6	..	..	5.4	2.2 × 2.7 (2.45)	Oblong	"	..	50 0
21.7	..	..	5.0 × 5.1 (5.05)	2.8	Round	Present	Embryonic shield.	45.0
21.8	..	..	5.0	2.6 × 2.8 (2.7)	Oblong	None	..	55.0
21.9	..	..	4.9	2.2 × 2.6 (2.4)	"	Present	Embryonic shield.	45.0
21.10	..	..	4.6 × 5.1 (4.85)	2.6 × 2.8 (2.7)	"	None	" "	50.0
21.11	..	..	4.1 × 4.6 (4.35)	2.15	Round	Present	" "	54.0
21.12	..	..	4.0 × 4.4 (4.2)	2.0	"	"	" "	55.0

Temperature range from 20.3° to 25.8°, — 5.5°. Average length of primitive streak, 0.99 mm. Average age, 3.33 hours, or 3.47 per cent of normal development. Average diameter of blastoderm, 4.99 mm. Average diameter of area pellucida, 2.53 mm. The large temperature range was due to the stopping of the gas for a few hours, during which time the incubator cooled to 20.3, but since the averages fit into the general curves this was not a matter of enough importance to exclude the data.

SERIES D. — INCUBATION 22.

Thirteen eggs, incubated at 25.22° from September 23, 3 P. M., to September 28, 3 P. M., 5 days.

No. of egg.	Length of primitive streak in mm.	Age in hours.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	General remarks.	Volume of egg in c.c.
22.1	1.70	15.3	6.0 × 6.6 (6.3)	2.5 × 3.1 (2.8)	Round	Present	..	47.0
22.2	1.50	13.6	5.0 × 5.4 (5.2)	2.4	"	"	..	58.0
22.3	1.30	10.7	6.2 × 7.0 (6.6)	3.2	"	"	..	55.0
22.4	1.20	9.6	6.2	2.8	"	"	..	52.0
22.5	1.20	9.6	5.8	2.2	"	"	..	55.0
22.6	1.15	9.2	6.3	3.0	"	"	..	47 0
22.7	1.00	8.0	5.4 × 6.1 (5.75)	3.1	"	"	..	53.0
22.8	0.90	7.2	6.0 × 6.9 (6.45)	2.9	"	"	..	50.0
22.9	0 45	3.6	5.4	2.0	"	None	..	52.0
22.10	..	..	5.6	2.8	"	Present	Embryonic shield.	49.0
22.11	..	..	5.4 × 5.7 (5.55)	2.9 × 3.2 (3.05)	Oval	None	" "	54.0
22.12	..	..	5.0 × 5.15 (5.08)	2.0	Round	Present	" "	52.0
22.13	..	..	4.7 × 5.0 (4.85)	2.4 × 2.6 (2.5)	"	"	" "	50.0

Temperature range from 24.6° to 25.6°, — 1.00°. Average length of primitive streak, 1.17 mm. Average age, 6.68 hours, or 5.57 per cent of normal development. Average diameter of blastoderm, 5.78 mm. Average diameter of area pellucida, 2.67 mm.

SERIES D. — INCUBATION 23.

Six eggs, incubated at 28.92° from October 1, 2 P. M. to October 6, 2 P. M., 5 days.

No. of egg.	Length of primitive streak and groove in mm.	Age in hours.	Diameter of vitelline area in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.	Diameter of area vasculosa in mm.	Vol. of egg in c c.
23.1	2.9	16	20.0 × 25.0 (22.5)	3.0 × 5.8 (4.4)	Pear	Present	8.0 × 10.0 (9.0)	56.0
23.2	2.9	16	21.0 × 22.0 (21.5)	3.2 × 5.3 (4.25)	"	"	7.4 × 8.4 (7.9)	47.0
23.3	2.9	16	15.6 × 17 2 (16 4)	3.5 × 5.15 (4 32)	"	"	11.6 × 12.0 (11.8)	46.5
23.4	2.7	16	15.8 × 18.3 (17.05)	3.8 × 5.0 (4.4)	"	"	9.8 × 10.4 (10.1)	48.5
23.5	2.0	16	20.5 × 22.3 (21.4)	3.4 × 5.0 (4.2)	"	"	7.1 × 8.8 (7.95)	48.0
23.6	2.0	16	13.7 × 14.8 (14.25)	2.7 × 3.8 (3.25)	"	"	6.6 × 8.1 (7.35)	50.0

Temperature range from 28.6° to 29.2°, — 0.6°. Average length of primitive streak, 2.57 mm. Average age, 16 hours, or 13.33 per cent of normal development. Average diameter of blastoderm, 18.85 mm. Average diameter of area pellucida, 4.14 mm.

VII.

SERIES E.—GROUP a.

Nine eggs, kept at 17.4° from May 15, 9 A. M. to May 21, 9 A. M.,— 6 days.

No. of egg.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.
a.1	5.0	2.9 × 3.3 (3.1)	Oblong
a.2	4.6 × 5.2 (4.9)	2.8 × 2.85 (2.82)	Nearly round
a.3	4.8	2.25 × 2.4 (2.32)	Oblong
a.4	4.4 × 5.0 (4.7)	2.5 × 2.6 (2.55)	Nearly round
a.5	4.5	2.3 × 2.4 (2.35)	" "
a.6	4.4	2.1 × 2.8 (2.45)	Oblong
a.7	4.3	2.1	Round
a.8	4.0	2.0 × 2.2 (2.1)	Nearly round
a.9	3.5	..	Not defined

Temperature range from 16.9° to 17.9°, — 1.0°. Average diameter of blastoderm, 4.46 mm. Average diameter of area pellucida, 2.47 mm.

Nine eggs kept in incubator room at 17.0° to 17.5° : one at 18.0°.

	Egg taken from nest.			Blastoderm fixed.			Egg kept.			Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.
	Month.	Day.	Hour.	Month.	Day.	Hour.	Days.	Hours.	Minutes.				
b.1	5	17	1.30 P. M.	5	26	10.20 A. M.	8	20	50	5.2	3.2	Round	None
b.2	5	17	10 A. M.	5	27	11.30 A. M.	10	1	30	5.0	2.8 × 3.4 (3.1)	Oblong	"
b.3	5	17	9 30 A. M.	5	18	5.40 P. M.	1	8	10	4.5 × 4.9 (4.7)	2.9 × 3.5 (3.2)	Oval	"
b.4	5	17	10 A. M.	5	18	5.30 P.M.	1	7	30	4.6	3.1	Round	"
b.5	5	17	1 P. M.	6	3	10 A. M.	17	21	0	4.1 × 4.6 (4.35)	2.7 × 2.9 (2.8)	Oblong	"
b.6	5	17	10 A. M.	5	29	10 A. M.	12	0	0	4.0 × 4.2 (4.1)	2.5	Round	"
b.7	5	17	1.30 P. M.	5	18	5.10 P. M.	1	3	40	3 6 × 4.0 (3.8)	2.3 × 2.4 (2.35)	Nearly round	"
b.8	5	17	12 M.	5	18	5 P. M.	1	5	0	3.5	2.1	Round	"
b.9	5	17	12 M.	5	25	1.20 P. M.	8	1	20	3.2 × 3.6 (3.4)	..	Not defined	Many present forming a foam-work.

One egg in incubator room at 18.0°.

b 10	6	7	9.30 A. M.	6	12	9 A. M.	4	23	30	5.0	2.6 × 2.85 (2.72)	Oblong	None

Average diameter of blastoderm, 4.37 mm. Average diameter of area pellucida, 2.79 mm.

In this group it was of interest to see if there would be any growth of the blastoderm at 17°–18° in eggs kept for periods of time varying from 1–17 days. The largest and smallest of the blastoderms (b.1 and b.9) were kept about the same length of time (between 8 and 9 days), the one (b.5) kept for the longest time (nearly 18 days) had a diameter (4.35 mm), slightly under the normal (4.41 mm.) while the measurements for the group show about the same variation as in the other groups of Series E; therefore it is evident that the given temperature does not influence growth either during short or long periods and that the physiological zero is above this temperature.

SERIES E. — GROUP c.

Twelve eggs, kept at 19.11° from July 12, 1.45 P. M. to July 19, 8.30 A. M., 6 days, 19 hours.

No. of egg.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Hydropic vesicles.
c.1	5.3	2.3	Round	Present
c.2	4.8 × 5.3 (5.05)	2.6	"	"
c.3	4.8	..	Not defined	None
c.4	4.7	..	" "	Present
c.5	4.6	2.3 × 2.6 (2 45)	Nearly round	"
c.6	4.4	3.0 × 3.1 (3.05)	" "	None
c.7	4.2 × 4.6 (4.4)	..	Not defined	Present
c.8	4.0 × 4.4 (4.2)	..	" "	None
c.9	3.8 × 4.4 (4.1)	2.1	Round	Present
c.10	4.0	2.45	"	"
c.11	3.6	..	Not defined	"
c.12	3.2 × 4.0 (3.6)	..	" "	"

Temperature range from 18.50° to 20.10°, — 1 80°. Average diameter of blastoderm, 4.39 mm. Average diameter of area pellucida, 2.49 mm.

SERIES E. — GROUP d.

Eggs 6, 8, 12, and 13, laid July 30; 1, 2, 3, 4, 5, 7, 9, 10, and 11, laid July 31. All kept in a refrigerator at 16° and fixed August 2.

No. of egg.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.
d.1	4.8 × 5.6 (5 2)	2.9	Round
d.2	4.6 × 5 0 (4 8)	2.2	"
d.3	4.4 × 5.0 (4.7)	2.9 × 3.2 (3 05)	Oblong
d.4	4.4	2.6	Round
d.5	4.3 × 4.5 (4.4)	2.0	"
d.6	4.3	2.4 × 2.8 (2 6)	Oval
d.7	3.9 × 4.6 (4.25)	2.6 × 3.1 (2.85)	Oblong
d.8	4.2	2.5	Round
d.9	4.1	2.6	"
d.10	4.1	2.3	"
d.11	3.6 × 4.0 (3.8)	2.3 × 2.8 (2.55)	Oblong
d.12	3.6 × 4.0 (3.8)	2.3	Round
d.13	3.8	2.0	"

Average diameter of blastoderm, 4.30 mm. Average diameter of area pellucida, 2.50 mm.

SERIES E. — GROUP e.

Eggs 2, 3, 4, 6, 9, 11, laid August 8; eggs 1, 5, 7, 8, 10, 12, 13, 14, and 15, laid August 9. All kept in refrigerator at 16° and fixed August 13.

No. of egg.	Diameter of blastoderm in mm.	Diameter of area pellucida in mm.	Form of area pellucida.	Volume of egg in c.c.
e.1	5.5	2.5	Round	65.0
e.2	4.7 × 5.1 (4.9)	2.8	"	57.0
e.3	4.9	2.2	"	60.0
e.4	4.6 × 5.0 (4.8)	2.6	"	53.0
e.5	4.6 × 4.9 (4.75)	2.5 × 2.6 (2.55)	Nearly round	60.0
e.6	4.4 × 5.0 (4.7)	2.6	Round	48.0
e.7	4.5 × 4.7 (4.6)	..	Not clearly defined	60.0
e.8	4.1 × 4.7 (4.4)	2.5 × 3.0 (2.75)	Oblong	50.0
e.9	4.4	2.5	Round	45.0
e.10	4.4	2.3	"	47.0
e.11	4.4	2.0	"	53.0
e.12	4.1 × 4.6 (4.35)	2.5	"	50.0
e.13	3.7 × 4.3 (4.0)	2.4	"	50.0
e.14	4.0	1.9	"	49.0
e.15	3.7 × 4.2 (3.95)	1.9 × 2.2 (2.05)	Oblong	50.0

Average diameter of blastoderm, 4.54 mm. Average diameter of area pellucida, 2.40 mm.

VIII. The Physiological Zero.

Prévost and Dumas,[1] using an ordinary incubator without thermostat, stated that development begins at from 28° to 30°. Dareste,[2] as we have seen, places the physiological zero at 28°. He says emphatically, "Every observation which I have mentioned shows that no development takes place below 28°, and that development already commenced is fatally arrested at a certain point between 30° and 34°." Kaestner[3] also adopted 28° as the physiological zero. Rauber[4] gave the physiological zero at 25°, without, however, presenting any evidence for his statement.

From my incubations, of those with average temperatures between 20°–21°, two, D.**16** at 20.13° and B.**13** at 20.92°, give no trace of the embryo, while two, C.**15** at 20.05° and B.**14** at 20.72°, give respectively 0.07 and 0.54 per cent of normal development. The four incubations between 21°–22° — D.**17** at 21.08°, B.**12** at 21.11°, B.**11** at 21.38° and B.**10** at 21.87° — give respectively 0.56, 1.07, 1.25, and 2.33 per cent of normal development. In the series of incubations, eggs were kept at 17.4° (E.a) and 19.11° (E.c), but did not show any development of the embryo.

Since there is in no case any trace of the embryo below 20°, and with four incubations between 21°–22°, each showing some percentage of embryonic development, I would place the physiological zero at the degree between 20°–21°. The two groups of blastoderms showing traces of the primitive streak at this range of temperature may be considered as due to the normal variation in the constitution of protoplasm.

Regarding the influence of temperature on protoplasm in general it has been shown by many authors that for some forms (plants, protozoa, amphibia, reptiles and others) the activities of metabolism and movement do not entirely cease until within a few degrees above zero. Early in the last century Macaire[5] showed that in the metabolic pro-

[1] Dumas: Article "Œuf," in Dictionnaire classique d'histoire naturelle, xii, p. 121.

[2] Dareste, C.: Recherches sur la production artificielle des monstruosités, ou Essais de tératogénie expérimentale. 2 éd. p. 129, Paris, 1891.

[3] *Loc. cit.*

[4] Rauber: Sitzungsberichte der Naturforschenden Gesellschaft zu Leipzig, 1884.

[5] *Cf.* Davenport, C. B.: Experimental morphology, part 1, p. 222, New York, 1897.

cesses resulting in phosphorescence in fireflies light appears just above 20°, while a few years later Artaud [1] demonstrated the same thing for marine organisms.

For homoiothermic animals Martin and Applegarth [2] showed that the isolated cat's heart may be cooled to 16.5° and will revive if soon warmed again, but that usually it dies at about 17° or 18°. So it is seen that the physiological zero for the egg of the common fowl, 20°–21°, is also near the lethal temperature for the mammalian heart, and for the production of phosphorescence in fireflies and other organisms.

IX. The Index of Development.

Réaumur,[3] and later Bonnet,[4] state that at a lower temperature than the optimum, development is retarded, while at a higher temperature it is accelerated. Dareste [5] says that from 40°–42° an embryo of 24–30 hours is equal to a 3 day chick of normal incubation, while on the other hand from 30°–33° [6] an embryo of 7 or 8 days is only equal to a normal 3-day chick. He gives the maximum for development at 43°,[7] 44° being fatal, and declares that there is very little development at 41°, 42°, and 43°.[8] Rauber [9] and Kaestner [9] agree with Dareste in all essential points.

Davenport [10] gives the following index of development for the embryo of the fowl, founded upon a paper by Féré.[11]

Temperature	34°	35°	36°	37°	38°	39°	40°	41°
Index of development .	0.65	0.80	0.72	?[1]	(1.00)	1.06	1.25	1.51

[1] "The stage at 37° is taken from too few observations to be trustworthy. The stages at 35° and 36° are irregular, doubtless because of too few observations. As we go beyond 41° the ratio must decline again with great suddenness to 0°."

[1] See note 5 on p. 387.

[2] Martin, H. Newell, and Applegarth, E. C.: Studies from the Biological Laboratory of the Johns Hopkins University, 1890, iv, p. 275; also Martin: Physiological Papers, p. 103, Baltimore, 1895.

[3] Réaumur: L'art de faire éclorre et élever en toute saison des oiseaux domestiques, foit par le moyen de la chaleur du fumier. Paris, 1749.

[4] Bonnet: Expériences pour servir à l'histoire de la génération des animaux et des plantes. Trad. de Sénebier, p. 188.

[5] *Loc. cit.*, p. 121.

[6] *Loc. cit.*, p. 129.

[7] *Loc. cit.*, p. 118.

[8] *Loc. cit.*, p. 128.

[9] *Loc. cit.*

[10] *Loc. cit.* Part II, p. 459.

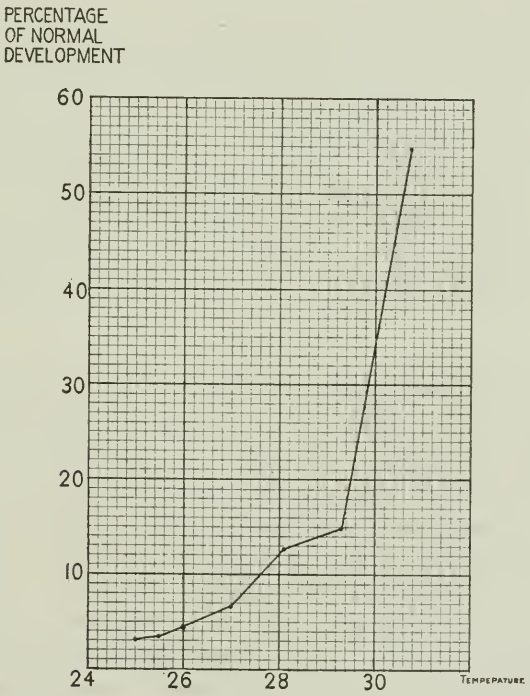
[11] *Loc. cit.*

After working over Féré's data, I get the results tabulated below.

Temperature	34°	35°	36°	37°	38°	39°	40°	41°
Index of development .	0.65	0.77	0.71	0.61	(1.00)	1.11	0.75	1.50
Number of embryos with age given . .	29	37	53	25	..	37	9	21

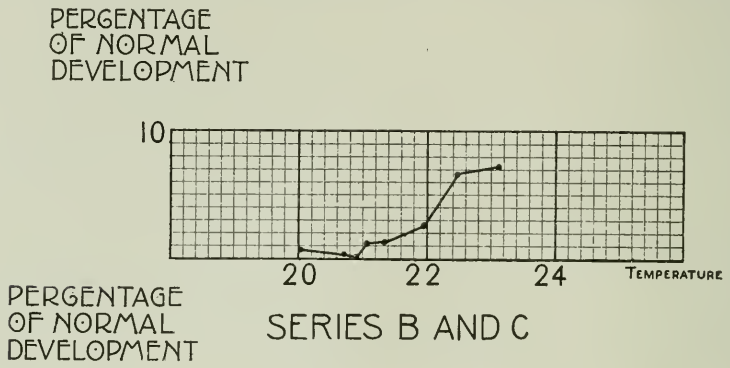
The differences in my table are mainly due to employing the full number of cases given by Féré and the addition of the index for 37°. If the objection given above in Davenport's foot-note should hold for 37° it could be urged with even greater force for 40° and 41° and almost as much for 34°.

In Series A, B, C, and D I have carried the index of development from 30.75° to the physiological zero, 20°–21° and present the results there found in the following graphic form.



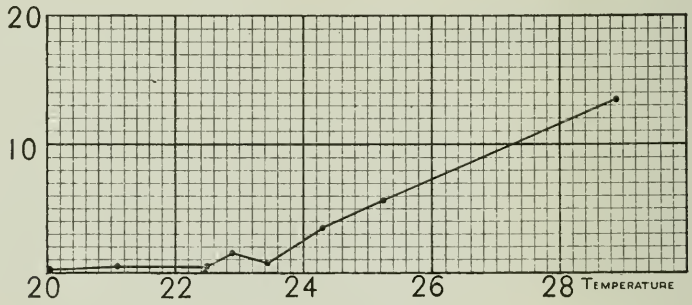
SERIES A

The break between Curves A and B represents a complete change in apparatus and environment from Cincinnati to Hartford. However, this gap is not of great importance since it involves merely the number of blastoderms with embryos of the same general development.



PERGENTAGE
OF NORMAL
DEVELOPMENT

SERIES B AND C



SERIES D

The last incubation of Curve A is 3 per cent less than the first of Curve B and C, but in both, the ontogenetic stage, that of the fully developed simple primitive streak, is practically the same. In Curve D, representing the incubations of Series D, I have given a continuous index of development from the minimum to 28.92°, which shows a general agreement with curves A and B–C and at the same time bridges over the gap between the first two. From the data shown in these curves two phases are distinctly represented. First between the physiological zero and 27°–28°, where only the primitive streak is found, the percentage of normal development not rising above 14 per cent. Second from 27°–28° to 30.75°, wherein the ontogenetic differentiation as shown by the appearance of mesodermic somites, nervous system and other features of the embryo, is marked by the abrupt rise from 14.91 to 54.83 per cent.

X. Normal Size and Variation of the Blastoderm and of the Area Pellucida.

V. Baer[1] noticed the great amount of variation in the early stages of the chick, but concluded that by the later stages each abnormal deviation had developed as nearly as possible back into the normal condition. Kupffer and Benecke,[2] as well as Keibel and Abraham,[3] have also called especial attention to this fact.

The diameter of the blastoderm of the unincubated egg of the domestic fowl is given by Foster and Balfour[4] at "about 4 mm.," by Dareste[5] from 3 to 5 mm., by Assheton[6] at 4.3 mm.; and from figures 33 and 35 of Duval[7] at 2.5 mm. and 3.09 mm. respectively. The last diameters, which are manifestly too small, are obtained from the magnifications as given.

I get 4.41 mm. as an average value from the exact measurement of the blastoderms of the fifty-nine cases included in Series E. Until a larger series has been measured this may be taken as the normal average diameter of the blastoderm. The standard deviation from this average is 0.4792 mm. and the coefficient of variability, 0.1087.

In regard to the form of the area pellucida there is considerable variation. Among the 136 blastoderms in which embryos have not developed, taken from the above series, there is a frequency of $59\frac{19}{34}$ per cent round, $12\frac{1}{2}$ per cent nearly round, $23\frac{9}{17}$ per cent oblong and $4\frac{7}{17}$ per cent oval.

From the measurement of fifty unincubated blastoderms I find that the average diameter of the area pellucida is 2.51 mm., with a standard deviation of 0.3382 mm. and a coefficient of variability of 0.1347. This may be taken as the normal until a larger series is obtained. The average from twenty measurements by Dursy[8] is

[1] Baer, C. E. v.: Ueber die Entwicklungsgeschichte der Thiere. Beobachtung und Reflexion. Königsberg, 1828 und 1837.

[2] Kupffer, C. und Benecke, B., Photogramme zur Ontogenie der Vögel. Nova Acta Acad. Leop. Carol., Bd. xli, 1879.

[3] *Loc. cit.*, p. 7.

[4] Foster, M. and Balfour, F. M.: The Elements of Embryology, p. 4, London, 1896.

[5] *Loc. cit.*: p. 287.

[6] Assheton, R.: Proceedings of the Royal Society, 1896, lx, p. 354.

[7] *Loc. cit.*

[8] Dursy, E.: Zeitschrift für rationelle Medicin, 1867, xxix, pp. 227, 229.

2.91 mm., while Moleschott[1] gives the diameter at "something over 3 mm."

XI. Growth of the Blastoderm Independently of the Appearance of the Primitive Streak.

Panum[2] observed blastoderms which had developed without an embryo. In eggs not incubated until twenty-seven days after being brought into the laboratory, Broca[3] found a transformation of the blastoderm with an absence of the embryo. Dareste[4] found that such cases could be produced by either the lowest or highest temperatures within the range determining development. This author also observed the continued growth of the blastoderm with a disorganization and disappearance of the embryo. Rabaud[5] demonstrated that in eggs kept at — 18° for one-half of an hour and afterwards incubated at 38° the large majority of blastoderms had extended to some distance over the yolk but without any trace of embryonic differentiation beyond that involved in cell proliferation.

Data from the above incubations are arranged according to temperature in the table on page 393. Even before a trace of the primitive streak appears there is usually a multiplication of lower layer cells toward the centre, or in the posterior part of the area pellucida, forming the embryonic shield. This stage, which occurs generally, I have noted as particularly prominent in A.**6**, A.**7**, B.**10**, B.**12**, B.**14**, D.**17**, D.**18**, D.**19**, D.**20**, D.**21**, and D.**22**.

Now, taking 4.41 mm. as the normal average diameter of the blastoderm of the unincubated egg, I find in A.**4** at 27.00°, the highest temperature showing a blastoderm without a trace of embryo beyond that of the indefinite proliferation of cells known as the embryonic shield, that there is an average diameter of 7.00 mm., or an average growth of 2.59 mm., the greatest shown. Toward the lower limits there is not the same close relation. However, the least diameter (4.44 mm. in D.**18**) is still above the normal, but the difference (0.02) is not large enough to be of importance. The relation of temperature

[1] Moleschott: Untersuchungen, X. Zur Embryologie des Hühnchens, I. (*cf.* Dursy, *Loc. cit.*).

[2] Panum: Untersuchungen über die Entstehung der Missbildungen, zunächst in den Eiern der Vögel. Kiel, 1860.

[3] Broca: Annales des sciences naturelles, 1862, xvii, p. 81.

[4] *Loc. cit.*, p. 284.

[5] Rabaud: Comptes Rendus, 1899. t. 128. 1899. pp. 1183–5.

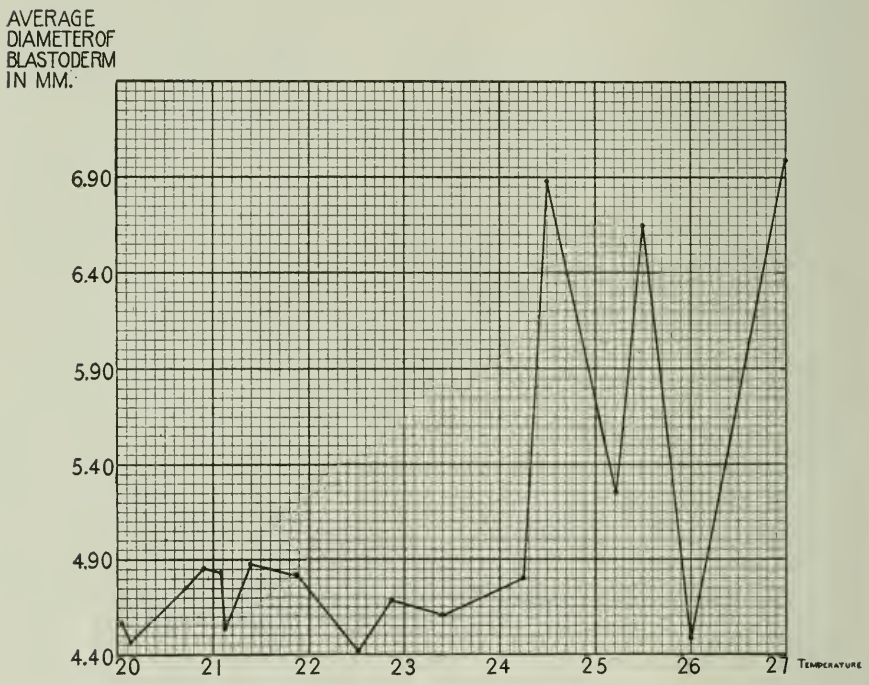
to the growth of the blastoderm without primitive streak is shown in the curve on p. 394. From 20.05° to 24.24° the average growth is confined within 0.5 mm., while in three out of the four cases above the latter temperature there is a marked increase of from 2.00 mm. to 2.59 mm.

Incubation.	Number of blastoderms without embryos.	Average temperature in degrees C.	Extreme limits of temperature in degrees C.	Range of temperature in degrees C.	Average diameter of blastoderm in mm.	Average diameter of area pellucida in mm.
A.4	1	27.00	26.75–27.25	0.50	7.00	2.00
A.5	2	26.00	25.80–26.20	0.40	4.50	1.50
A.6	6	25.50	24.80–26.28	1.40	6.67	2.38
D.22	4	25.22	24.60–25.60	1.00	5.27	2.59
A.7	5	24.50	24.75–25.25	0.50	6.90	2.35
D.21	7	24.24	20.30–25.80	5.50	4.82	2.45
D.20	8	23.40	23.20–24.70	1.50	4.63	2.27
D.19	7	22.89	22.50–23.20	0.70	4.70	2.34
D.18	10	22.51	22.30–22.90	0.60	4.44	2.23
B.10	5	21.87	20.70–22.05	1.35	4.86	2.03
B.11	8	21.38	21.10–21.55	0.45	4.90	2.90
B.12	9	21.11	20.65–21.50	0.85	4.55	2.66
D.17	11	21.08	20.80–21.70	0.90	4.84	2.41
B.13	12	20.92	20.60–21.40	0.80	4.89	2.49
B.14	10	20.72	18.00–21.50	3.50	4.78	2.73
D.16	11	20.13	19.70–20.60	0.90	4.49	2.52
C.15	6	20.05	19.14–20.94	1.80	4.59	2.72

The normal average diameter of the area pellucida is 2.51 mm. In ten out of the seventeen groups the average diameter of the area pellucida is less than the normal. This would indicate that the cells of the area which form the embryo are not especially influenced by a temperature which is sufficient to cause growth in that part of the blastoderm from which the embryo is not formed.

In the above seventeen groups, of the 183 blastoderms given, 122,

or 63.39 per cent, failed to differentiate a primitive streak. In all of these groups the average diameter of the blastoderms without primitive streaks is greater than the normal. Thus cell division without embryonic organization occurs in the majority of blastoderms of eggs incubated at from 20° to 27°. In the table founded upon Duval, given on page 355, the greatest length of primitive streak is 1.93 mm.



RELATION OF TEMPERATURE TO GROWTH OF BLASTODERM

Before the appearance of notochord, or mesodermic somites, this stands for the sixteen hour stage. In A **3**.10 the length of primitive streak is 3.00 mm.; in A **6**.1, 2.0 mm.; A **6**.2, 2.0; B **8**.1, 2.4; and D **22**.1, **22**.2, and **22**.3, 2.9 mm. This shows that continued growth may take place in the primitive streak stage as well as in the simple blastoderm when apparently there is not enough warmth to allow of further ontogenetic differentiation.

XII. Normal Size and Variation in the Volume of the Egg and the Relation of Diameter of Blastoderm to Volume of Egg.

In order to determine whether the diameter of the blastoderm varies directly with the volume of the egg, the following data were secured. From the one hundred cases of Series D and E.e, it is found that the average volume of the egg taken from the displacement of water is 51.67 c.c. with a standard deviation of 4.6499 c.c. and a coefficient of variability of 0.0900. The only group of unincubated eggs in which I ascertained the volume is E.e. Of the seven blastoderms having a larger than normal diameter, six are in eggs having a greater than normal volume, while of the eight blastoderms below the normal seven are in eggs of less than the normal volume. Thus in nearly 87 per cent of these fifteen cases the diameter of the blastoderm varies directly with the volume of the egg.

For incubated eggs, considering only those in which there has not been any development of the primitive streak, the following data are presented.

Series D.	Temperature in degrees.	Percentage of blastoderms that vary directly with the volume of the egg.
16	20.13	54
17	21.08	54
18	22.51	40
19	22.81	43
20	23.40	13
21	24.24	14
22	25.22	50

It is obvious that as the temperature rises the percentage of blastoderms which vary directly with the volume of the egg decreases so that the average for the whole of Series D is only 38 per cent. This of course is due to the growth of the blastoderm at the higher temperatures, a fact which is best shown by the next table.

The ratio of the normal average volume of the egg to the normal average diameter of the blastoderm is 51.67 c.c.: 4.41 mm., or 11.7 c.c.: 1 mm.

Series D	Temperature in degrees.	Number of cases in which the volume of egg is proportionately greater than the diameter of blastoderm, or where their ratio is above the normal.	Number of cases in which the volume of egg is proportionately less than the diameter of blastoderm, or where their ratio is below the normal.
16	20.13	7	6
17	21.08	3	8
18	22.51	3	7
19	22.81	2	5
20	23.40	2	6
21	24.24	2	5
22	25.22	0	4
	Total	19	41

When the ratio is above the normal the blastoderm is proportionately smaller in relation to the volume of the egg, while when the ratio is below the normal the blastoderm is proportionately larger. So in this last table it is seen that this growth of the blastoderm follows directly with the rise of temperature, as shown in another way in Section XI. Since in E.e in relation to volume the eggs are almost evenly distributed about the average, I do not think that the general averages given in this paper can be especially affected by this element, which might easily affect a few measurements.

XIII. Summary.

The following conclusions have been derived from the study of the foregoing data embracing 238 incubated and fifty-nine unincubated eggs of the domestic fowl, Gallus domesticus.

1. The physiological zero, or the temperature below which there is no development, previously given by most authors at 28°, and by one at 25°, is established at the degree included between 20° and 21°.

2. The index of development is given for temperatures from 20°–21° to 30.75°. The first phase shows a very gradual rise in the percentage of development of the embryo to 14 per cent at 27°–29°, the primitive streak alone showing. The second phase, beginning with notochord, neural plate and groove, and mesodermic somites presents an abrupt rise to 54.83 per cent of normal development at 30.75°.

3. The normal average diameter of the blastoderm of the unincubated egg, as determined from the measurement of fifty-nine individuals, is 4.41 mm. with a standard deviation of 0.4792 mm. and a coefficient of variability of 0.1087.

4. The normal average diameter of the area pellucida of the unincubated egg as determined from the measurement of fifty individuals is 2.51 mm. with a standard deviation of 0.3382 mm. and a coefficient of variability of 0.1347.

5. From 136 blastoderms in which embryos have not developed, the form of the area pellucida is $59\frac{19}{34}$ per cent round, $12\frac{1}{2}$ per cent nearly round, $23\frac{9}{17}$ per cent oblong and $4\frac{7}{17}$ per cent oval.

6. The normal average volume of the egg, as determined from the measurement of 100 individuals, is 51.67 c.c. with a standard deviation of 4.8602 c.c. and a coefficient of variability of 0.0942. In 85 per cent of fifteen unincubated eggs where the volume was noted the diameter of the blastoderm varies directly with the volume of the egg, but the variates are so evenly distributed about the average that the general averages of the measurements in this paper would not be especially affected by this element.

7. The introduction of successively higher stages, and the increased growth of blastoderms without primitive streaks as the temperature rises, together with a continued growth of the primitive streak with the non-appearance of other features of the embryo at a low temperature, 20°–21° to 27°–29°, would indicate a direct dependence of ontogenetic organization upon warmth.

THE EXCRETION OF NITROGEN DURING NERVOUS EXCITEMENT.

By FRANCIS GANO BENEDICT.

[*From the Chemical Laboratory of Wesleyan University.*]

SO far as I am aware, the influence of psychical processes, especially that form commonly termed nervous excitement, on the excretion of nitrogen has never been studied in man. In examining the results of metabolism experiments made in this laboratory certain anomalies were observed in the periodic excretion of nitrogen by subjects subsisting on a diet containing a constant amount of protein. A possible explanation, and one that seemed not unreasonable when the conditions of the individual cases were considered, involved the assumption of an increased nitrogen elimination following or during nervous, mental, or psychical excitement.

In spite of the vast amount of research on the relation of muscular work to the excretion of nitrogen in both man and animals the number of experiments in which nervous excitement intentionally or unintentionally formed a condition of the experiment is extremely small. This is the more surprising when the effect of nervous excitement on the secretions is considered. It is, for example, well known to obstetricians that the milk of a woman affected by some strong emotion may be materially altered in quality.

In animal physiology the experiments of Hagemann[1] on the sexual phases of a female dog may perhaps be cited as bordering on this question. Unfortunately at the most interesting portion of the experiment, *i. e.*, that during which the animal was laboring under the sexual excitement (heat), the data are insufficient as the analyses of the urine were not reported. The remainder of the experiment included the period of gestation and lactation, and was consequently one of sexual rest.

The only experiments on man that can be considered as coming under this head are those made during hypnosis. The most striking

[1] HAGEMANN: Virchow's Archiv für pathologische Anatomie, 1890, cxxi, p. 557.

experiment of this kind is that reported by Hoover and Sollmann[1] on the excretion of nitrogen during hypnotic sleep. On the last day of an eight-day fast in hypnotic sleep an enormous increase in the nitrogen metabolized was found, the nitrogen rising from 14.5 gms. on the seventh day to 21.5 gms. on the eighth day. The authors make no comment on this variation. There is nothing in the article to indicate that any suggestion of nervous activity was made at any time.

Other experiments during hypnosis made by Brock, Gürtler, and A. Voisin and Haraut are mentioned by Moll.[2] The inaccessibility of the original papers prevents personal examination of the data, but according to Moll they are insufficient to warrant drawing any conclusions. It would thus appear that this field has as yet not been entered by accurate investigators. In no instance therefore do we find recorded an experiment primarily designed to study the effect of nervous excitement on the metabolism of nitrogen.

When it is considered that the amount of blood distributed to the liver and kidneys is regulated by the vasomotor nerves their influence on metabolism might be expected to be appreciable.

In connection with the influence of nervous excitement on the bodily functions it may be noted that a strong desire to micturate during excitement is a common experience. This need not necessarily indicate a change in the nature of the secretion though the demonstration of this point has not yet been undertaken.

The two experiments here reported were made to determine the effect of prolonged nervous excitement on the excretion of nitrogen.

The ideal conditions for such an experiment demand a preliminary period of several days in which the subject is given a diet with constant nitrogen supply. All nervous excitement should be carefully avoided. When the system is about in nitrogen equilibrium the subject should be placed under strong nervous excitement, the diet remaining the same. To secure this last condition the writer attended on two successive years one of the great intercollegiate football games.

Experiment of 1900.

The experiment began at 7 A.M., Nov. 20 and ended at 12 M. Nov. 25. The subject was thirty years of age, six feet high, weighing 195 pounds and of active habits.

[1] Hoover and Sollmann: Journal of experimental medicine, 1897, ii, p. 405.

[2] Moll: Hypnotism, London, 1900, p. 128.

Two weeks before the experiment proper began, the results of a dietary study showed that the subject was living on a diet containing approximately 120 gms. of protein and furnishing 3600 calories of energy per day. Subsequently analyses were made of the total twenty-four hour amounts of urine for several days and it was then seen that 13.22, 12.30, and 12.94 gms. of nitrogen were actually excreted on three successive days. These observations as well as those of the dietary study were made on a mixed diet, varying in amounts, eaten as desired.

In planning the diet for the experiment proper it was calculated that 15.5 gms. of nitrogen in the form of protein and 3600 calories of energy would suffice to prevent any appreciable loss of material from the body.

To determine whether or no there was an actual loss or gain of nitrogen in the body during the experiment the fæces were collected and analyzed, thus giving the remaining data for striking a nitrogen balance and making therefore a complete nitrogen metabolism experiment. The separation of the fæces for the experimental period was made in the usual manner by means of lampblack. Two gelatine capsules containing lampblack were taken with the supper of Nov. 19, and two were taken with the midday meal of Nov. 25. All fæces colored with lampblack were rejected. In this plan therefore the fæces corresponded to the food eaten during breakfast of Nov. 25, in addition to that eaten during the five preceding days, and hence the total amount of nitrogen excreted in the fæces is for a period of five and one-third days. Assuming no material differences in the assimilation of the food we can subdivide the total nitrogen eliminated, *i. e.*, 4.73 gms. and consider the elimination per day as 0.89 gm. The elimination of nitrogen through this channel has, however, no direct bearing on the general question under consideration and the data are given simply to show whether the body was in nitrogen equilibrium.

The diet contained whole milk, beef, butter, soda and graham crackers, gingersnaps, parched cereal, and sugar.

The last five articles were of the ordinary commercial quality, and as similar materials had been often used in the laboratory in previous experiments their composition was sufficiently well known to serve for calculating the diet. All samples were subsequently analyzed. Their composition is shown in Table I.

The butter was practically non-nitrogenous and was of a standard

creamery brand. The nitrogen content was determined by estimating the casein and dividing by the factor 6.25.

The beef, which furnished a considerable quantity of nitrogen on the first two days of the experiment was freed from fat, cooked, and especially canned by a Western packing house for use in metabolism experiments. It was very dry and became so unpalatable that the menu was changed and sufficient whole milk substituted to furnish

TABLE I.

Weight and nitrogen content of foods.

Food material.	First Period. November 20 and 21.			Second Period. November 22, 23, and 24.		
	Weight per day. Grams.	Per cent of nitrogen.	Weight of nitrogen. Grams.	Weight per day. Grams.	Per cent of nitrogen.	Weight of nitrogen. Grams.
Beef	100	5.58	5.58	..	..	..
Butter	100	0.22	0.22	100	0.22	0.22
Milk	1250	0.55	6.88	2280	0.55	12.54
Parched cereal .	40	2.07	0.83	40	2.07	0.83
Graham crackers .	50	1.26	0.63	50	1.26	0.63
Soda crackers . .	50	1.68	0.84	50	1.68	0.84
Gingersnaps . .	60	0.87	0.52	60	0.87	0.52
Sugar	125	..	..	125	..	..
Total per day . .	..	..	15.50	..	..	15.58

an amount of nitrogen equivalent to that in the beef. The diet of the last three days (including the day of the football game) therefore contained no meat.

Arrangements were made with a local dairyman whereby the morning milk from five selected cows was mixed and the milk for this experiment was obtained from the mixture. In this way milk of a remarkably constant nitrogen and fat content was obtained. The nitrogen of the milk was determined every day and the milk fat was also determined daily by the Babcock method.

The nitrogen determinations in both food and urine were made according to the Kjeldahl method.

A careful diary was kept of all muscular work and habits of life during the experiment, and an attempt was made to estimate the degree of nervous excitement on the different days of the experiment. The first day was coincident with the performance of a test experiment with the Atwater-Rosa respiration calorimeter and as a result the nervous excitement was estimated to have been three or four times as great on this as on the subsequent days up to the day of the football game. No attempt was made to compare this day with the others. The daily routine was purposely the same on each day save the last when it was entirely changed.

In general the day began with breakfast at half past seven o'clock; the morning was spent at the chemical laboratory in experimental work; dinner was served at one; the afternoon was spent at the laboratory; supper was at six and the subject went to bed about ten.

On the last day of the experiment the routine was broken by taking the train at half past ten for New Haven, to witness the football game which took place at 2 P. M. Middletown was reached again at half past seven in the evening. During the evening further excitement was furnished by the celebration of a local football victory.

It is fair to say that especially during the period from 12 M. to 9 P. M. of Nov. 24 the subject was laboring for a greater part of the time under strong nervous excitement.

The morning of Nov. 25 was spent at the laboratory, where at 12 o'clock the last urine was passed and the experiment ended.

The urine was collected in twenty-four-hour periods on the first two days of the experiment. On the last three days the urine for each day was collected in three periods; the first from 7 A. M. to 12 M.; the second from 12 M. to 9 P. M., this period being intended to cover the time of greatest nervous excitement on Nov. 24; and the night period from 9 P. M. till 7 A. M.

The body weight, without clothes, was taken each morning on rising and was as follows; Nov. 20, 86.00 kilos; Nov. 21, 85.15 kilos; Nov. 22, 85.10 kilos; Nov. 24, 84.80 kilos; Nov. 25, 84.80 kilos.

The nitrogen balance during the whole experiment is shown in Table II in which the daily amounts of nitrogen in the food, fæces, and urine are given.

The amount of nitrogen in the food was purposely made somewhat larger than that which would be expected to be necessary to maintain

nitrogen equilibrium when about thirteen gms. of nitrogen (the amount found in the preliminary experiment) was being excreted per day.

It is seen that in spite of this increase in the amount of ingested nitrogen an actual loss of nitrogen from the body was experienced. This loss is apparently entirely independent of any nervous excitement and is accounted for with difficulty. It is possible that the energy of the diet was not sufficient to maintain energy equilibrium. The energy has been calculated from previous determinations of

TABLE II.

Income and outgo of nitrogen.

Date. November, 1900.	Nitrogen.			
	In food. Grams.	In fæces. Grams.	In urine. Grams.	Gain + or loss −. Grams.
20–21	15.50	0.89	15.20[1]	−0.59
21–22	15.50	0.89	15.56	−0.95
22–23	15.58	0.89	16.55	−1.86
23–24	15.58	0.89	15.67	−0.98
24–25	15.58	0.89	14.93	−0.24
Total, 5 days . .	77.74	4.45	77.91	−4.62
Average per day .	15.55	0.89	15.58	−0.92

[1] For data regarding the analysis of urine, see Table III.

similar materials and found to be 3750 Calories per day. This is not appreciably different from that found in the dietary study made previous to this experiment, as indeed the values there obtained served as a basis for the calculation of this menu. The common experience is that in a diet insufficient in energy the body will draw on its body fat, and as soon as this store has been partially depleted body proteid will be metabolized at a greater rate. It is seen therefore that the energy in the diet would be considered sufficient to maintain the body. The panniculus adiposus of the subject was well developed and hence

a considerable loss of fat would normally be expected to take place before the body proteid would be appreciably attacked.

In considering the elimination of nitrogen it is important to bear in mind that on the third day the diet was somewhat changed, milk

TABLE III.

Amount and composition of urine excreted.

Date. November, 1900.	Period.	Weight of urine. Grams.	Specific gravity.	Per cent of nitrogen.	Weight of nitrogen. Grams.
20–21	7 A. M.–7 A. M.	1038.5	1.0290	1.46	15.20
21–22	7 A. M.–7 A. M.	790.5	1.0350	1.97	15.56
22	7 A. M.–12 M.	187.0	1.0305	1.94	3.62
22	12 M.–9 P. M.	346.0	1.0345	1.98	6.85
22–23	9 P. M.–7 A. M.	284.6	1.0320	2.14	6.09
				Total	16.55
23	7 A. M.–12 M.	194.3	1.0300	1.83	3.55
23	12 M.–9 P. M.	350.3	1.0340	1.73	6.06
23–24	9 P. M.–7 A. M.	390.0	1.0300	1.55	6.06
				Total	15.67
24	7 A. M.–12 M.	240.5	1.0280	1.38	3.32
24	12 M.–9 P. M.	372.0	1.0335	1.62	6.03
24–25	9 P. M.–7 A. M.	292.0	1.0325	1.91	5.58
				Total	14.93
25	7 A. M.–12 M.	187.0	1.0300	1.59	2.97

being substituted for beef. On this day the excretion of metabolized nitrogen was much higher than on any previous or subsequent day. This may be explained by the lag[1] in the nitrogen elimination of the

[1] SHERMAN and HAWK: This journal, 1900, iv, p. 42.

meat eaten at noon of the preceding day. The readily assimilated proteid of the milk would be absorbed rapidly while the less digestible meat proteids eaten the preceding day would lag and possibly explain a part of the sudden rise in the nitrogen excretion of the third day. After this day there is a gradual falling off to the end of the experiment.

Table III. gives the elimination of nitrogen by periods. It will be observed that on Nov. 24 the excretion for the last two periods (those of greatest nervous excitement) is no larger, but on the other hand slightly smaller, than during the corresponding periods of the preceding day. It was thought that there might be a lag in the excretion of nitrogen and accordingly the diet was strictly adhered to for breakfast of Nov. 25 and the urine for the first period collected. The amount of nitrogen excreted during this period was slightly lower than that excreted during the corresponding period on the previous days.

Experiment of 1901.

In the second experiment the most important changes introduced were a lengthening of the whole experimental period, an increase in the number of daily periods, and an adjustment of the diet insuring constancy during the whole experiment. The experiment began at 7 A. M. Nov. 19 and ended at 7 A. M. Nov. 26.

Inasmuch as with a diet containing 15.5 gms. of nitrogen in the form of protein the subject lost nitrogen during the first experiment the diet for the experiment of 1901 was selected so as to furnish a somewhat larger amount, *i. e.*, 16.7 gms. of nitrogen. The calculated energy was 3640 Calories as compared with 3750 Calories in the first experiment, a change necessitated by the unpalatability of the long continued diet.

To determine the complete balance of income and outgo of nitrogen the fæces were collected and analyzed. Those collected for the whole period of seven days contained 5.81 gms. of nitrogen, or 0.83 gm. per day.

The articles in the diet were substantially the same as those used in the first experiment, save that the beef was replaced by gluten crackers and the milk was taken from a large herd rather than from five selected cows. The nitrogen content of the milk was determined each day. The changes in composition were so slight from day to day as to be considered negligible for the purpose of this experiment.

All the samples were analyzed except the butter, the nitrogen content of which was assumed to be the average of a number of samples from the same creamery previously analyzed. The amounts of the different foods consumed per day and their nitrogen content are shown in Table IV.

TABLE IV.

Weight and nitrogen content of foods.

Food material.	Weight per day. Grams.	Per cent of nitrogen.	Weight of nitrogen. Grams.
Milk	1700	0.59	10.03
Butter	50	0.22	0.11
Gluten crackers . .	100	4.32	4.32
Graham crackers .	50	1.29	0.64
Soda crackers . .	50	1.69	0.85
Gingersnaps . . .	60	1.25	0.75
Sugar	125	..	..
Total per day . .	..	..	16.70

The daily routine of life was much the same as that previously outlined and no noticeable period of nervous excitement was experienced until 8 A. M. Nov. 23. On this day the subject took a railroad journey of 116 miles to Boston and owing to heavy traffic was delayed all along the line, not reaching the football field until five minutes after the game had begun. The anxiety necessarily resulting from the vexatious delays accompanied by the ever threatened breakdown of the engine, which would have delayed the journey beyond the time of the football game, added not a little to the nervous excitement.

During the progress of the football game the excitement was noticeably greater than in the first experiment, the difference being an overwhelming victory for the team in which the subject was interested as against, in the first experiment, an overwhelming defeat. The return train was taken at 6 P. M., Middletown being reached at 11 P. M.

The ordinary routine was observed on the two following days.

The urine was collected in four periods; the first from 7 A. M. to

12 M.; the second from 12 M. to 6 P.M.; the third from 6 P.M. to 11 P.M.; and the fourth from 11 P.M. to 7 A.M.

The time of greatest nervous excitement on Nov. 23 was during the second period. The collection of the urine in periods continued during the entire seven days of the experiment. The changes noted in the body weight from day to day were insignificant.

While the amount of energy supplied by the food was somewhat lower than in the former experiment the amount of nitrogen was larger by about one gm. The amount of nitrogen lost in the fæces

TABLE V.

Income and outgo of nitrogen.

Date. November, 1901.	Nitrogen.			
	In food. Grams.	In fæces. Grams.	In urine. Grams.	Gain + or loss −. Grams.
19–20	16.70	0.83	14.88	+0.99
20–21	16.70	0.83	15.63	+0.24
21–22	16.70	0.83	17.18	−1.31
22–23	16.70	0.83	16.36	−0.49
23–24	16.70	0.83	16.57	−0.70
24–25	16.70	0.83	15.92	−0.05
25–26	16.70	0.83	16.02	−0.15
Total, 7 days . .	116.90	5.81	112.56	−1.47
Average per day .	16.70	0.83	16.08	−0.21

per day was slightly less. This is contrary to expectation, as a considerable amount of the nitrogen in the diet was in the form of the vegetable protein of gluten crackers. The use of this material to increase the amount of protein in a diet in experiments of this nature is strongly to be recommended. The crackers are coarsely ground and eaten cold or hot with milk and sugar, as are many of the prepared breakfast foods.

The balance of income and outgo of nitrogen is shown in Table V.

It will be seen that in general the body was not far from being in nitrogen equilibrium and though actually experiencing a slight loss in nitrogen it was less than in the experiment of 1900.

Why the body with the increased nitrogen content of the diet should lose nitrogen during the experiment, is hard to state. As a matter of fact a number of experiments have shown that during the year the subject metabolizes nearer 14 gms. of nitrogen per day than the 16 actually metabolized during this experiment. In any case we should not be justified in assuming that nervous excitement could influence the whole experimental period and we must assume therefore that the persistent loss of a small amount of nitrogen during the latter portion of the experiment probably has no direct bearing on the question under consideration.

The metabolized nitrogen was highest in amount on Nov. 21, 22, and 23, the greatest quantity appearing on the first of these days. On the day following the football game, Nov. 24, there was a diminution in the quantity of metabolized nitrogen excreted amounting to 0.65 gm. That this is insignificant is obvious, however, when the marked increase of 1.55 gm. from Nov. 20 to Nov. 21 is noticed.

The periodic excretion of nitrogen is shown in Table VI.

Excluding the first day, Nov. 19, which must be considered as preliminary, we see that on Nov. 23 the one noticeable variation in the excretion of nitrogen by periods is the fact that the amounts excreted during the first two periods covering the time of greatest excitement are larger than in the corresponding periods of any other day. On the other hand the amounts excreted during the two later periods of the day, *i. e.*, 6 P. M. to 11 P. M. and 11 P. M. to 7 A. M. are smaller than in the corresponding periods of any other day.

It is thus suggested that the nervous excitement caused a more rapid excretion of nitrogen (a phenomenon not observed in the experiment of 1900) but that the increased elimination of the earlier part of the day was immediately compensated by a diminished elimination during the remainder of the day, the total elimination for the day not being excessive.

The elimination on the two days following the day of greatest nervous excitement is not characterized as being in any way abnormal and consequently needs no discussion.

The results of these two experiments would seem to indicate that nitrogen metabolism undergoes no noticeable change during periods of intense nervous excitement. It is by no means certain, however,

TABLE VI.

Amount and composition of urine excreted.

Date. November, 1901.	Period.	Weight of urine. Grams.	Specific gravity.	Per cent of nitrogen.	Weight of nitrogen. Grams.
19	7 A. M.–12 M.	289	1.028	0.851	2.46
	12 M.–6 P. M.	580	1.017	0.843	4.89
	6 P. M–11 P. M.	312	1.024	1.238	3.86
19–20	11 P. M.–7 A. M.	251	1.023	1.462	3.67
				Total	14.88
20	7 A. M.–12 M.	218	1.020	1.498	3.27
	12 M.–6 P. M.	234	1.031	1.832	4.11
	6 P.M.–11 P.M.	234	1.029	1.725	4.04
20–21	11 P.M.–7 A.M.	195	1.032	2.162	4.22
				Total	15.64
21	7 A. M.–12 M.	155	1.028	2.021	3.13
	12 M.–6 P. M.	244	1.031	1.978	4.83
	6 P. M.–11 P.M.	260	1.026	1.774	4.61
21–22	11 P. M.–7 A. M.	318	1.020	1.449	4.61
				Total	17.18
22	7 A. M.–12 M.	176	1.027	1.962	3.45
	12 M.–6 P. M.	216	1.032	2.073	4.47
	6 P. M.–11 P. M.	200	1.030	1.971	3.94
22–23	11 P. M.–7 A. M.	222	1.026	2.037	4.50
				Total	16.36

TABLE VI (*continued*).

Date. November, 1901.	Period.	Weight of urine. Grams.	Specific gravity.	Per cent of nitrogen.	Weight of nitrogen. Grams.
23	7 A. M.–12 M.	302	1.019	1.416	4.27
	12 M.–6 P. M.	340	1.022	1.534	5.22
	6 P. M.–11 P. M.	187	1.027	1.764	3.30
23-24	11 P. M.–7 A. M.	201	1.028	1.879	3.78
				Total	16.57
24	7 A. M.–12 M.	265	1.021	1.371	3.63
	12 M.–6 P. M.	361	1.020	1.301	4.70
	6 P. M.–11 P. M.	267	1.023	1.411	3.77
24–25	11 P. M.–7 A. M.	245	1.023	1.556	3.81
				Total	15.91
25	7 A. M.–12 M.	208	1.022	1.579	3.29
	12 M.–6 P. M.	362	1.019	1.265	4.58
	6 P. M.–11 P. M.	281	1.016	1.373	3.86
25-26	11 P. M.–7 A.M.	378	1.018	1.136	4.30
				Total	16.03

that long continued anger, sorrow, or fear would not produce disturbances of nitrogen metabolism other than those that could properly be ascribed to a derangement of digestive functions. It is further to be said that the effect of nervous excitement accompanying personal effort such as for example the feeling of sudden and great responsibility should be thoroughly investigated before drawing any final conclusions regarding nitrogen metabolism during nervous excitement.

The difficulties attending such experimentation are considerable though not insurmountable, and it is to be hoped that the question may receive further consideration.

STUDIES ON THE PHYSIOLOGICAL EFFECTS OF THE VALENCY AND POSSIBLY THE ELECTRICAL CHARGES OF IONS. I. — THE TOXIC AND ANTITOXIC EFFECTS OF IONS AS A FUNCTION OF THEIR VALENCY AND POSSIBLY THEIR ELECTRICAL CHARGE.[1]

By JACQUES LOEB.

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I. Introduction.

FIVE years ago I published a series of papers on the physiological effects of the electric current which impressed upon me the long known fact that the galvanic current is the most universal and effective stimulus for life phenomena. This fact suggested to me the idea that it should be possible to influence life phenomena just as universally and effectively by the electrically charged molecules — the ions — as we can influence them by the electric current. From that time on the whole working force of my laboratory was devoted to the investigation of the physiological effects of ions.

My first aim was to find out whether or not it is possible to alter the physiological properties of tissues by artificially changing the proportion of ions contained in these tissues. In this way originated the investigations on the effect of ions upon the absorption of water by muscles,[2] the effects of ions upon the rhythmical contractions of muscles, and medusæ,[3] the heart of the turtle,[4] and the lymph hearts[5] of the frogs, the rôle of ions in chemotropic phenomena[6] and the in-

[1] A preliminary report of these experiments appeared in Pflüger's Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 68.

[2] Loeb, J.: Archiv für die gesammte Physiologie, 1897, lxvii, p. 1; 1898, lxxi, p. 457; 1899, lxxv, p. 303.

[3] Loeb, J.: Festschrift für Professor Fick, 1899, p. 101. This journal, 1900, iii, p. 327; 1900, iii. p. 383; 1901, v, p. 362; Archiv für die gesammte Physiologie, 1900, lxxx, p. 229.

[4] Lingle, D. J.: This journal, 1900, iv, p. 265.

[5] Moore, A.: This journal, 1900, iv, p. 386.

[6] Garrey, W. E.: This journal, 1900, iii, p. 291.

fluence of ions upon embryonic development,[1] and the development of unfertilized eggs (artificial parthenogenesis).[2] Those who have followed my work on artificial parthenogenesis may have noticed that from the start I aimed at bringing about artificial parthenogenesis through ions. It seemed to me that I could not find any better test for my idea that the electrically charged ions influence life phenomena most effectively than by causing unfertilized eggs to develop by slightly altering the proportion of ions contained in them. I believe that all these experiments proved what I expected they would prove, namely, that by slightly changing the proportion of ions in a tissue we can alter its physiological properties.

The next step taken consisted in proving that it was indeed the electrical character of the ion that determined its specific efficiency. I succeeded in doing this three years ago. It was known that a frog's muscle gives rise to twitchings or rhythmical contractions when immersed in certain solutions. I showed that such contractions, occurred *only in solutions of electrolytes, and not in solutions of non-conductors* (distilled water, various sugars, glycerine, urea).[3] Soon after I showed the same to be true also for the rhythmical contractions of the medusæ.[4] From observations made in my laboratory, the same fact was shown to hold for the turtle's heart by Mr. Lingle,[5] and for the lymph hearts of the frog by Miss Moore.[6] I am confident that this fact will be proved universally.

In the physiology of the heart one frequently encounters the statement that calcium is the stimulus for the contraction of the heart. I had found that a muscle is able to twitch rhythmically when immersed in the solution of salts with a monovalent kation, — I obtained contractions in Na-, Li-, Rb-, and Cs- salts, — but that the addition of a small quantity of a bivalent kation — Ca, Mg, Sr, Ba, Be, Mn, Co — inhibits these rhythmical contractions.[7] This seemed to be a direct contradiction to the statement that calcium salts are the "cause" of the heart-beat. The significance of the calcium had to

[1] Loeb: Archiv für Entwicklungsmechanik, 1898, vii, p. 631. This journal, 1900, iii, pp. 327, 434.

[2] Loeb: This journal, 1899, iii, p. 135, and 1900, iii, p. 327; 1900, iv, p. 178, and 1901, iv, p. 423. Archiv für die gesammte Physiologie, 1901, lxxxvii, p. 594.

[3] Loeb: Festschrift für Fick, 1899, p. 101.

[4] Loeb: This journal, 1900, iii, pp. 328, 383.

[5] Lingle: This journal, 1900, iv, p. 265.

[6] Moore: This journal, 1900, v, p. 87.

[7] Loeb, Festschrift für Fick, 1899, p. 101.

be looked for, then, in another direction. It was soon found that the muscle, the apex of the heart, and a medusa contract rhythmically in a pure sodium chloride solution, but that they soon come to a standstill. If, however, a trace of a soluble calcium salt is added to the sodium chloride solution, the contractions continue much longer. I concluded from this that the pure sodium chloride solution acts, in the long run, as a poison, — that is to say, brings about definite but at present unknown physical changes in the protoplasm — but that a trace of a calcium salt annihilates this toxic action. The amount of calcium necessary for this antitoxic effect is of course much smaller than the amount necessary to inhibit the rhythmical contractions. Soon after I succeeded in demonstrating conclusively the poisonous effect of a pure sodium chloride solution, and the annihilation of this effect by calcium.[1] The eggs of a marine fish (Fundulus) develop normally in sea-water, but they can develop just as well, as I had previously found, in distilled water. The addition of ions from the outside is consequently not necessary to the development of this animal. I found, now, that, if the freshly fertilized eggs of this fish are put into a pure sodium chloride solution having a concentration equal to the concentration of the sodium chloride in the sea-water (about $\frac{5}{8}$ *m*), not a single egg can develop into an embryo. If, however, a trace of a calcium salt is added to the sodium chloride solution, as many eggs develop and in just as normal a manner as in ordinary sea-water. The calcium ions in this case undoubtedly serve the purpose of annihilating the poisonous effects of a pure sodium chloride solution.

In the meantime I had become familiar with the brilliant experiments of Hardy upon the influence of ions and the galvanic current upon colloidal solutions.[2] They indicated to me that the next step I had to take was to see whether or not the valency and the sign of the electrical charge of an ion determine its physiological effects. I suspected that the antitoxic effect of the calcium ion in the above mentioned experiment was due to its electrical charge and decided to investigate in a more systematic way whether or not the sign and quantity of the electrical charge influence life phenomena. My experiments carried on at Woods Hole this summer showed conclusively that this is the case for the antitoxic effects of ions and

[1] Loeb: This journal, 1900, iii, p. 327 ; Archiv für die gesammte Physiologie, 1900, lxxx, p. 229.

[2] Hardy: Proceedings of the Royal Society, 1900, lxvi, p. 110.

probably for the production of rhythmical contractions through ions. It seems at least possible that it is true also for artificial parthenogenesis.

II. The Antitoxic Effect of Ions as a Function of their Electrical Charges and Valency.

1. The development of an embryo from the freshly fertilized egg of the before-mentioned fish, Fundulus, served as a test for the toxic and antitoxic effects of ions. I chose this particular animal for two reasons. First, the process of development in this form is to an astonishing degree independent of the osmotic pressure of the surrounding solution. The egg will develop not only in sea-water, the osmotic pressure of which is about equal to that of a $\frac{5}{8}$ m[1] sodium chloride solution, but also in distilled water, or in sea-water the concentration of which has been doubled (by the addition of NaCl). In the following experiments, therefore, we need not at all consider the osmotic pressure of the surrounding solution. Secondly, since enormous numbers of the eggs can be obtained, it is an easy matter to perform the experiments upon hundreds and thousands of eggs at once.

The eggs were artificially fertilized in the laboratory by the addition of sperm, and then immediately distributed into the various solutions. The embryo forms in from about twenty-six to forty-eight hours — varying with the temperature — and twenty-four hours later the heart begins to beat, and the circulation is established. Usually about two hundred eggs were put into a solution, and after two or three days the developed embryos were counted and the percentage of the eggs which had developed was determined. The eggs were kept under observation as long as the embryos remained alive. Usually when an embryo was once formed, development went further, and the circulation was established.

2. First of all, the toxic effects of a pure sodium chloride solution at various concentrations were tested. In a $\frac{m}{4}$ NaCl solution every egg produced an embryo, which died, however, before, or immediately after emerging from the egg. (The embryo hatches from between twelve to twenty days after fertilization.) Upon the other hand, in a $\frac{3}{8}$ m NaCl solution only a few of the eggs give rise to embryos, — about 1 to 5 per cent. In a $\frac{4}{8}$ m NaCl solution an embryo forms but rarely,

[1] m represents that degree of dilution of a solution which contains one gram-molecule of the substance in 1 litre of the solution.

and in a $\frac{5}{8}$ *m* NaCl solution, the formation of embryos is rendered impossible. The egg goes through the first stages of segmentation, but dies when it reaches the 32- or 64-cell stage. The concentration of a $\frac{5}{8}$ *m* sodium chloride solution is indeed so high above the point of the fatal concentration of sodium chloride, that a slight decrease in the degree of dissociation of the NaCl solution brought about through the addition of a small amount of another salt having a common ion, could be entirely disregarded. In the following experiments, however, salts with different ions were combined, wherever this was possible.

If to a pure sodium chloride solution a trace of a calcium salt is added, as many eggs develop as in ordinary sea-water, as shown by Table I.

TABLE I.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}$ *m* NaCl	0
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{64}$ $CaSO_4$	3
100 c.c. $\frac{5}{8}$ *m* NaCl + 1 c.c. $\frac{m}{64}$ $CaSO_4$	3
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{m}{64}$ $CaSO_4$	20
100 c.c. $\frac{5}{8}$ *m* NaCl + 4 c.c. $\frac{m}{64}$ $CaSO_4$	75
100 c.c. $\frac{5}{8}$ *m* NaCl + 8 c.c. $\frac{m}{64}$ $CaSO_4$	70

This series of experiments does not show whether it is the Ca or the SO_4 ion that has the antitoxic effect. To determine this point the same series of experiments was twice repeated, with certain modifications. In the first of these $Ca(NO_3)_2$ was added to the $\frac{5}{8}$ NaCl solution instead of $CaSO_4$. The result was practically that given in Table I. In the second Na_2SO_4 was added to the sodium chloride solution. The addition of Na_2SO_4 did not inhibit the toxic action of the sodium chloride, and the eggs developed no better than in the pure sodium chloride solution. We shall return to this point later. However, in order to eliminate entirely the effect of the anions in the antitoxic effects produced, a series of experiments were instituted in which the toxic and antitoxic salt both had the same anion.

TABLE II.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}\,m$ $NaNO_3$	0
100 c.c. $\frac{5}{8}\,m$ $NaNO_3$ + $\frac{1}{2}$ c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	1
100 c.c. $\frac{5}{8}\,m$ $NaNO_3$ + 1 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	10
100 c.c. $\frac{5}{8}\,m$ $NaNO_3$ + 2 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	15
100 c.c. $\frac{5}{8}\,m$ $NaNO_3$ + 4 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	15
100 c.c. $\frac{5}{8}\,m$ $NaNO_3$ + 8 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	70

It is undoubtedly true, therefore, that the addition of even a small amount of Ca ions diminishes the toxic action of a pure sodium chloride solution. It can further be shown that the concentration of the Ca ions necessary to abolish the poisonous effects of a sodium chloride solution increases as the concentration of the latter increases (see Table III).

Tables II and III show clearly that the amount of calcium necessary to annihilate the poisonous effect of a solution of a sodium salt increases with the concentration of the sodium salt in the solution.

The embryos formed in these solutions, rendered harmless through the addition of calcium, developed a normal circulation and lived several weeks. As a rule, however, they did not hatch. It was further found that the addition of 5 c.c. of a $\frac{m}{64}$ $CaSO_4$ solution could annihilate absolutely the toxic effect of a $\frac{3}{8}\,m$, $\frac{4}{8}\,m$, or $\frac{5}{8}\,m$ NaCl solution. These experiments leave no room for doubt that the presence of a trace of Ca ions is capable of rendering inert the poisonous effects of a pure sodium chloride solution.

3. It was next shown that Sr, Ba, and Mg ions are also capable of annihilating the poisonous effects of a pure NaCl solution in a way similar to that of Ca ions (see Table IV).

TABLE III.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{3}{8}$ *m* $NaNO_3$	5
100 c.c. $\frac{3}{8}$ *m* $NaNO_3$ + $\frac{1}{2}$ c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	48
100 c.c. $\frac{3}{8}$ *m* $NaNO_3$ + 1 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	40
100 c.c. $\frac{3}{8}$ *m* $NaNO_3$ + 2 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	63
100 c.c. $\frac{3}{8}$ *m* $NaNO_3$ + 4 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	66
100 c.c. $\frac{3}{8}$ *m* $NaNO_3$ + 8 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	70
100 c.c. $\frac{4}{8}$ *m* $NaNO_3$	1
100 c.c. $\frac{4}{8}$ *m* $NaNO_3$ + $\frac{1}{2}$ c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	8
100 c.c. $\frac{4}{8}$ *m* $NaNO_3$ + 1 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	9
100 c.c. $\frac{4}{8}$ *m* $NaNO_3$ + 2 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	45
100 c.c. $\frac{4}{8}$ *m* $NaNO_3$ + 4 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	42
100 c.c. $\frac{4}{8}$ *m* $NaNO_3$ + 8 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	70
100 c.c. $\frac{6}{8}$ *m* $NaNO_3$	0
100 c.c. $\frac{6}{8}$ *m* $NaNO_3$ + $\frac{1}{2}$ c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	0
100 c.c. $\frac{6}{8}$ *m* $NaNO_3$ + 1 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	2
100 c.c. $\frac{6}{8}$ *m* $NaNO_3$ + 2 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	7
100 c.c. $\frac{6}{8}$ *m* $NaNO_3$ + 4 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	10
100 c.c. $\frac{6}{8}$ *m* $NaNO_3$ + 8 c.c. $\frac{m}{64}$ $Ca(NO_3)_2$	50

TABLE IV.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}$ *m* NaCl	0
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{3}{4}$ c.c. *m* $BaCl_2$	75
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. *m* $BaCl_2$	90
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. *m* $MgCl_2$	75
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{5}{16}$ *m* $SrCl_2$	90
100 c.c. $\frac{5}{8}$ *m* NaCl + 1$\frac{1}{2}$ c.c. $\frac{5}{4}$ *m* $Ca(NO_3)_2$	80

That the threshold for the antitoxic effects of Ba, Mg, and Sr has the same magnitude as that of Ca may be indicated by a single experiment with Ba (Table V).

TABLE V.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}$ *m* NaCl	0
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{32}$ $BaCl_2$	8
100 c.c. $\frac{5}{8}$ *m* NaCl + 1 c.c. $\frac{m}{32}$ $BaCl_2$	4
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{m}{32}$ $BaCl_2$	27
100 c.c. $\frac{5}{8}$ *m* NaCl + 4 c.c. $\frac{m}{32}$ $BaCl_2$	76
100 c.c. $\frac{5}{8}$ *m* NaCl + 8 c.c. $\frac{m}{32}$ $BaCl_2$	75

It can be seen that the threshold of the antitoxic effect of barium is almost identical with that for Ca under similar conditions.

Since all these ions are related chemically, the objection was possible that we were dealing here not with the effects of the valence or the electrical charge of the ions, but with a specific chemical effect. It was, therefore, necessary to show that the same effect can be produced by bivalent kations which lie outside of the calcium group. My first experiments failed me, since I at first employed too large amounts of the antitoxic salts. I discovered only gradually that the poisonous effects of a sodium chloride solution may be annihilated by a bivalent kation in quantities much smaller than are given in Table IV, which presents the results of one of my first experiments. My experiments now succeeded.

4. A large number of experiments were performed with $ZnSO_4$ as the antitoxic substance for NaCl. The NaCl solution used was somewhat more concentrated than that usually employed, namely $\frac{11}{16}$ *m* instead of $\frac{5}{8}$ *m*.

TABLE VI.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{11}{16}$ *m* NaCl	0
100 c.c. $\frac{11}{16}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{128}$ $ZnSO_4$	$\frac{1}{2}$
100 c.c. $\frac{11}{16}$ *m* NaCl + 1 c.c. $\frac{m}{128}$ $ZnSO_4$	2
100 c.c. $\frac{11}{16}$ *m* NaCl + 2 c.c. $\frac{m}{128}$ $ZnSO_4$	22
100 c.c. $\frac{11}{16}$ *m* NaCl + 4 c.c. $\frac{m}{128}$ $ZnSO_4$	50
100 c.c. $\frac{11}{16}$ *m* NaCl + 8 c.c. $\frac{m}{128}$ $ZnSO_4$	75

To supplement these results the following table dealing with the effects of a more concentrated $ZnSO_4$ solution and a more dilute NaCl solution than that of the previous table may be given.

TABLE VII.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{4}{8}$ *m* NaCl	5
100 c.c. $\frac{4}{8}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{32}$ $ZnSO_4$	90
100 c.c. $\frac{4}{8}$ *m* NaCl + 1 c.c. $\frac{m}{32}$ $ZnSO_4$	80
100 c.c. $\frac{4}{8}$ *m* NaCl + 2 c.c. $\frac{m}{32}$ $ZnSO_4$	86
100 c.c. $\frac{4}{8}$ *m* NaCl + 4 c.c. $\frac{m}{32}$ $ZnSO_4$	88

The remaining experiments showed a similar agreement in the results obtained. It is worthy of note that these embryos remained alive over a week, possessed an entirely normal circulation, and moved in the egg.

The experiments with freshly prepared $FeSO_4$ yielded as striking results as the above. Only in these experiments the transformation of the bivalent into the trivalent Fe ion introduces a disturbing element. We shall see later that the ferric ion is apparently extremely poisonous. The addition of $\frac{1}{2}$ c.c. or 1 c.c. of a freshly prepared $\frac{m}{4}$ $FeSo_4$ solution to 100 c.c. of $\frac{5}{8}$ *m* NaCl solution annihilates the

poisonous effect of the pure sodium chloride solution just as completely as the addition of the Zn ions in the previous experiment.

Then I tried whether cobalt ions are capable of annihilating the antitoxic effects of a pure sodium chloride solution. The results were very clear indeed.

TABLE VIII.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}$ *m* NaCl	0
100 c.c. $\frac{5}{8}$ *m* NaCl + 1 c.c. $\frac{m}{64}$ $CoCl_2$	6
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{m}{64}$ $CoCl_2$	2
100 c.c. $\frac{5}{8}$ *m* NaCl + 4 c.c. $\frac{m}{64}$ $CoCl_2$	2
100 c.c. $\frac{5}{8}$ *m* NaCl + 8 c.c. $\frac{m}{64}$ $CoCl_2$	50
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{m}{8}$ $CoCl_2$	88
100 c.c. $\frac{5}{8}$ *m* NaCl + 5 c.c. $\frac{m}{8}$ $CoCl_2$	62

Since the amount of a bivalent kation capable of exhibiting its antitoxic properties was so extraordinarily small, I risked the attempt to annihilate the poisonous effects of a pure sodium chloride solution through the addition of Pb, Cu, and Hg ions. Had I not before demonstrated the antitoxic effects of so poisonous an ion as the zinc ion, such an attempt would have appeared to me only ridiculous. With copper acetate and mercuric chloride I obtained negative results throughout, for these two ions are so poisonous indeed that the small amounts necessary to render inert the poisonous effects of a sodium chloride solution are sufficient to kill the egg or cause its coagulation. With lead ions, however, I had a distinct success. For the antitoxic salt lead acetate was used, and for the toxic salt, sodium acetate. It was proved that the latter was slightly more toxic than NaCl.

TABLE IX.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{4}{8}$ *m* CH_3CO_2Na	1
100 c.c. $\frac{4}{8}$ *m* CH_3CO_2Na + $\frac{1}{2}$ c.c. $\frac{m}{64}$ Pb acetate	8
100 c.c. $\frac{4}{8}$ *m* CH_3CO_2Na + 1 c.c. $\frac{m}{64}$ Pb acetate	12
100 c.c. $\frac{4}{8}$ *m* CH_3CO_2Na + 2 c.c. $\frac{m}{64}$ Pb acetate	23
100 c.c. $\frac{4}{8}$ *m* CH_3CO_2Na + 4 c.c. $\frac{m}{64}$ Pb acetate	34

In another case 40 per cent of the eggs formed embryos. The objection was here again at hand that the decrease in the degree of the dissociation of the sodium acetate had played a rôle. Although lead chloride is only very slightly soluble, I tried to see if the few lead ions that go into solution when lead acetate is added to sodium chloride would still suffice to weaken the poisonous effects of a pure NaCl solution. Such was indeed the case.

TABLE X.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{4}{8}$ *m* NaCl	3
100 c.c. $\frac{4}{8}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{64}$ Pb acetate	7
100 c.c. $\frac{4}{8}$ *m* NaCl + 1 c.c. $\frac{m}{64}$ Pb acetate	17

In the remaining solutions the number of embryos could not be determined, since the eggs had been rendered opaque by the precipitation of the lead salts.

5. Experiments were now made to see if it were possible to annihilate the toxic effects of a sodium chloride solution through the addition of salts having a trivalent ion. $AlCl_3$, $Cr_2(SO_4)_3$ and $FeCl_3$ were used. The experiments with $FeCl_3$ all yielded negative results. No concentration could be found at which this salt exhibited antitoxic properties. Perhaps the strongly acid character of this solution had something to do with this result. The experiments with the two other salts, however, yielded positive results.

TABLE XI.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}$ *m* NaCl	0
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{1}{4}$ c.c. $\frac{m}{192}$ $AlCl_3$	0
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{192}$ $AlCl_3$	4
100 c.c. $\frac{5}{8}$ *m* NaCl + 1 c.c. $\frac{m}{192}$ $AlCl_3$	25
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{m}{192}$ $AlCl_3$	39
100 c.c. $\frac{5}{8}$ *m* NaCl + 4 c.c. $\frac{m}{192}$ $AlCl_3$	25

Two other series of experiments yielded the same results. It is worthy of note that the amount of a trivalent kation capable of exerting a certain antitoxic effect is considerably less than the amount of a bivalent kation necessary for the same purpose. At the same time one notices, however, that the number of eggs forming embryos is, even at the best, lower than when bivalent kations are employed. The reason for this lies, as I believe, in the fact that the trivalent ion causes readily a coagulation of the egg contents, as direct observation shows. But this coagulation is not exclusively a function of the valency of the ions, for Cu, Hg, and to a slight extent Pb have the same influence upon the egg. The influence of the Cr ion in bringing about coagulation is much more marked than is the case with Al, and its antitoxic effects are correspondingly slight, but yet definite.

TABLE XII.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{8}$ *m* NaCl	0
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{1}{4}$ c.c. $\frac{m}{96}$ $Cr_2(SO_4)_3$	3
100 c.c. $\frac{5}{8}$ *m* NaCl + $\frac{1}{2}$ c.c. $\frac{m}{96}$ $Cr_2(SO_4)_3$	8
100 c.c. $\frac{5}{8}$ *m* NaCl + 1 c.c. $\frac{m}{96}$ $Cr_2(SO_4)_3$	8
100 c.c. $\frac{5}{8}$ *m* NaCl + 2 c.c. $\frac{m}{96}$ $Cr_2(SO_4)_3$	10
100 c.c. $\frac{5}{8}$ *m* NaCl + 4 c.c. $\frac{m}{96}$ $Cr_2(SO_4)_3$	6

6. Since traces of trivalent kations, and small amounts of bivalent kations suffice to thus annihilate the poisonous effects of a sodium chloride solution, experiments were made to ascertain if the same could also be brought about by monovalent kations. The experiments have thus far led to no positive results. I tried to see if the poisonous effects of a pure sodium chloride solution could be done away with by the addition of potassium salts (KCl and K_2SO_4). Small amounts of potassium salts were entirely without effect. The addition of $\frac{1}{2}$ to 2 cc. of *m* KCl or K_2SO_4 occasionally yielded results, in that 1 per cent to 5 per cent of the eggs formed embryos. Lithium salts showed themselves to be even less active. I occasionally obtained a slight antitoxic action by the addition of large amounts of NH_4 salts. Whether hydrogen ions can yield better results must be determined through further experiments.

7. Not only can the poisonous effects of a pure sodium chloride solution be annihilated through the addition of small amounts of bivalent or trivalent kations, but it seems as though the same holds for *all* salts which, like NaCl, have a univalent kation and anion. No embryos develop in a $\frac{3}{8}$ *m* LiCl solution. By the addition of small amounts of $Ca(NO_3)_2$, $BaCl_2$, $SrCl_2$ or $MgCl_2$, 50–60 per cent of the eggs were caused to form embryos, which developed normally. Other kations of a higher valency were not tested. I obtained entirely similar results in regard to KCl. In a $\frac{6}{8}$ *m* or even a $\frac{7}{8}$ *m* KCl solution, an egg may occasionally develop. When a small quantity of $MgCl_2$, $Ca(NO_3)_2$, $SrCl_2$, $BaCl_2$ or $FeSO_4$ was added, the poisonous effects of the pure KCl solution were annihilated. Of salts having other bivalent kations, only $ZnSO_4$ (a single experiment) was used. An effect was obtained in this case also, but it was less striking than in the case of the other bivalent kations.

NH_4Cl seems to be the least toxic of all the salts mentioned thus far. Even in a $\frac{7}{8}$ *m* NH_4Cl solution an embryo could form occasionally. This immunity of the Fundulus egg against NH_4Cl is perhaps related to its great immunity against urea. I cannot get rid of the suspicion that a percentage of the NH_4 ions is perhaps done away with in the metabolism of the egg. I obtained striking antitoxic effects with small amounts of $SrCl_2$ and, although less definite, of $FeSO_4$. $Ca(NO_3)_2$ increased the number of embryos formed, though not as greatly as the other salts with a bivalent kation, but the life of the embryos was very considerably prolonged.

The shortness of the spawning season limited the number of my

experiments, so that I decided to bring my experiments upon the annihilation of the poisonous effects of a pure sodium chloride solution to a close, and to carry the remaining experiments only far enough to decide if we are dealing here, in the main, with the same condition of affairs. That I believe, is undoubtedly the case, so that I feel myself justified in making the following statement: *The salts of monovalent kations (Na, Li, K, NH_4) with monovalent anions (Cl, NO_3, CH_3COO) exert a toxic effect at certain concentrations. This toxic effect can be annihilated through the addition of a small amount of a salt having a bivalent kation. For NaCl, proof has been brought forward that trivalent kations exhibit even a much more energetic antitoxic effect than bivalent kations.* Further experiments are yet to be made, to decide if the poisonous effects of the other salts (LiCl, KCl, NH_4Cl) can also be done away with through the addition of such small amounts of trivalent kations as suffice for NaCl.

8. While the preceding experiments show an undoubted influence of the valency of the ions upon their antitoxic effects, it was now necessary to prove that the sign of the electrical charge was the second determining variable. I instituted a large number of experiments in which I attempted to annihilate the poisonous effects of a $\frac{5}{8}$ *m* NaCl and a $\frac{7}{8}$ *m* KCl solution by the addition of salts having a univalent or bi- or trivalent anion. The antitoxic effects of the following salts were investigated: KOH, NaBr, NaI, $NaHCO_3$, Na_2CO_3, Na_2So_4, Na_2HPO_4, sodium citrate, K_2SO_4. Extensive quantitative experiments were made with Na_2SO_4, K_2SO_4, $NaHCO_3$ and Na_2HPO_4. The results were negative throughout. In the best cases one per cent of the eggs formed embryos. *It followed from these experiments that the toxic effects of salts with a monovalent kation and a monovalent anion can be annihilated only by bi- or trivalent kations but not by mono-, bi-, or trivalent anions.* If we correlate this fact with that previously found, that spontaneous, rhythmical contractions of muscles, medusæ and hearts are possible only in solutions of electrolytes, then the idea can certainly not be repudiated, that the antitoxic effect of salts in the above mentioned experiments may be a function of the magnitude and the sign of the electrical charges of the ions.

9. If the toxicity of a pure $CaCl_2$, $MgCl_2$, $BaCl_2$ or $SrCl_2$ solution is compared with the toxicity of a solution of a chloride of a monovalent kation,then it is found that the former are the more poisonous. In a $\frac{m}{8}$ $Ca(NO_3)_2$ solution no embryo develops. This same toxic concentration is reached in a $MgCl_2$ solution at the dilution of $\frac{m}{2}$. Can

the toxic effects of these solutions also be overcome? One can indeed easily overcome the poisonous effects of a $\frac{m}{8}$ $Ca(NO_3)_2$ solution by adding large amounts of a KCl or NH_4Cl solution. NaCl and LiCl solutions are almost without effect.

TABLE XIII.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + $\frac{1}{2}$ c.c. $2\frac{1}{2}$ m KCl	15
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 1 c.c. $2\frac{1}{2}$ m KCl	34
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 2 c.c. $2\frac{1}{2}$ m KCl	40
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 4 c.c. $2\frac{1}{2}$ m KCl	55
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 8 c.c. $2\frac{1}{2}$ m KCl	67

As one can see, the number of embryos formed shows a definite increase with an increase in the concentration of the KCl. I tried still stronger solutions of KCl in further experiments, and found that in a mixture of 100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 20 c. c. $2\frac{1}{2}$ m KCl a still larger number of eggs formed embryos than in the preceding experiments. Upon the other hand it could be shown that the addition of small amounts of KCl was without effect.

TABLE XIV.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + $\frac{1}{2}$ c.c. $\frac{m}{32}$ KCl	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 1 c.c. $\frac{m}{32}$ KCl	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 2 c.c. $\frac{m}{32}$ KCl	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 4 c.c. $\frac{m}{32}$ KCl	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 8 c.c. $\frac{m}{32}$ KCl	2
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 2 c.c. $2\frac{1}{2}$ m KCl	12

The size of the antitoxic dose of KCl in Ca $(NO_3)_2$ poisoning is in fact extraordinarily larger than the antitoxic dose of $Ca(NO_3)_2$ in the case of KCl poisoning. Similar relations exist for the antitoxic effect of NH_4Cl upon $CaCl_2$ poisoning.

TABLE XV.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$	0
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 1 c.c. $2\frac{1}{2}$ m NH_4Cl	9
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 2 c.c. $2\frac{1}{2}$ m NH_4Cl	8
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 4 c.c. $2\frac{1}{2}$ m NH_4Cl	16
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 8 c.c. $2\frac{1}{2}$ m NH_4Cl	21
100 c.c. $\frac{m}{8}$ $Ca(NO_3)_2$ + 16 c.c. $2\frac{1}{2}$ m NH_4Cl	16

The antitoxic effects of NH_4Cl are not as great as those of KCl. Similar experiments with NaCl and LiCl as antitoxic substances were without positive result.

Similar experiments were then performed with $MgCl_2$. By the addition of $MgSO_4$ the toxic effects of $MgCl_2$ could not be done away with. But through the addition of large amounts of KCl, NH_4Cl or small amounts of $SrCl_2$ this was possible, as also — to a slight extent — through the addition of $Ca(NO_3)_2$. The dilution at which a $MgCl_2$ solution hinders the development of an embryo is $\frac{5}{16}$ m $MgCl_2$. Table XVI shows a series of antitoxic experiments. NaCl and LiCl were just as unable to annihilate the toxic effects of the $MgCl_2$ solution as they were unable to annihilate the poisonous effects of a $Ca(NO_3)_2$ solution. When less than $\frac{1}{2}$ cc. of a $\frac{m}{16}$ $SrCl_2$ solution was added, not a single egg could develop.

10. If, in these experiments, only the kations have an antitoxic effect, and this the greater, the greater their electrical charge; and if in these antitoxic effects we are dealing only with electrical effects, then it is to be logically expected that the *toxic* effects which are inhibited in these cases, are also electrical effects, and indeed the effects of the negative electrons. If the antitoxic ions are the strongly charged positive ions, then the toxic ions in the sodium chloride solution must be the Cl ions. But in a pure sodium chloride solution we

have just as many kations as anions, and in consequence just as many positive as negative electrical units. It is therefore not at once intelligible why the negative charges of the chlorine ions should be able to call forth poisonous effects in a sodium chloride solution. If it is necessary for us to accept the fact that we are here dealing with electrical effects, then we are forced to further conclude that, for some reason or other, the negative charges of the chlorine ions attain a greater activity than the positive charges of the sodium ions. Nernst has pointed out the fact that the metallic ions tend to bind their

TABLE XVI.

Solution.	Percentage of eggs yielding embryos.
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$	0
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 1 c.c. $2\frac{1}{2}$ *m* NH_4Cl	16
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 2 c.c. $2\frac{1}{2}$ *m* NH_4Cl	22
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 4 c.c. $2\frac{1}{2}$ *m* NH_4Cl	34
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 8 c.c. $2\frac{1}{2}$ *m* NH_4Cl	9
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 16 c.c. $2\frac{1}{2}$ *m* NH_4Cl	3
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 1 c.c. $\frac{5}{16}$ *m* $SrCl_2$	25
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 2 c.c. $\frac{5}{16}$ *m* $SrCl_2$	22
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 4 c.c. $\frac{5}{16}$ *m* $SrCl_2$	9
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 8 c.c. $\frac{5}{16}$ *m* $SrCl_2$	0
100 c.c. $\frac{5}{16}$ *m* $MgCl_2$ + 16 c.c. $\frac{5}{16}$ *m* $SrCl_2$	0

electrical charges more strongly than the anions, and he brings this into connection with the fact that we are acquainted with kathode rays but not with anode rays. Another possibility may be thought of. The egg — and all protoplasm — is a system with various phases; we have solid parts (membranes), and liquid parts which are either rich or poor in colloids. It is conceivable that the coefficient of distribution for the positive and negative ions is unequal in the various phases, and that this fact leads to the toxic effects of the negative ions which can be annihilated by the addition of a small number of positive ions holding a double or triple charge.

I was long inclined to look upon the sodium ions as the toxic ions

in a pure sodium chloride solution, and I have upheld this view in my preliminary communication concerning these experiments. What led me to this conclusion was the following experiment. I tested the relative toxicity of H and OH ions for the eggs of Fundulus. As was to be expected, it came to light that the hydrogen and hydroxyl ions differ in their toxicity. In a $\frac{n}{200}$ KOH solution the eggs developed and formed embryos, while a $\frac{n}{1000}$ HCl solution killed the eggs almost immediately. The hydrogen ions are therefore at least as much as five times as poisonous as the hydroxyl ions. But I do not believe that we are forced to conclude from this that the poisonous effects of a sodium chloride solution necessarily originate from the positively charged ions. Besides the electrical charge other factors may have to be considered in the toxicity of ions for the determination of which physical chemistry and physics must first furnish us the data. Into this category belongs, for example, the fact that the toxic effects of the sodium salts of the halogens upon fish eggs (perhaps upon protoplasm in general) increases in the following order NaCl, NaBr, NaI, NaF. In addition we find that the bivalent anions are in general more poisonous than the monovalent, and the trivalent more poisonous than either. The same also holds true to a certain extent for the poisonous effects of kations.

In order to make the bulk of this paper no greater than it is already, I shall discuss my experiments on the toxic effects of ions no further at this point.

III. Experiments upon the Influence of the Sign and Quantity of the Electrical Charge of Ions upon their Antitoxic Effects upon Muscle.

A series of experiments upon muscle which have been conducted in part by myself, in part by my assistant, Mr. Neilson, have thus far yielded the following results.

1. The duration of life of the frog muscle in a sodium chloride solution is considerably lengthened if a small but definite amount of a salt with a bivalent kation is added thereto, as, for example, Ca, Sr, Mg. This antitoxic effect can be shown, for example, when to 100 c.c. of a $\frac{m}{8}$ NaCl solution, 1 c.c. of a $\frac{m}{32}$ calcium solution are added. The muscle reacts then to stimulation with induction shocks 14 to 20 hours longer than in a pure sodium chloride solution.

2. The same holds true when, instead of the sodium chloride

solution, the chlorides of other monovalent kations are used (Li, NH_4, K).

3. When we compare the poisonous effects of sodium salts with anions of different valencies it can be observed that, in general, the toxicity increases with the increase in the valency of the anion. I have already called attention to this rôle played by valency.[1] I have also emphasized the fact, and repeat it at this point, that valency is only one of a series of variables which are of importance for the toxic effect of ions. I tried now to determine if and in what proportions the amount of $CaCl_2$ necessary to overcome the toxic effects of a sodium salt varies with the valency of the anion. Sodium acetate, sodium sulphate, and sodium citrate were chosen for comparison. In a $\frac{m}{8}$ solution of sodium acetate the muscle lost its faradic irritability in about 24–25 hours. By the addition of 1–4 c.c. of a $\frac{m}{32}$ $CaCl_2$ solution to 100 c.c. of a $\frac{m}{8}$ solution of sodium acetate, the duration of the irritability of the muscle was lengthened to 48–51 hours! In a $\frac{m}{8}$ Na_2SO_4 solution the muscle lived 17–19 hours. In order to increase the duration of its life, 1–4 c.c. of a $\frac{m}{8}$ $CaCl_2$ solution had to be added to 100 c.c. of a $\frac{m}{8}$ $NaSO_4$ solution. Under such circumstances the muscle remained irritable for about thirty-six hours. The amount of $CaCl_2$ which was most favorable for annihilating the effects of a sodium acetate solution was ineffective when added to a sodium sulphate solution, and the amount which was most favorable for the latter acted harmfully when added to 100 c.c. of a $\frac{m}{8}$ solution of sodium acetate. In an $\frac{m}{8}$ sodium citrate solution the muscle lost its irritability in less than three hours. Through the addition of $\frac{1}{2}$–2 c.c. of an *m* solution of $CaCl_2$ to 100 c.c. of the sodium citrate solution, the irritability of the muscle was maintained more than seven hours. The amount of calcium necessary to annihilate the poisonous effects of sodium salts having anions of different valencies increases therefore more rapidly than the increase in valency. For the acetate, sulphate, and citrate the antitoxic doses stand in about the following relation: 1 : 4 : 16. These figures leave no room for doubt that the calcium in this case serves to do away with the poisonous effects of the anions and not of the sodium ions. In the poisonous effects of a sodium chloride solution we must therefore, in all probability, consider the Cl ions as poisonous, and not the Na ions, as I stated in my former papers.

These experiments, therefore, speak for the fact, that the poisonous

[1] Loeb: Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 68.

effects of salts with a univalent kation are due to the negative charges (or negative electrons) of the anions, and that a small amount of a salt with a bivalent kation, by means of its positive charges, acts antitoxically to the latter.

The question can now be raised, if salts with kations of a higher valency and monovalent anions may exert poisonous effects through their positive charges. The poisonous action of a $CaCl_2$ solution might perhaps be caused by the Ca ions. I have, therefore, tried to see if the toxic effects of a $CaCl_2$ solution cannot be overcome by the addition of trivalent anions, for example, citrate ions. Thus far, however, I have not yet succeeded in reaching any definite positive results. These experiments will be continued.

IV. Theoretical Considerations.

1. We have to attempt to answer the question, How can the electrical charges of ions produce a toxic or antitoxic effect? The answer to this question must be preceded by the answer to the more general question, How can the electrical charges of ions as well as of an electric current influence life phenomena? The basis for the answer to this question will undoubtedly be found in the work of Hardy,[1] as well as that of Bredig,[2] on the rôle of the electrical charges of the particles in a colloidal solution. Hardy has shown that living protoplasm is to be considered as a colloidal solution, a hydrosol. Such hydrosols are suspensions of solid particles in a fluid (water). These particles are at the highest about 1,000 to 10,000 times as large as the dimension which the kinetic theory of gases assumes for the molecules. The forces which keep these particles in solution are of an electrical nature. There exists, according to Helmholtz and Quincke, a double electrical layer at the limit between particle and surrounding water.

When the colloidal particles have a positive charge, the surrounding particles of the water have an equal negative charge. It agrees with this assumption that the colloidal particles move under the influence of an electrical current in the same way as ions. They move to the anode when they carry a negative charge, and to the kathode when they carry a positive charge. Hardy has made it probable that these charges keep the particles in solution, inasmuch as through these charges they must repel each other. Hardy has shown that as soon

[1] Hardy: Proceedings of the Royal Society, 1901, lxvi, p. 110. Journal of physiology, 1899, xxiv, pp. 182, 288.

[2] Bredig: Anorganische Fermente. Leipzig. 1901.

as these charges are taken away from them the colloidal particles will no longer be held in suspension, but either fall down or rise to the surface. In this case the hydrosol is transformed into a hydrogel. These charges can either be taken away from them through the oppositely charged electrode of a battery, or through oppositely charged ions which easily give off their charge. Solutions whose colloidal particles have a negative charge can be caused to coagulate (go into the gel stage) by one of two means: either by positively charged ions, or by the positive electrode of a battery. The coagulating effect of ions increases with their valency, and much more rapidly than the valency. The most valuable among Hardy's discoveries is the fact that in a solution of white of egg the colloidal particles can be rendered either positively or negatively electric by the addition of hydrogen or hydroxyl ions. When the neutral or isoelectric point is reached, the slightest change — one feels almost inclined to use the word "stimulus" — is sufficient to transform the solution into a gel.

But long before the critical point of a colloidal solution is reached the variation in the charge of the colloidal particles alters their physical properties. An increase in their charge has the same effect as if the viscosity of the liquid were increased.

The bulk of our protoplasm consists of colloidal material, and the physical manifestations of life, such as muscular contractions and protoplasmic motions, and the innervations,[1] are due to changes of the

[1] The idea that the phenomena of innervation as well as those occurring in the central nervous system are due to changes in the colloidal solutions of the nerves and ganglions has been held by me for several years and found its expression not only in my lectures but also in the English edition of my book on the "Comparative Physiology of the Brain and Comparative Psychology" which appeared in 1900. On page 14, for example, the reader will find the following passage: "It becomes evident that the unravelling of the mechanism of associative memory is the great discovery to be made in the field of brain-physiology and psychology. But at the same time it is evident that the mechanism cannot be unravelled by histological methods, or by operations on the brain, or by measuring reaction times. *We have to remember that all life phenomena are ultimately due to motions or changes occurring in colloidal substances.* The question is, which peculiarities of the colloidal substances can make the phenomenon of associative memory possible? For the solution of this problem the experience of physical chemistry and of the physiology of the protoplasm must be combined. From the same sources we must expect the solution of the other fundamental problems of brain physiology, namely the process of conduction of stimuli." On pages 19-21 and other places of the same book I have discussed the idea that the influence of ions upon rhythmical contractions is due to the effect of ions on the physical qualities of the colloidal solutions. At that time Hardy's earlier work was known to me.

condition of these colloidal solutions. We now may be able to understand why the electrical current is the universal form of stimulation. The reason may be that the particles in colloidal solutions are electrically charged, and that every alteration of the charge of the particles will result in a process of innervation or a contraction or protoplasmic motion, etc. We likewise understand why the ions, on account of their electrical charges, are equally well capable of altering the physiological properties of the tissues, as the galvanic current.

But how can the ions cause toxic and antitoxic effects through their electrical charges? In my preliminary notice on these experiments which appeared in Pflüger's Archiv in November, 1901, I pointed out the possible relation of the electrical charges to the viscosity of the protoplasm. Phenomena of cell division are, as I believe with Bütschli and Quincke, phenomena of protoplasmic streaming. Such phenomena require, as Quincke has shown, a definite degree of viscosity. If the viscosity is too great, no protoplasmic motion is possible, and the same is true if the viscosity is too small. It may be possible that the toxic charges — presumably the negative one in the case of sodium salts — alter the viscosity of the protoplasm by either making it too liquid or too viscous, thus preventing the protoplasmic motions necessary for cell division or the muscular contraction. Small quantities of oppositely charged ions with a higher valency, which give off their charge sufficiently readily, will act as antitoxic substances.

2. The thermodynamical theory of life phenomena has utterly failed to show how the thermal energy produced through the splitting up and oxidation of foodstuffs can lead to muscular contraction. Engelmann's well-known attempt at an explanation is based on a physical impossibility, in view of the fact that some muscles, especially those of the wings of insects, are capable of relaxing and contracting a large number of times in a second. The facts mentioned at the beginning of this paper point distinctly towards the possibility that part of the chemical energy in our body is transformed into electrical energy,[1] or, in other terms, the ions formed in metabolism seem

[1] This view is in harmony with a view expressed by d'Arsonval, who, to my knowledge was the first one to claim that the muscle is not a thermal motor. He assumes that changes in surface tension produce protoplasmic motions, and shows how, on the basis of Lippmann's observations, electrical changes must lead to changes in surface tension. Hardy's experiments and my own observations are, as I believe, in harmony with d'Arsonval's view. D'Arsonval: Archives de physiologie, 1889, V. series, i, p. 460.

to play a rôle in the dynamics of life phenomena. From the facts mentioned in this paper we can see that these ions, or rather, their electrical charges, may be responsible for such physical manifestations of life as the muscular contractions and others. It remains to be explained how the electrical energy of the ions may be transformed into the mechanical energy produced by the contracting muscle. This will be discussed in the second paper, but I will point out here that I believe that the electrical energy of the ions is transformed into surface energy. It will now become necessary to pay more attention to the production of ions in metabolism than has been done before. The CO_3 and PO_4 ions, as well as the H ions, can no longer be considered as mere waste products of metabolism.

3. The fact that ions may act toxically through their electrical charges, and that ions with the opposite charge may act antitoxically, may open a new and very fertile field for pathology and therapeutics. As I have stated in previous papers, especially certain neuroses, and perhaps certain mental diseases, may now find their explanation.[1] Two years ago I pointed out that we must realize the existence of physiologically balanced salt solutions, that means salt solutions in which the ions are so combined that the toxic effects of the one are counteracted by the antitoxic effects of some other ion. Any disturbance in the right proportion of monovalent ions and ions of higher valency must lead to more or less pronounced modifications of the life phenomena.

[1] Loeb: This journal, 1901, v, p. 362, and Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 68.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 128.

A CONTRIBUTION TO THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF THE MEDUSA GONIONEMUS MURBACHII. PART I.—THE SENSORY REACTIONS OF GONIONEMUS.

BY ROBERT M. YERKES.

CONTENTS.

I. CHARACTERISTICS, DISTRIBUTION, AND HABITS OF GONIONEMUS.

GONIONEMUS MURBACHII, one of the Trachomedusæ, has four radial canals from which the gonads are suspended in irregular folds. The tentacles vary in number from thirty to eighty. They are usually not more than four centimetres in length, and each has near its distal end a viscid or suctorial body by means of which the animal is able to attach itself to objects. The portion of the tentacle distal to this body frequently makes a sharp angle with the other part and is much lighter in color. On the margin of the swimming bell, at the base of each tentacle, there is a body which appears greenish yellow when seen from the aboral side and brownish from the oral, whose function is supposed to be sensory. And between each pair of tentacles, rather irregularly situated with reference to them,

is a small oval sac, the lithocyst,[1] which contains a spherical body, the lithite. The lithocyst is thought to have to do with the orientation of the organism.

The manubrium is quadrate in cross section, with a short stalk. The lips, normally four, are very irregularly folded. When the animal is at rest the bell is almost hemispherical and has a marginal diameter of from one to two centimetres.[2]

According to Murbach, the species of medusa on which the studies of this paper were made was first noticed on the North Atlantic Coast at Wood's Hole in 1894, and it was described by him from this locality in 1895 (Murbach, :95). It was thought by him to be identical with G. vertens, described by A. Agassiz from the Gulf of Georgia, Washington, in 1862 (Agassiz, :62, p. 128). But recently differences in these two forms have been noted, which cause Mayer (:01, p. 5) to regard them as distinct species. He therefore proposes to give to the Wood's Hole species, which until a few months ago was known as G. vertens, the name G. murbachii, after Murbach, the describer.

Gonionemus murbachii has been found at various points on the Atlantic Coast, but it is by no means common. At Wood's Hole it occurs from June to October in a small pond connected with the harbor, the Eel Pond. Rarely it has been found elsewhere in the vicinity.

Apparently the most favorable habitats for these medusæ are quiet protected harbors or ponds which are affected by the tides, but whose currents are not sufficiently strong to tear the animals from their attachments and carry them seaward. Such places usually abound in vegetation, to which the animals are found attached, and have a rich food supply. In the Eel Pond there is a great deal of eel grass, and it is clinging to the leaves or roots of this grass that Gonionemus is most commonly found. Any disturbance in the water, such as stirring the grass with an oar or dip net, causes the animals to free themselves from the object to which they are attached, — either by the viscid bodies of the tentacles or by the lips of the manubrium, — and to swim to the surface. A convenient mode of capturing them is to disturb the water and then dip them up as they appear at the surface. Upon reaching the surface they at once turn over, the mouth of the

[1] This organ is often called the otocyst, but since this term is associated with the sense of hearing, for the existence of which there is no evidence in Gonionemus, it seems desirable to use lithocyst.

[2] For fuller description and drawings of Gonionemus, see Hargitt, :01, p. 294, and :01[a], p. 593.

bell thus becoming uppermost, and begin to sink by force of gravity. Under ordinary circumstances the medusæ are not seen at the surface of the water. It would appear, therefore, that they do not migrate upward in any definite way for the purpose of feeding or in response to light. Surface towing at night proves that there are few, if any, more at the surface then than in the daytime.

The food of Gonionemus consists of small fishes, crustaceans, larvæ of various kinds, and such dead organic material as comes within its reach. Often a Gonionemus is found attached by a few tentacles to a weed, with the exumbrellar surface of the bell against the weed and the manubrium swinging free in the water. It is evident that the animal can in this position seize food, if any chances to pass within reach. It seems probable that the restricted distribution of Gonionemus is due to the distribution of the food supply, for within the Eel Pond those regions in which the animals are abundant are frequently found to contain masses of decomposing organic matter. It should be remarked, too, that the Eel Pond receives a great deal of refuse during the spring and summer, and for this reason is probably a favorable habitat for Gonionemus.

II. Problems of Neural Physiology.

The physiological problems whose solution was sought in the following experiments naturally fall into two groups: (1) Those concerning the functions of the sense organs, and (2) those which have to do with the rôle of the so-called central nervous system.[1]

Chief among the problems of the first group are the following:

1. Has Gonionemus a sense of taste (*i. e.*, a chemical sense) distinguishable from the tactual sense?

2. If there is such a sense, where is it located?

3. Are all parts of the body equally sensitive to all forms of stimuli, and if not, what is the localization?

4. Do different qualities of stimuli call forth different kinds of reactions, or is intensity of stimulus alone significant?

5. Do any stimuli have a directive influence upon the movements of the medusa?

Of the second group may be mentioned:

1. Do the special reactions of parts of the medusa, such as the tentacles or manubrium, depend upon the central nervous system?

[1] This paper deals with the first group of problems only.

2. Is there any evidence of nerve centres?

3. What is spontaneity, and what is its relation to the nervous system?

4. Is coördination dependent upon the central nervous system?

5. Are there any evidences of the functional importance of the central nervous system?

III. Reactions to Stimuli.

A. To chemical stimuli. 1. *Foods.* — Since in nature the feeding reactions of Gonionemus are inseparably connected with reactions to mechanical stimuli, it is not possible to decide without careful study whether any particular reaction is a response to taste or to touch. Food is obtained by contact; the animals swim about and as soon as the tentacles touch an object of nutritive value they adhere to it and close around it. If the object be a living organism, the nematocysts are discharged for the paralyzing of the prey. The lips of the manubrium, probably by means of a secretion, hold food which comes in contact with them, thus enabling the manubrium to surround it. Hungry Gonionemi swim about almost constantly with their tentacles extended. Well fed individuals are more frequently found at rest with contracted tentacles. Obviously both locomotion and the extension of the tentacles increase the animal's chances of obtaining food.

An experimental study of the reactions of Gonionemus to chemical stimuli was begun by observation of the manner in which normal animals react to fish-meat. A small piece of fresh fish placed upon the tentacles causes a reaction which usually presents five fairly well marked phases: (1) Those tentacles that have been touched by the meat contract, twisting about one another in such fashion as to hold the food and carry it along with them; (2) the group of contracting tentacles bends in toward the mouth; (3) that portion of the margin of the bell bearing the contracting tentacles contracts, thus drawing the tentacles nearer to the manubrium; (4) the manubrium bends over toward the side from which the food is being brought, until finally the lips touch the food; and (5) the meat, adhering to the lips, is slowly surrounded by the manubrium.

If the piece of meat be placed near, instead of in contact with, the animal, there is at first no reaction; but after a few seconds the tentacles nearest the food begin to move about, and unless they happen

to reach the meat there soon follows a general contraction, or series of contractions, of the bell, which may take the animal either toward or away from the source of the stimulus. To all appearances the tentacle movements and the swimming are not definitely directed "food-seeking" movements, but simply motor reactions to a stimulus, — reactions, moreover, which cannot be distinguished from those in response to touch, light, electricity, and other stimuli. By the chemical stimulus of meat the medusa is aroused to activity, and usually in swimming about sooner or later comes in contact with the food. The stronger the stimulus, within limits, the quicker, more violent, and persistent the reaction. In these reactions to food at a distance, which evidently cannot be interpreted as "food-seeking" in the psychological sense, there is excellent evidence of a sense of taste.

In order to determine whether the food-taking reaction described is a response to all kinds of stimuli, or a specific reaction to nutritive substances, experiments were made with solutions of meats, acids, alkalies, and salts. In these experiments a Gonionemus was put into a two-inch Stender dish containing sea-water, and the stimulating substance applied to it with a capillary pipette. After each test the dish was washed out and refilled. Solutions of fish-meat were made by macerating fishes, allowing the mass to stand for an hour and then twice filtering.

To a strong solution of fish-meat applied to the tentacles the "food-taking" reaction is uniformly given. In order to be certain that the response was not due to mechanical stimulation, these experiments were checked by testing the animal's reaction to a current of sea-water from the pipette. To this stimulus, even though it were sufficiently strong to move the tentacles mechanically, there was seldom any more than a slight contraction of the tentacles in the region of the disturbance.

If a fish-meat solution to which the "feeding reaction" is regularly given be diluted, it will finally cease to call forth the complete five-phase reaction, and instead only the first, or first and second phases will appear. It is thus possible by using different strengths of the solution to get partial reactions.

Comparison of the reactions to food and to a current of water shows that quality of stimulus is of importance, and experiments with different solutions of fish-meat prove that intensity is also significant.

A drop of a solution of acetic acid ($\frac{n}{25}$ was used) applied to the

tentacles invariably causes their sudden contraction, and in most cases a subsequent contraction of the bell, which is frequently continued into a long swimming bout. The stronger the stimulus the quicker and more prolonged the reaction. Very weak acid causes only a slight local contraction of the tentacles. Other acids, alkalies, and salts produce similar reactions. These observations prove that foods cause a special "feeding reaction," which is not given normally in response to other stimuli.

Before finally deciding, however, that the "feeding reaction" is given only in response to nutritive substances, it is necessary to inquire whether some strength of almost any stimulus may not produce this result; in other words, whether the intensity of the stimulus, rather than the quality, is not the determinant of the reaction. With this point in mind, the reactions of Gonionemus to a series of strengths of HCl, KOH, NaCl, and several other chemicals were noted. In no instance were "feeding reactions," as in the case of foods, regularly given. Now and then a partial food-taking reaction appeared, but to no intensity of the chemicals tested was this given frequently enough to make it significant.

In the description of the "feeding reaction" mention was made of the peculiar twisting of the tentacles. This "corkscrew" movement is given in response to most foods, and especially to gelatine and meats. It is evidently serviceable for the holding of the food while it is being carried to the lips. That this twisting is a highly specialized reaction is proved by the fact that it is given only in response to foods and to "motile touch." To all other forms of chemical and mechanical stimulation, to light, and to electricity, the usual reaction is a straight contraction.

It seems well to consider here the effect of "motile touch" stimuli, applied to the tentacles, even though it does not naturally come in a section devoted to chemical stimuli. If the tentacles of an animal which is somewhat hungry be stimulated by quickly drawing a glass rod along them, they will suddenly twist about one another, just as when gelatine or meat is used. The twisting is usually followed by phases two and three of the "feeding reaction" (p. 437). Since this reaction is caused in so definite a manner by no other mechanical stimulus, there must be something in the character of the touch which determines the reaction. It seemed possible that the greater intensity of the "motile touch," as compared with an ordinary contact or pressure stimulus, might be the important factor; but com-

parison of the reactions to different kinds and intensities of stimuli proves that this is not true. Undoubtedly the stimulus given by a moving object is of vast significance in the life of Gonionemus, while simple contact, unless it be with food, is of less importance. For presumably the medusa lives in great part upon small, free-swimming animals which come in contact with the tentacles and whose capture is facilitated by a quick, twisting response of these organs. It would seem, therefore, as if this phase of the "feeding reaction" occurred in response to a particularly significant kind of tactual stimulus because of its importance to the animal. The reaction-time to "motile touch" is short as compared with that to food or to any other tactile stimulus, it being about 0.30–0.35 of a second as compared with 0.40–0.50. In the capture of rapidly moving animals speed of reaction is all-important; hence, the development of this unusually quick, special reaction to a particular kind of mechanical stimulation. Ordinarily the chemical sense determines that the tentacles shall twist in their response to foods, and this because the nutritive substance can thus be brought to the mouth. But if, in case of the "motile touch" given to Gonionemus by a passing animal, the twisting reaction of the tentacles were given only as a result of the chemical stimulus of food, the probability is that the prey would have escaped before the reaction could occur. A "motile touch" stimulus initiates the "feeding reaction" by calling forth the first phase; but unless it is supplemented by a taste stimulus later, the "feeding reaction" is not continued.

In the typical "feeding reaction" the manubrium bends toward the food. If during such a movement the piece of food be moved to the opposite side of the bell, the manubrium, too, in a few seconds will bend in the opposite direction, that is, again toward the food. The motor reactions of this organ are therefore definitely determined and directed by the source of the stimulus. Strong stimulation of any part of the bell usually causes the manubrium to point to the region of disturbance. This is a reaction which is probably to be explained on the basis of its value as a part of the "food-taking" activity; for again, as in the case of "motile touch," the stimulus usually indicates the presence of food, and the most serviceable reaction for the organism would seem to be a movement of the manubrium toward the stimulated area.

According to their effects upon Gonionemus, chemicals may be classified in three categories: (1) those that cause the special "feed-

ing reaction," which in its perfect form consists of five phases, any one or more of which may appear without the others; (2) those which call forth "locomotor reactions." The locomotor reaction may be preceded by contraction of the tentacles and by movements of the manubrium; but to all strong stimuli it is a quick, sharp contraction of the bell, which causes locomotion of the animal by forcing water out of the cavity of the bell. Weak stimuli often cause movements of the tentacles, which may or may not be followed by bell contrac-

TABLE I.

Reactions of Gonionemus to chemical stimuli.

Indifferent.	Feeding reaction.	Motor reaction.
Sugar [1]	Fish meat	Decomposing meats
Starch [1]	Crab meat	NaCl (10 n solution)
NaCl	Shrimp meat	KCl ($\frac{n}{4}$ solution)
CaCl	Clam	KOH
Filter paper	Beef	
Wood	Bread	HCl
Sand	Gelatine	HNO_3
		H_2SO_4
		Tannin
		Acetic acid
		Alcohol
		Chloroform

[1] Either starch or sugar may occasionally give partial feeding reactions.

tions. And, finally, (3) there are substances to the chemical influence of which the animals are apparently indifferent. Such substances may influence the organism, but they cause no visible reactions. According to their effects upon Gonionemus a number of substances that have been tested are classified in the accompanying table. It is of course uncertain whether wood, sand, and filter paper furnish chemical stimulation. But, however that may be, the table shows discrimination between foods and non-foods, and proves the presence of a sense of taste in addition to the tactile sense.

2. *To acids and alkalies.* — From reliable solutions of the chemicals used the desired strengths were made by adding distilled water. To solutions of hydrochloric, nitric, sulphuric, or acetic acids of $\frac{n}{100}$ or stronger, locomotor reactions were always given. Rarely partial feeding reactions resulted from acid stimuli, but in no case was a complete reaction noticed.

TABLE II.

Reactions of Gonionemus to different strengths of HCl and KOH.

Strength of solutions.	HCl.	KOH.
	Response.	Response.
1 n	Quick motor reaction.	Quick motor reaction.
2 $\frac{n}{25}$	Less violent motor reaction.	Less violent motor reaction.
3 $\frac{n}{50}$	Local reaction of tentacles, followed by motor reaction.	Slower motor reaction.
4 $\frac{n}{100}$	Delayed motor reaction.	Slower motor reaction.
5 $\frac{n}{150}$	Delayed motor reaction.	Contraction of tentacles, followed by motor reaction.
6 $\frac{n}{200}$	Contraction of tentacles, followed by motor reaction after a few seconds.	Contraction of tentacles, followed by motor reaction.
7 $\frac{n}{300}$	Contraction of tentacles, and delayed motor reaction.	Occasionally partial "feeding reaction," but usually motor reaction after delay.
8 $\frac{n}{400}$	Slight tentacle reaction. Motor reaction very slow.	Tips of tentacles alone react. Motor reaction to large quantity of reagent.
9 $\frac{n}{600}$	Tips of tentacles alone react. Motor reaction to large quantity of reagent in bell.	No reaction which could be certainly traced to the alkali.
10 $\frac{n}{1200}$	Indefinite reaction of tips of tentacles.	No reaction.

With a view to testing the limits of effectiveness of acids and alkalies, observations were made with a series of solutions of HCl and KOH. These solutions were made up with distilled water, but as this is itself a stimulus, the results of tests with these solutions were compared with those gotten with solutions made up with sea-water. In the experiments the solution was applied with a capillary pipette first to the tentacles and then, if there was no response, to the margin and bell. The results of these experiments are given in Table II.

Concerning the limits of sensitiveness Kahlenberg (:98, p. 17) states that $\frac{n}{800}$ HCl is slightly astringent to the human taste, and

Richards ('98, p. 122) claims that for sulphuric, nitric, and hydrobromic acids the limit of taste for man is about $\frac{n}{1000}$. The experiments with Gonionemus show reactions to $\frac{n}{1200}$ HCl. Kahlenberg is authority for the statement that man is unable to distinguish from distilled water $\frac{n}{800}$ KOH, while $\frac{n}{400}$ can be faintly tasted. Gonionemus is extremely sensitive to KOH, but the limit is a less dilute solution than that for acids.

In general it may be said that placing Gonionemus in solutions of acids from n to $\frac{n}{150}$ at first causes a marked acceleration of the rhythmic contractions of the bell. There is almost continuous swimming accompanied by movements of the tentacles and manubrium. If the animals are left in the solution for a few seconds, coördination is lost, and this is soon followed by paralysis of the entire organism.

3. *Localization of the chemical sense.*— Among animals related to Gonionemus the sense of taste and its localization have been studied by Pollock and Romanes, Nagel, Loeb, and Parker. Pollock and Romanes ('82, p. 474) studied the reactions of the sea anemone to chemical stimuli. They believed the reactions to be due to smell, but later investigations have proved this conclusion erroneous. Until 1891 it was generally believed that the tentacles contained the taste organs; Loeb ('91, pp. 69–70) then discovered that sea anemones without tentacles will accept food and reject other substances. From this he concluded that the tentacles are not the only bearers of taste organs. A year later Nagel ('92, p. 334) studied taste and its distribution in several actinians and found them all provided with a delicate and definitely localized chemical sense. According to him the tentacles are very sensitive to all stimuli, the edge of the mouth is insensitive and the localization is the same for taste, touch, and temperature. To settle a point of disagreement between Nagel and Loeb concerning the distribution of the organs of taste, Parker ('96, p. 107) made a careful study of Metridium. The results of his work agreed with Loeb's, in that a certain zone of the mouth region, in addition to the tentacles, is chemically sensitive. Parker was able to distinguish taste and touch in Metridium and to note specific reactions to foods.

An investigation of the localization of the chemical-sense organs of Gonionemus by means of stimuli given with a capillary pipette [1] en-

[1] Although chemicals thus applied diffuse rapidly, it is possible with care and numerous repetitions to get fairly accurate results. I used colored solutions in most cases, so that the diffusion could be watched.

ables me to arrange the important regions of the body in order of increasing sensitiveness, thus: (1) Exumbrella (insensitive); (2) Velum (insensitive); (3) Subumbrella; (4) Margin; (5) Manubrium; (6) Lips; (7) Tentacles (increasingly sensitive toward the suctorial body); (8) Tips of tentacles.

Of all regions the tips of the tentacles are by far the most sensitive. Their reaction-time is shorter than that of any other region, 0.40–1.00 second. The subumbrellar surface of Carmarina hastata is said by Nagel ('94, p. 517) to be absolutely insensitive to chemical stimuli and very sensitive to mechanical stimuli. This is not true of Gonionemus, for even after the margin of the bell and the whole of the manubrium have been cut away, so that a stimulus can affect only the subumbrella, contractions (beats of the bell) occur in response to stimuli. The velum and exumbrella of Gonionemus are quite insensitive to all chemicals. Even destructive acids may be poured upon them without causing any reaction.

B. To mechanical stimuli. — As it has been found convenient in describing the reactions to chemical stimuli to make frequent comparisons with the reactions to mechanical stimuli, it will not be necessary in this section to do more than describe the methods of experimentation and summarize the results.

A fine glass rod was employed to give *tactile* stimuli. With this, fairly accurate localization was possible. The tentacles, if touched lightly, contract independently, whereas the *tactile* stimulation of any other portion of the organism usually causes a "locomotor reaction." It is noticeable that the tentacles react much more quickly than the bell. This is probably because they contain within themselves the necessary mechanism of response, whereas the bell contracts only after the transmission of impulses to and from the nerve ring. Since transmission is comparatively slow in medusæ,— it being for Aurelia, according to Romanes ('85, p. 88), only 18 inches per second, it is probable that the slowness of response on the part of the bell is in part attributable to this cause. Weak tactile stimuli on the bell frequently cause no reaction, or they may be reacted to after two or three seconds' delay.

The localization of the sense of touch is similar to that for chemical stimuli, the only difference being that to chemicals the manubrium is more sensitive than the margin of the bell, whereas the reverse is true for mechanical stimuli. If anything, the subumbrellar surface is more sensitive to chemicals than to touch. In order of

increasing sensitiveness to touch, the parts, then, would be: (1) Exumbrella (insensitive); (2) Velum (insensitive); (3) Subumbrella; (4) Manubrium; (5) Margin of bell; (6) Tentacles (base); (7) Tentacles (tip).

Currents of water if locally applied cause some form of the "motor reaction," never the "feeding reaction." If the animals are forced to swim in a current, their tendency is to move against it—*i. e.*, a positively rheotactic reaction. The probable cause of this will be mentioned in the section on the directive influence of stimuli. Any disturbance in the water causes the animals to move about. This is a motor reaction whose significance would appear to rest on the fact that such disturbances usually indicate the presence of food, which random swimming movements may enable the medusa to capture.

Jarring, pinching, and all other forms of mechanical stimulation tried, invariably induce motor reactions. In the life of Gonionemus mechanical stimuli are of almost, if not quite, as much importance as chemical. The tactile sense enables it to obtain food just as often, it would seem, as the chemical. For higher animals mechanical stimuli serve as warnings of danger and frequently precede sudden motor reactions whose end is escape from the stimulus, but in the medusa the "feeding reaction" is fundamental, the escape from danger a secondary and relatively unimportant matter.

C. To photic stimuli. — The reactions of Gonionemus to light consist of (1) tentacle contractions, (2) movements of the lips and manubrium, and (3) contractions of the bell (swimming). The reaction-time to light is much longer than that to any other stimulus studied.

According to Berger (:00, p. 6), Gonionemus is active in "ordinary evening light," but strong light (electric) causes the inhibition of movement.

Romanes ('85, p. 39) has observed that Sarsia, one of the naked-eyed medusæ, is extremely sensitive to light. A flash of light usually causes one or two contractions of its bell. This is sometimes the case in Gonionemus, but it is not a predictable reaction, except to very strong light. Romanes proved the dependence of this reaction upon the presence of light, instead of the change from light to darkness or the reverse. The change from light to darkness, he claims (p. 40), is inhibitory of action. His statement is not very apt, however, for what we have in that case is merely the absence of any motion-producing stimulus. His further observation that Sarsia is

more active in light than in darkness holds also of Gonionemus. Furthermore, Gonionemus always settles down in a shaded region, — in other words, it is negatively photokinetic or photopathic.

When a number of the medusæ are placed in a glass vessel before a window they usually collect in the darkest region of the vessel. A simple test of this was made by putting a number of the animals in a dish having a bottom 16 × 10 inches and a depth of $3\frac{1}{2}$ inches, one-half of which was covered with a black cloth. By way of illustration, the results of one test were as follows: eight animals were put into the dish in the afternoon at four o'clock; within fifteen minutes all were in the light half of the vessel, and there they remained with some changes of position until nine o'clock in the evening. At seven o'clock the next morning only one was in the light region, and of the others several were attached to the sides and bottom of the dark region of the dish. Similar results were gotten with several lots.

Again, when Gonionemi in a glass collecting pail are disturbed by agitation of the water, they swim about rapidly and in a few minutes most of them are found on the more intensely illuminated side of the vessel. If, now, they are allowed to remain undisturbed for an hour, they will be found either equally distributed throughout the vessel or collected in the darker region.

There are here two questions to be answered. First, why do the animals at first come to the light? Secondly, why is it that they are later found in the shaded regions? The following statement of the relation of the motor reaction of Gonionemus to stimulation by light accounts for the facts. In ordinary daylight they are, *when swimming*, positively phototactic; in very weak light, on the contrary, they are not directed by the stimulus to any considerable degree, and therefore appear to be indifferent. They come to rest in an intensity of light which is below that necessary to direct their movements to any important extent and are therefore negatively photopathic. These statements may at first seem contradictory, but I believe they are not. It is known that some animals swim toward a source of light (*i. e.*, continue a positively phototactic reaction) until they are in an intensity of illumination far above that of their normal habitat, but, as soon as the effectiveness of the unusual stimulus wears off, or any combination of conditions destroys the directive influence of the light, they wander back to that intensity to which they are accustomed. It might be said, therefore, that they are positively phototactic to intensities of light to which normally they are nega-

tively photopathic. Such I believe to be the case for Gonionemus. Intense light directs its movements and forces it to go toward the source of the stimulus; but it comes to rest in relatively dark regions only.

D. The directive influence of stimuli. — Are the movements of Gonionemus definitely directed by stimuli? The observations which have been described enable us to classify the reactions of Gonionemus as follows.

A. Motor Reactions (Swimming).
  1. General reactions ("Locomotor reactions"), which are due to the stimulation of the organism as a whole.
  2. Special reactions, which are due to the stimulation of certain parts of the organism.
     a. Tentacle movements.
     b. Manubrium movements.
     c. Bell contractions, which result from a local stimulus of the margin or bell. These are directed.

B. Feeding Reactions.
  1. Tentacle reaction (twisting).
  2. Manubrium and lip movements.
  3. Margin and bell contractions.

Under *A* 1 come all movements that are not determined by local stimulation. They are such reactions as are caused by changes in osmotic pressure, or the chemical constitution of the medium in which the medusa exists. Under *A* 2 are classed all reactions to stimuli which affect only certain organs, or which stimulate symmetrical points of the organism unequally. All such stimuli have a directive value. For if one region only of the margin is stimulated, there occurs a quicker and more forceful contraction in that region than elsewhere; hence the body is given an impetus in a certain direction. If, for example, a strong chemical be applied to a portion of the margin, there follows a sudden contraction of the bell which carries the animal away from the stimulating substance. Observation shows that in case of harmful stimuli the movement is usually away from the side stimulated; the reverse is true for foods. Chemical stimuli evidently determine the direction of the movements of Gonionemus. From this it is probable that the chemical sense is of value in obtaining food at a distance from the organism. Mechanical stimulation is likewise directive in a similar way, as is clearly shown by the reactions to a touch on one side of the bell.

Light also, it would appear, directs the animal's movements by unequal stimulation of symmetrical points. It is impossible, because of the form of the medusa and its mode of locomotion, that the direction of its movements be as accurately determined by light stimulation as are those of certain Entomostraca, of the larvæ of some worms, and of other animals whose structure permits of more accurate orientation in reference to the source of light.

We are now in a position to say that Gonionemus neither seeks nor avoids things in the human sense of these terms. Its reactions are definitely determined by the quality, intensity, and location of the stimulus and not by the end to be attained. In general the quality of stimulus determines the kind of reaction to be given (whether motor or feeding, etc.); the intensity determines the quickness, duration, and extent of the reaction; the location of the stimulus determines the part or parts to react, and the direction of the movement. Food is found by movements which, although apparently fortuitous, because they are very imperfectly directed and determined by the unequal stimulation of symmetrical points in the body, are not wholly so.

IV. Summary.

1. Gonionemus has a delicate chemical sense.

2. All portions of the body, except the exumbrella and the velum, are sensitive to both chemical and mechanical stimuli.

3. The tentacles are the most sensitive portions of the organism to chemical, mechanical, and photic stimuli.

4. Gonionemus gives two important kinds of reaction to chemicals: (1) the "feeding reaction," to all nutritive substances; and (2) the "locomotor reaction," to substances which are harmful.

5. The kind of reaction given by the organism, or by any part of it, to a stimulus depends upon the quality of the stimulus.

6. Intensity of stimulation determines the quickness, duration, and extent of a reaction.

7. When chemical, mechanical, or photic stimuli affect symmetrical points of the body unequally, they have a directive influence upon the movements of the organism.

8. Gonionemus is positively photo*tactic* in daylight.

9. It is negatively photo*pathic* to daylight and to greater intensities of light.

It is a pleasure to acknowledge my indebtedness to Professors E. L. Mark and G. H. Parker for assistance in this investigation.

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THE LIBERATION OF VOLATILE SULPHIDE FROM MILK ON HEATING.[1]

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MILK is a highly complex liquid, and a great deal still remains to be known about the changes which occur in it under various conditions. Of special interest are the changes occurring in connection with the pasteurization and sterilization of milk for infants. It is well known that milk frequently contains micro-organisms in such varieties and numbers as to be injurious to children fed on it. On the other hand, it is claimed by many that the heating of milk to destroy the more dangerous bacteria also has a detrimental effect. This detrimental effect of heating must depend on changes in the composition of the milk.

Whether this transformation is due to a change in the composition of the casein, milk sugar, fat, lecithin, or other substances in the milk, or to several of these combined, is a matter of considerable interest. It has been often observed that after long boiling milk acquires a brown color, in all probability due to a partial decomposition of milk sugar. Again, the characteristic odor of boiled milk is in itself suggestive of chemical change; and further, the formation of a film on milk when the latter is heated, even at a temperature far below the boiling point, is significant of altered composition. Though some investigators (1) claim that this film is due merely to a process in which particles of matter carried to the surface with the milk fat become dried, the evidence[2] is strongly in favor of the idea that the formation of the film involves a chemical process as well as a physical one.

Baginsky (2) demonstrated by a series of experiments that in the complete sterilization of milk several changes occur. The casein is less readily precipitated by rennin, and its digestibility in gastric

[1] This research was carried on with the aid of an appropriation from the Rockefeller Institute for Medical Research.

[2] To be discussed in a subsequent paper.

juice is diminished. A change occurs also in the other phosphorus-containing substances; and finally, there is a partial decomposition of the milk sugar, and a slight loss in fat. According to Baginsky and others there is a decrease in the amount of organic phosphorus, and a corresponding increase of inorganic phosphorus.

Of special interest are the changes in the proteids of the milk, and it is with these that this paper deals. The decomposition of proteids by boiling alkali and acids, superheated water, etc., which many investigators have repeatedly demonstrated, is well established.[1]

The first intimation that the proteids in milk liberate sulphur compounds on boiling came from Niemann (9). In sterilized market milk which he obtained for physiological experiments, he observed the odor of sulphuretted hydrogen. During the analysis of the milk he found that on warming it colored lead acetate paper black. He further found that the sulphide occurred in considerable quantity: 3–6 mg. per 300 c.c. Of sixteen guinea-pigs fed with this milk, fourteen died in three to six days. It is very probable that this large amount of hydrogen sulphide was not due to mere sterilization of the milk, but to previous bacterial action, or to some other foreign influence. This suggestion is strongly urged by Biedert and Oppenheimer.

Very recently Oppenheimer (10) demonstrated that volatile sulphide is given off from milk on ordinary boiling. His report on the subject is very brief. Several flasks were partly filled with samples of milk. Strips of lead acetate paper were suspended in the necks of the flasks by means of cotton plugs, and the flasks heated in a boiling water-bath for different periods varying from five to forty-five

[1] By the action of acidulated water on casein, at one hundred degrees Centigrade, Chittenden and Meara (3) obtained caseoses and casein-peptone, which had the general properties of the bodies formed by the action of gastric juice on proteids. And recently E. Fischer (4) undertook a study of the decomposition products of casein obtained by concentrated hydrochloric acid, and demonstrated, among other things, the presence of amido-valerianic acid, α-pyrrolidine carbonic acid, and phenyl-alanin. Superheated steam has a similar action on proteids. Krukenberg (5), Neumeister (6), Salkowski (7), and others have obtained a number of decomposition products on heating proteids and albuminoids (meat, blood-fibrin, keratin, spongin, etc.) in water, at one hundred and thirty to one hundred and seventy degrees Centigrade. Among these were hydrogen sulphide, ammonium sulphide, peptone, tryosine, and leucine. Chittenden and Mendel (8) have shown that on heating a solution of hemp seed globulin to boiling, so as to bring about coagulation, the reaction of the liquid gradually changes, and becomes alkaline; due perhaps to a gradual decomposition of the globulin and the formation of ammonia.

minutes. Slight browning occurred on five minutes' heating. The browning increased with the heating, and in forty-five minutes the paper was colored intensely dark.

At the instance of the Rockefeller Institute for Medical Research, and with the assistance of Professor L. B. Mendel, I have undertaken an investigation of this subject, with a view to verifying the results of Oppenheimer, and making a more extended study of the subject.

Oppenheimer's experiment was repeated, and similar results were obtained. The heating was done in a tall water bath. In common flasks of 150 c.c. capacity were placed 100 c.c. of milk, and strips of lead acetate paper were suspended from the cotton plugs in the necks of the flasks, which were then put into the boiling bath. At different intervals the flasks were taken out and the paper strips removed. The milk used was fresh, as bacterial counts showed. During five minutes' heating, the lead paper was perceptibly colored. In ten minutes it became distinctly yellow-brown, increasing in intensity with the time of heating, until after forty-five to fifty minutes, when it became intensely black.

The intensity and rate of the coloring depend on the concentration of the milk, the reaction, the degree of heat, and the time of exposure. Fresh milk diluted with an equal amount of water required almost twice the time to blacken lead paper as the same milk undiluted; and with increased dilution the liberation of sulphide decreased proportionally. This behavior suggests that the blackening is necessarily due to the milk itself, and not to foreign substances.

The addition of weak alkali hastens the decomposition, while weak acids retard it. Samples of milk treated with ten cubic centimetres $\frac{n}{10}$ potassium hydroxide per one hundred cubic centimetres yielded much more volatile sulphide in a given time than the same milk without the alkali; and milk to which ten cubic centimetres $\frac{n}{10}$ sulphuric acid per one hundred cubic centimetres were added colored lead paper much more slowly than normal milk. Addition of more acid almost entirely prevented the coloration. The alkali milk was still acid to litmus, and the acid milk not acid to lacmoid. Addition of too much alkali, so as to render the milk alkaline to litmus, tended, of course, to hold the sulphide in solution to a great extent, as non-volatile alkali sulphide. Alkaline potassium phosphate (K_2HPO_4) also facilitates the liberation of sulphide; while acid potassium phosphate (KH_2PO_4) greatly retards it, and sometimes almost entirely prevents it. For example, five cubic centimetres of a four per cent

solution of acid phosphate per one hundred cubic centimetres of milk permit of very little blackening of lead acetate paper; and the same amount of alkaline phosphate (though much weaker as an alkali) quite perceptibly increases the production of volatile sulphide. Hence the reaction of the milk is of special importance in this decomposition.

Finally, the higher the temperature, the more rapid is the liberation of sulphide. The lowest temperature at which blackening of the lead paper occurs is eighty-five to eighty-six degrees Centigrade. Above this, the rate of decomposition increases rapidly with increase in temperature.

In order to obtain a more definite idea as to the amount of volatile sulphide liberated, an apparatus of the following description was devised (Fig. 1):

Fig. I.

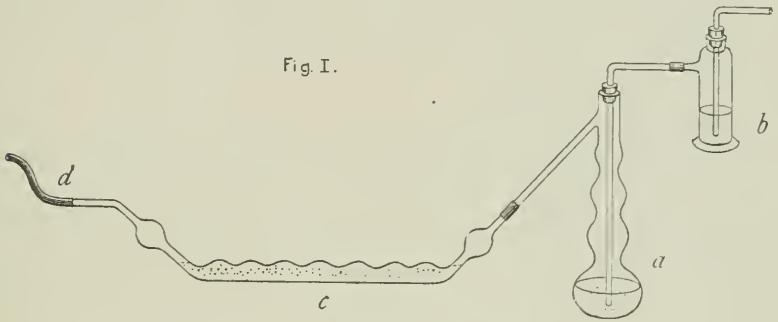

FIGURE 1.

A three hundred cubic centimetre Ladenburg distillation flask (*a*) is connected, on the one hand, with a wash bottle containing a strong solution of potassium permanganate[1] (*b*); and on the other, with an absorption apparatus (*c*) consisting of a glass tube with a bulbous upper surface (to facilitate absorption), and a bulb at each end. The horizontal portion of the tube has a capacity of ten cubic centimetres, and is filled with a very dilute solution ($\frac{n}{5000}$) of potassium permanganate. Two hundred cubic centimetres of milk are introduced into the flask, and the latter plunged into a boiling water bath. The passage of gas through the absorption tube is facilitated by connecting the end of the tube (*d*) with a suction pump, and aspirating cautiously. By regulating the amount of air drawn through, the time required to decolorize the permanganate can be fairly definitely determined, and comparative results very easily obtained. Thus,

[1] To free the air drawn through from substances that reduce permanganate solution.

Two hundred cubic centimetres of fresh normal milk decolorized the potassium permanganate in nineteen to twenty minutes.

The same amount of milk plus twenty cubic centimetres $\frac{n}{10}$ potassium hydroxide decolorized the solution in fourteen to fifteen minutes.

Two hundred cubic centimetres of milk plus twenty cubic centimetres $\frac{n}{10}$ sulphuric acid required thirty-five to thirty-eight minutes to decolorize.

Two hundred cubic centimetres of milk plus ten cubic centimetres of a four per cent solution of acid potassium phosphate required forty to forty-five minutes.

And one hundred cubic centimetres of milk, plus one hundred cubic centimetres of distilled water completely reduced the permanganate in thirty to thirty-four minutes.

No decolorization of the permanganate occurs at the ordinary room temperature, even after air has been drawn through the milk and tube for five hours. Hence the sulphide liberated on heating must be formed during the heating.

That the reduction of the permanganate is due to volatile sulphide and not to volatile fats and fatty acids, is indicated by the fact that the specimens of milk to which the largest amount of acid had been added, decolorized the solution least rapidly. And further, the blackening of lead acetate paper is an evidence that there is a liberation of sulphur.

The reaction of the samples of milk was as follows: ten cubic centimetres of milk required 1.5–1.8 cubic centimetres $\frac{n}{10}$ potassium hydroxide to make it neutral to litmus; while ten cubic centimetres of milk required 4.5–4.9 cubic centimetres $\frac{n}{10}$ sulphuric acid to make it neutral to blue lacmoid paper.

The older the milk, as a rule, and the more acid the reaction, the longer it requires to decolorize the permanganate. For example, a specimen of milk (two hundred cubic centimetres) which had stood exposed at room temperature for twenty-four hours required fifty minutes for the decolorization. In general, the fresher the milk, the greater the amount of sulphide liberated within a given time.

A third way of demonstrating the liberation of volatile sulphide from milk is by means of "lead cotton," a method which Habermann (11) employed in identifying hydrogen sulphide in tobacco smoke. This method consists in drawing the liberated gas through a glass tube containing dry absorbent cotton which has previously been soaked in neutral solution of lead acetate, and dried. A Ladenburg

flask (Fig. 2, *a*,) of three hundred cubic centimetres capacity is connected with a glass tube (*b*) which contains the lead cotton. To prevent condensation water from entering this tube a bulb (*c*) is suspended between the flask and tube. As in the previous method, the passage of the gas through the tube is facilitated by cautious aspiration. In order to regulate the current of air, the latter is bubbled through a wash bottle containing water (*d*) which is placed between the cotton tube and the suction pump. The air drawn through the milk is made to pass through a wash bottle (*e*) containing a strong solution of potassium permanganate.

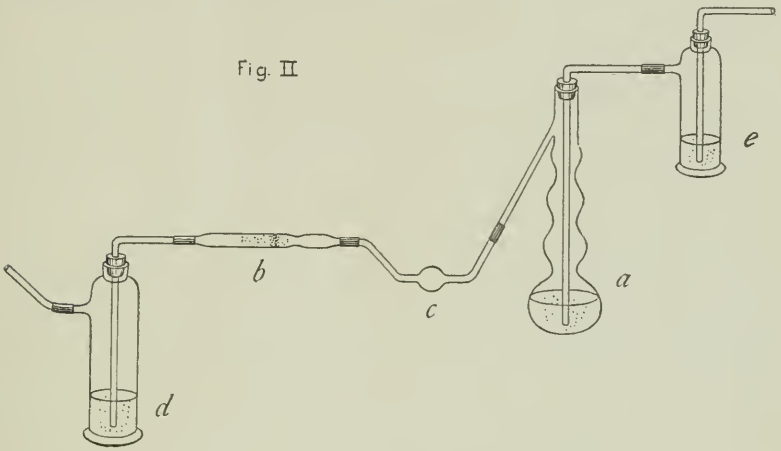

FIGURE 2.

Two hundred cubic centimetres of milk are introduced into the flask, and the latter plunged into a boiling water-bath. Within five minutes the lead cotton begins to blacken. In ten minutes an intensely black band is seen in the tube. The intensity and width of the band increases with the heating, so that after forty-five to fifty minutes the band in the small tube is fully half an inch broad.

By far the greater part of the blackening occurs within the first hour, while at the end of the second hour there is little change. This is a very satisfactory method for qualitative study, and is also of value in making comparative studies of different samples of milk, the intensity of the blackening and the width of the band affording an indication as to the amount of sulphide liberated.

Pasteurized and condensed milk deport themselves peculiarly towards heat, the amount of sulphide liberated being much smaller than with normal milk. Different samples of so-called pasteurized

milk required thirty to thirty-five minutes to decolorize the permanganate (ten cubic centimetres); and ordinary milk (two hundred cubic centimetres) which had been heated at sixty-five to seventy degrees Centigrade for one hour and cooled, did not completely reduce the permanganate in sixty minutes. Condensed milk[1] liberates very little sulphide on heating, even after the addition of small quantities of alkali.

This difference in the quantity of sulphur liberated is in part due to a change in the reaction of the milk during the pasteurization and concentration. Milk heated at sixty-five and seventy degrees Centigrade gradually becomes more acid; while when heated at about one hundred degrees it gradually becomes more alkaline, owing perhaps to small quantities of ammonia formed. But the slight increase in acidity is not sufficient to account for such a decrease in the amount of sulphide liberated. The idea that some rearrangement of the sulphur-containing molecule occurs, even at so low a temperature, and thus renders the sulphur more stable, suggests itself.

The proteids in the milk are the source of the sulphur given off on heating, the casein playing a particularly prominent part.

Purified commercial casein was dissolved in water with the aid of dilute potassium hydroxide, and filtered. The filtrate, containing from two to three per cent of casein, was acidified with acid potassium phosphate. Then a small quantity of alkaline phosphate was added, leaving the solution still acid to litmus. On heating this solution in a boiling water-bath, it liberated enough sulphide in forty-five minutes to color lead acetate paper distinctly brown.

Lactalbumin also gives off sulphide on heating. A fresh sample of milk was diluted with an equal volume of water, and the casein precipitated by means of dilute hydrochloric acid. To different portions of the filtrate containing the lactalbumin, varying quantities of alkaline potassium phosphate were added, to neutralize partially the strong acid reaction. On heating, volatile sulphide was liberated from the less acid portions.

Egg albumin was also found to give off sulphur in considerable quantity as volatile sulphide, on heating. This is a common reaction in hard-boiled eggs, in which the hydrogen sulphide odor often brings the freshness of the eggs into question.

As might be expected, pure cream does not yield more than a small

[1] Two different commercial brands were used.

amount of volatile sulphide, which comes from the milk proteids in it. And on the other hand, skimmed milk, when perfectly fresh, gives off more sulphur than whole milk.

Summary.

On heating normal milk above eighty-five degrees Centigrade a partial decomposition of the milk proteids occurs, which is indicated by the liberation of volatile sulphide, in all probability hydrogen sulphide.

The amount of sulphur thus given off is very small, but is sufficient in quantity to be easily recognized by the blackening of lead acetate paper and lead acetate cotton, and the decolorization of dilute potassium permanganate solution.

Alkalies and alkaline-reacting phosphates facilitate this decomposition, while acids and acid-reacting phosphates retard it. Hence the amount of sulphide liberated depends largely on the reaction of the milk.

What part this liberation of sulphide may take in rendering milk injurious is a question of practical significance. The reaction is in itself conclusive evidence that the milk suffers a change in composition. The important question arises: "Does sterilization and pasteurization render it injurious for prolonged use?" There is some evidence that scurvy in infants results from the use of milk sterilized by boiling. May this result be connected with alterations in the proteids, permitting the liberation of volatile sulphide?

In conclusion I wish to thank Professor L. B. Mendel for his valuable assistance in carrying out the work on which this paper is based.

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THE INFLUENCE OF TEMPERATURE, ODORS, LIGHT AND CONTACT ON THE MOVEMENTS OF THE EARTHWORM.

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Influence of Temperature.

ALLOLOBOPHORA FŒTIDA, the common manure worm, lives in damp soft soil, and in heaps of well-rotted or decomposing manure. In the laboratory the animals were kept in tin pails half full of manure, where they lived all winter in apparently perfect health and produced great numbers of cocoons and eggs. The average temperature of this manure was 18°C., a little below the room temperature.

When a number of worms were placed on a wet pane of glass, and the latter slowly warmed, the worms did not creep away from the warm place until it became uncomfortably hot to the finger, although when the heat was intense enough to cause the water on the plate to steam, the worms crawled rapidly away, and many died after a brief exposure to such temperatures.

Preliminary experiments to determine the effect of temperature were made with the following apparatus. A large sheet of glass twelve inches square was supported at two corners at a height of eleven inches above the table. It was kept sprinkled with water, and two thermometers were laid on it. An alcohol lamp stood on the table beneath the plate, and by raising or lowering this lamp, and by moving it under different portions of the glass, the temperature of the plate was varied as much as was necessary. The worms were placed in the centre of the plate either over the flame or at a little distance from the hottest area. If a number of worms are put down near each other they soon crawl together and coil into a heap. Such a heap of worms on the plate is not disturbed by a rise of temperature from 20° to 30° C. If the temperature of the plate rises above 30° C., where the worms lie, there is at first more active squirming, which changes to a streaming if the temperature reaches 33° to 34° C.

Individuals rarely separate from the heap, but the whole mass moves away from the heated area. If the flame is brought nearer while this streaming is in progress, the rate is greatly accelerated, until 36° to 37° C. is reached, while temperatures from 36° to 40° C. prove fatal.

These experiments are open to the objections that the worms are crawling over an unwonted surface, exposed to the air, and the apparatus was accordingly modified as follows. A half-inch layer of manure was spread between two plates of glass twelve inches square. The manure was moistened until it was very sticky and was spread evenly, but not pressed down. Three thermometers (t^1, t^2, t^3) were thrust into the manure, one at each of the free corners, and one in the middle. The apparatus was supported by two corners as before; an alcohol lamp under one free corner served to raise the temperature, and a dish of ice-water, with ice floating against the lower plate, to lower the temperature. Readings from the three thermometers were taken from time to time, and when there was a marked change of temperature the upper plate was removed and the manure was divided into three sections (each containing a thermometer) by transverse cuts with a ruler. The worms in each section were then counted.

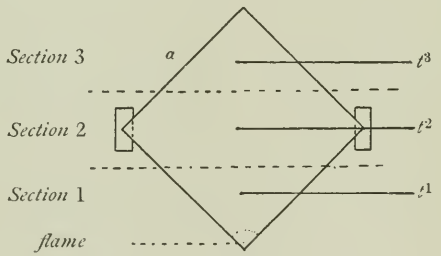

FIGURE 1.

Experiment 1. — The apparatus was that described above, heated at one corner (Section 1) for ninety minutes by an alcohol lamp placed just below the plate. Twenty worms were introduced into the manure at *a* (Fig. 1). The results are given in Table I. (The fact that t^1 registered a lower temperature at 12 M. than at 10.50 A. M. is due to the circumstance that the lamp burned out a few minutes before the cover was removed.)

Experiment 2. — Thirty-two worms were put into the manure in the centre of the plate. Below the middle of the lower plate was a dish of ice-water with a large lump of ice floating against the plate. The experiment continued until t^2 (Fig. 1) registered its lowest temperature and began to rise. The results are given in Table II.

TABLE I.

Section.	Temperature at 10.30. C.	Temperature at 10.50. C.	Temperature at 12.00. C.	Position of worms at 12 00
1	41°	51°	48°	0
2	25°	30°	34°	5
3	23°	28°	30°	15

TABLE II.

Section.	Temperature at beginning (10.25). C.	Temperature at end (11.40). C.	Position of worms at 11.40.
1	18.0°	19.0°	7 = 22%
2	17 0°	8.5°	12 = 37%
3	17.5°	19.0°	13 = 41%

Experiment 3. — Thirty-two worms were put into the manure over t^1 (Fig. 1). Under Section 1 was the flame, under Section 3 the lump of ice. The results are given in Table III.

TABLE III.

Section.	Temperature at beginning. C.	Position of worms at beginning.	Temperature at end. C.	Position of worms at end.
1	18°	32	70°	3 (dead)
2	20°	0	30°	21
3	20°	0	10°	8

If the heat is applied only to the tail, the worms crawl rapidly away in a straight line. When the head touches a hot place or object, the worm contracts sharply, turns aside or sometimes crawls backward for an inch or two. If the heat is too intense the worm squirms violently before crawling away. If the entire worm is laid on a warm place (30°–35°C.) the worm crawls as quickly as possible. That these

differences in reaction are due less to differentiation between the two ends of the body than to the fact that the animals habitually move forward rather than backward, and consequently if stimulated on the head end are obliged to progress in a less usual way to escape the stimulus, is evidenced by the following facts. Worms from which three, four or five anterior segments have been removed respond to the stimulus of heat like normal worms. If beheaded and normal worms are placed together on a hot area, they cannot be distinguished by their reactions.

Summary. — The preceding experiments point to the conclusion that a rise in temperature of ten degrees (*i. e.*, 18° – 28° C.) above that in which A. fœtida lives does not cause the retreat of the worms. A rise from 29° to 35° causes retreat, and violent squirming if the animal is retained in this temperature, while a rise from 35° to 40° is fatal, although such a temperature rarely causes progressive crawling. It is interesting to note that when a number of worms are coiled together, they respond not as individuals, but as a whole, the movement resembling that of a single organism.

Influence of Odors, and Effect of Drying.

In his book on "The Formation of Vegetable Mould by the Action of Earthworms" Darwin describes in the section on "Habits," some experiments in which he tested the sense of smell of earthworms. From their finding bits of food buried in the soil at a distance from their burrows, he concludes that earthworms possess the sense of smell in a slight degree. The behavior of Allolobophora fœtida when accidentally dropped near a pile of wet fresh sawdust led to some experiments with cedar oil, turpentine, ether, and similar substances. If a glass rod dipped in cedar-oil is held at a distance of one quarter of an inch from the head, there is usually a slight recoil, although the reaction is not constant. Xylol similarly held one quarter to one eighth of an inch from the head causes a slight recoil, and if the stimulation continues the worm turns and crawls away. Turpentine causes a similar recoil and change of direction. Either cedar-oil, xylol, or turpentine, if allowed to touch any part of the body, causes a violent squirming and the production of the offensive-smelling yellow secretion to which A. fœtida owes its name. At the same time the worm retreats as quickly as possible; if touched on or near the head it squirms backward for a short distance before turning aside, but if

touched on the tail-end, it crawls forward at once, squirming actively at the same time. A drop of ether on a glass rod held one quarter of an inch from the head produces a marked reaction. The worm recoils instantly and crawls quickly backward, usually accompanying this retreat by lifting fifteen or twenty anterior segments into the air and waving them about. If the worm is pursued with the rod, violent squirming results, and by quickly coiling and uncoiling, in opposite directions, the animal springs clear from the paper several times in succession. If the rod is touched to any part of the worm the squirming and springing are at first very violent, but after several springs the worm stiffens. When these substances are held near the tail (provided the animal is not bent so that the head also is affected) there is either a faint response or none. In this respect there is a slight individual difference.

When these worms are removed, for a longer or shorter time, from the manure in which they live, and are then returned, they at once burrow into the manure. When placed on soil with no admixture of manure, they crawl over the top for a time before burrowing. If removed from the manure to wet filter paper or some similar damp surface, they can crawl over it for an hour without exhibiting any sign of discomfort[1] except restlessness, which is probably due to the unwonted light. If, however, they are placed on a dry surface, the skin becomes dry in a few minutes, the worms crawl more and more slowly, and frequently lift half the body into the air, waving it from side to side with a peculiar rotary motion. At the end of two or three minutes the irritation has become so great that the worm squirms violently, produces the offensive odor, and after several springs it stiffens. If returned to moisture at this time the worms recover. If they are allowed to remain dry, one or two additional springs are made, the animals becoming stiffer after every such effort, and death soon follows. The early stages of this drying up are easily recognized by the "tight" appearance of the skin and the apparently unwarranted irritability, for at this period the slightest touch produces violent squirming.

The sensitiveness to drying led to some experiments to determine

[1] In using words like "discomfort" and "choice," which occur throughout this paper, it is perhaps necessary to state expressly that no psychic element is thereby introduced. The words are used to avoid long and awkward circumlocutions, and with this understanding furnish a readier, simpler, and more direct vocabulary for describing the reactions of these animals than more exact but longer expressions.

whether the animals perceive moisture at a short distance from the body, and whether any factor other than contact is involved in their burrowing. A twelve-inch square of filter paper laid on a plate of glass was made very wet in the centre. In a few minutes there was a wet spot, surrounded by a damp ring, with an outer margin of dry paper. When worms were laid on the dry paper and allowed to move freely they crawled around and around the wet spot with its damp ring until the skin dried, but unless the head and a few segments crossed the edge, they never crawled into the wet places. They even crawled along the edge of the damp ring without turning into it. But if the worm once crossed the edge of the damp region it crawled in at once, and remained in the wettest part, even when exposed to full sunlight.

The filter paper was now completely moistened, and in the centre was a little heap of tiny shreds of wet filter paper. The object of this arrangement was to provide a wet heap into which the worms could crawl if contact and moisture alone stimulated them to burrow. When laid on the paper the worms crawled around the heap as before. If their course accidentally brought them against the heap, they crawled over it without burrowing. In one case a worm burrowed a short distance, then withdrew and crawled over the top. The heap of paper was then moistened with a decoction of the manure in which the worms lived, instead of with tap-water. The results showed an interesting variation. The worms were no more apt to find the heap than before, but if they did find it, they burrowed at once and remained there. Seven out of ten burrowed at once, and only one left the heap after entering. A heap of wet sawdust was now substituted for the paper, but the worms turned aside from this as soon as the head came in contact with it. They not only did not enter, but did not crawl over the heap, except in one case out of ten. Finally a small pile of manure was put in the centre of the plate, and the worms were allowed to crawl around it. As in the other cases, there was no indication that the manure attracted the worms from a distance, for unless gently headed toward it by the experimenter they crawled by it even when very close. One worm turned aside just before reaching the manure, and skirted the edge in such a way that in the middle of the body the back touched the manure, and still the worm did not enter the heap. But if the head touched the manure, or any outlying fragment thereof, the worms burrowed at once, and remained in it until removed. When the tail was in contact with manure I saw no tendency to burrow,

although when worms in manure are disturbed, they travel through it forward or backward indifferently.

Summary. — The above experiments indicate that while these animals are not stimulated by certain odors at a distance from the body, they still possess a sense of smell. In this respect there is a difference between the anterior and posterior ends of the body, the anterior end being more sensitive. This sense is one of the factors in the burrowing of A. fœtida, contact and moisture alone being insufficient to call out the burrowing reaction.

Directive Influence of Light.

Preliminary experiments to determine the reactions of A. fœtida to light were made in the following ways.

Worms were laid on a piece of wet filter paper lighted mainly from one side, and with screens on the other sides to cut off most of the extraneous light. Under such circumstances the animals generally crawled to the darker side of the paper. A tin box, two inches in height, two inches wide, and eighteen inches long, painted black inside and having one glass end, was next used. Wet filter paper covered the floor of the box, and the entire apparatus except the glass end, which faced a sunless window, was covered with black oilcloth. A worm was placed on the filter paper, its length across the box, and the apparatus was covered. After two minutes the cover was lifted and the position of the worm noted. Of twenty experiments made in this way, fifteen showed in general a negative reaction, that is, the worm had moved to the darker end of the box, and away from the light, while five reactions were positive. This method is objectionable practically because one cannot watch the worm, and theoretically because the narrowness of the box inevitably brings the worms against one side, and introduces a new factor, thigmotaxis. The apparatus was therefore abandoned.

In these preliminary experiments the most marked features were the absence of regularity of response and a certain individual difference in reaction. Therefore I isolated fifteen large, normal, apparently healthy worms, keeping each in its own dish of manure, and distinguishing them by letters. An attempt was made to have this number include two series of worms, one sexually active and the other not active, using the swollen clitellum as the criterion. But this phase appears so suddenly and passes so quickly in individual worms, that it was found impossible to conduct long series of experiments on

worms in the sexual condition. Three sets of preliminary experiments were made on each of these fifteen worms. A sheet of wet gray filter paper eighteen inches square was placed on black oilcloth, giving a dark surface. The oilcloth lay on a table in front of a sunless window, and light from the other sides was carefully cut off by black screens. The worm to be experimented on was taken from the manure with as little disturbance as possible, an ordinary flexible section lifter serving the purpose better than forceps, and laid carefully on the filter paper. It was placed in the middle so that the head and more than half the length of the body were either (a) across the paper parallel to the surface of the window, or (b) pointing toward the window, or (c) pointing away from the window. In these three sets there was no attempt to make a definite number of experiments on each of the three directions, but we endeavored to see how the worms behaved when headed once and then left free to crawl. A "set" refers to all the movements made by one worm during one experiment, and not to a succession of experiments in which the worm was headed the same way each time. Each worm was allowed to crawl freely for ten minutes in Set 1, and for five minutes in Sets 2 and 3, and the results recorded by drawing the course of the worm on a reduced plan of the apparatus, with marginal notes where they were necessary to explain the sketches. Whenever the worm entirely left the filter paper, it was replaced carefully, and this new position indicated as the beginning of the second, third, or fourth advance in that set, by the appropriate numeral. The worm was usually replaced in or near the centre of the plate, in approximately the same relative position in which it had just crawled away; that is, if a worm crawled over the back edge, it was replaced in the centre with the head toward the back. If in crawling the worm partially left the paper, then returned tail first and assumed a new direction by turning sharply aside, that position was similarly indicated as the beginning of an advance.

Under such circumstances worms differ greatly in their manner of crawling. Some move deliberately, with much testing of the regions around the head. Others crawl quickly and "impetuously," leaving the wet filter paper for the dry oilcloth without seeming to appreciate the difference in surface, although the oilcloth soon dries their skins. Some begin to crawl rapidly the instant they are placed on the filter paper, but soon reduce their speed; others contract when first put down, and are quiet for some seconds (15–60) before crawling, but when they move, turn at once in a definite direction and crawl with

increasing speed in a straight line. Most of the worms move the head in various directions before beginning to crawl, as if exploring and "testing" the neighborhood, and it was not at all common for the first movement of the head to be in the direction in which the worm ultimately crawled. With such differences in habit it follows that there is a varying number of advances for each worm, for the duration of the experiment, and that the records are widely different. Four entire records are given, which represent the extreme variations in response.

Worm C. Set 2. March 8, 1901. Sluggish individual. When put down, contracted, lifted head slightly, moved head against paper and was still sixty seconds. Turned head, crawled slowly with many stops toward back; moved more quickly when body was straight. At edge of paper it paused, explored, half left paper and withdrew to it; turned on itself and crawled toward front across paper. Half-way across it turned to left, crawled to edge of paper, withdrew once from oilcloth, then crawled off toward back on oilcloth. It withdrew tail first to filter paper, turned and crawled toward back.

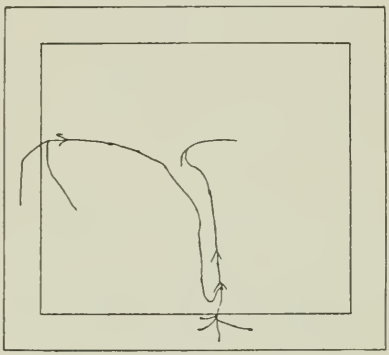

FIGURE 2. — Worm C, Set 2.

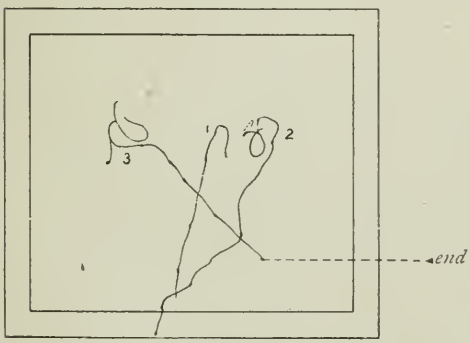

FIGURE 3. — Worm K, Set 2.

Worm K. Set 2. March 9, 1901. Worm turned and crawled at once, going quickly over back edge. When replaced at 2, turned its head to the left and remained quiet ten seconds; turned toward back, crawled (more slowly than before) in diagonal line and went over edge. Replaced at 3, turned head at once but slowly toward back and crawled diagonally back.

Worm O. Set 2. March 9, 1901. Worm quiet twenty seconds, then crawled quickly forward diagonally to right. Left paper without recoil, crawled more slowly on the oilcloth, moving toward front and then to left, and finally crawled on the filter paper again. Crossed filter paper and

left it by left back corner. Replaced at 2, worm coiled, crawled toward front, left paper by front edge, turning slightly to right on the wood.

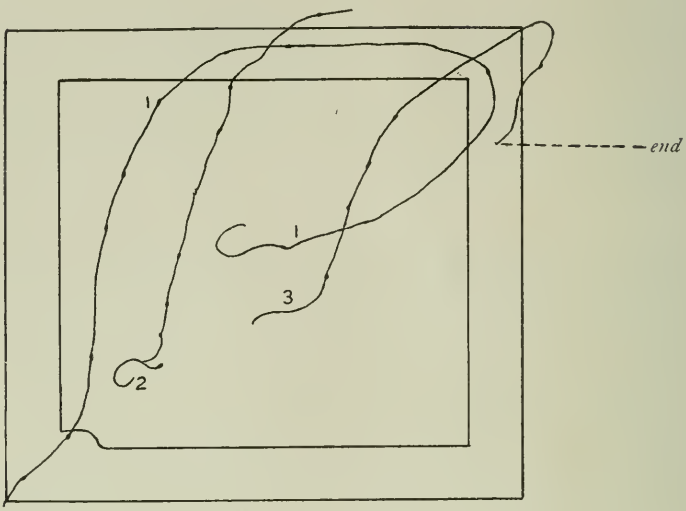

FIGURE 4.— Worm O, Set 2.

Replaced at 3 it crawled diagonally to front, crawled out on wood, keeping tail on filter paper. After twenty seconds it withdrew, crawled toward back on narrow strip of exposed oilcloth.

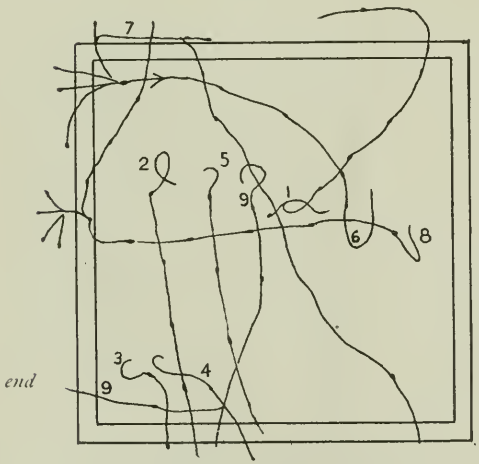

FIGURE 5.— Worm H, Set 1.

Worm H. Set 1. March 7, 1901. Worm moved head at once lifting fifteen segments; crawled quickly to right front corner, and left filter paper for wood without recoil. Replaced at 2, it crawled straight toward back and over edge with one recoil. Replaced at 3 and at 4, it went over the back edge at once; replaced at 5, crawled to back edge, recoiled from wood, turned to left and crawled over left edge without recoil. Replaced at 6, it was quiet ten seconds, crawled quickly toward front, bending to left, and at left front corner touched wood and recoiled four times. Crawled backward, turned to right and moved slowly along front edge of paper (= beginning

of advance 7). Short pause, then it crawled diagonally across paper to right back and left plate without recoil. (Its course was deflected near centre of plate by small bit of manure.) Replaced at 8, worm crawled straight forward across plate, explored wood and recoiled four times, turned toward front and over edge after three recoils.

The results of the preliminary experiments are given in Table IV.

From the detailed results quoted, and from Table IV, the following conclusions are apparent.

1. The position in which the worm is placed on the paper is a factor in determining the direction in which the worm crawls. Thus the table shows that 79 per cent of the advances are positive when the worms are headed toward the window (col. 2), while when started away from the window (col. 3), 82 per cent are negative. When the worms are placed parallel to the surface of the window (col. 1) 17 per cent of the movements are indifferent (*i. e.*, directly forward) as compared with 2 per cent indifferent from the positive position and none from the negative position (col. 3).

2. When placed in the indifferent position, the worms are more likely to crawl away from the window than toward it. Thus of fifty-nine responses in column 1, thirty-six (61 per cent) are in a generally negative direction, thirteen (22 per cent) are generally positive and ten (17 per cent) are indifferent. The negative movement includes more than half the entire number in the first column, and, speaking generally, A. fœtida reacts negatively to light stimuli under these conditions.

3. When A. fœtida reacts to light it is more likely to crawl in a diagonal than in a straight or direct path. If crawling positively it is more likely to crawl diagonally than directly. A series of "diagonal" paths necessarily includes many different angles, and theoretically the line of a diagonal advance may lie anywhere between the indifferent path, parallel to the surface of the window, and the exactly negative or positive path at right angles to the former. As a matter of fact, the diagonal advances do lie at almost every angle.

4. Although *in general* A. fœtida exhibits under these conditions a negative response, there is neither constancy nor regularity in the reaction, and it is impossible to predict with certainty whether a given worm placed on the paper in the indifferent position will crawl negatively, positively, or indifferently.

The great irregularity of response in the preliminary experiments

TABLE IV.[1]

Preliminary experiments on lettered worms. Sets 1, 2, 3.

Worm.	Worm headed across plate (‖ to window).	Worm headed +.	Worm headed −.
A	−, ‖−, ‖	‖+	
B	‖+, ‖−; +, ‖−	‖+; ‖+, ‖+,‖+	−; −
C	−; −	+; ‖−	−
D	‖; +; ‖−, ‖−	+; ‖+	‖−
E	+, ‖, ‖+, ‖−; ‖−; ‖ −, ‖−, ‖+	‖; +	‖+, −, −; +
F	‖, ‖+, −; ‖−, ‖+; ‖−	‖+, ‖+; ‖−; +	−
G	−, ‖−, ‖−; ‖+; ‖−	‖+; +; +	−
H	‖+; −, ‖−	‖+, ‖+	−, −, −, −, ‖−
I	‖−; ‖−	+; +; ‖+	−; −, −
J	‖−, ‖−; ‖−; −	‖; ‖+	−; −
K	‖	‖+; −, −, −; +	
L	‖−; ‖; ‖−, ‖, ‖	‖+; ‖+	
M	‖−, ‖	−; −	
N	+, −, −; ‖; −, −	+; +	−
O	‖+, ‖−, −, ‖+	‖+, +, +, ‖+, ‖+, ‖+, +	‖+, ‖+
	+ = 4 ‖− = 24 − = 12 ‖+ = 9 ‖ = 10 Generally − = 36 = 61% Generally + = 13 = 22% Indifferent 10 = 17% 59 = 100%	+ = 14 ‖− = 2 − = 5 ‖+ = 20 ‖ = 2 Generally + = 34 = 79% Generally − = 7 = 16% Indifferent 2 = 5% 43 = 100%	+ = 1 ‖− = 2 − = 17 ‖+ = 3 ‖ = 0 Generally − = 19 = 82% Generally + = 4 = 17% 23 = 99%

SUMMARY OF ALL REACTIONS IN PRELIMINARY EXPERIMENTS.

Generally − = (36 + 7 + 19) = 62 = 50%
Generally + = (13 + 34 + 4) = 51 = 41%
Indifferent (‖) = (10 + 2 + 0) = 12 = 9%
125 = 100%

[1] In this and all following tables of light reactions, the same abbreviations are used. + indicates a movement directly toward the source of light; − a movement directly away from the source of light; ‖ a movement parallel to the surface of the window; ‖+ a diagonally positive movement to right or left; ‖− a diagonally negative movement to the right or left.

led to the adoption of the following apparatus (see Fig. 6). A tin box, eight inches wide, four inches deep, and twelve inches long, which was painted black inside and had one end of glass, was faced against a sunless window opposite which were no buildings. The floor of the box was covered with wet gray filter paper. On this were placed thick microscopic glass slides arranged to form two narrow paths of the same width as a worm's body, one running parallel to the glass end, the other at right angles to the first and communicating with it. The slides were almost or quite as thick as the diameter of the worm, and the paths were widened or narrowed according to the size of each individual worm. The slides were then covered with a glass plate, which completed the fourth side of the paths. This device provided narrow moist passages, and had the further advantage of cutting off currents of air, and preventing undue drying. The method of using it was as follows. A worm was taken up with the section-lifter, and placed carefully on the filter paper with the head end lying in the indifferent path (‖). The glass cover was quickly lowered with as little shaking as possible, and a thick black cover was drawn over the head of the observer and over the entire apparatus except the glass end. Light was thus cut off except from the front. Preliminary experiments showed that illumination of the right side of the worm produces the same effect as illumination of the left. For convenience' sake, therefore, the worms were introduced from the right, and consequently illuminated from the right side except in Set 8. A worm introduced into a narrow chink like the paths in this apparatus is almost certain to crawl forward. It is obvious that when the worm reaches the end of the indifferent path it is forced to crawl either directly front[1] or directly back, and a record of the path "chosen" was made simply as + or −. The worm was allowed to crawl entirely out of the indifferent path before the record was made. Five experiments (that is, five choices) were made in a set, that number being selected to avoid the danger

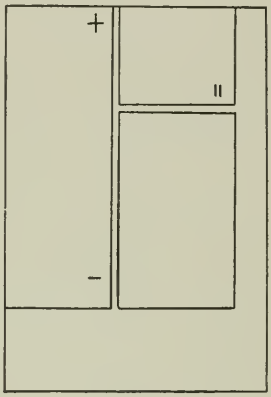

FIGURE 6.

[1] The words "front" and "back" refer always to the light and dark ends of the box respectively, while "forward" and "backward" refer to the manner of crawling, head first or tail first respectively.

of fatigue, and five sets were made for each worm (Set 4, Set 6, Set 8, Set 10, Set 12). In Set 4, the results of which are given in Table V,

TABLE V.

Worm.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Total.
+	0	0	0	1		0	0	0	0	1	0	0	0	1	0	3
−	5	5	5	4		5	5	5	5	4	5	5	5	4	5	67

record was made only of the path chosen. It was noticed during these experiments, however, that while the worms sometimes crawled immediately into one of the paths, there was frequently a hesitation, and a testing of the two paths, before a decision was made. In some cases the worms crawled so far forward into the plus path that half the body lay in that direction, then contracted, crawled backward into the indifferent path until it was possible to turn back and finally crawled through the negative path. Therefore a third reaction was recorded with ± indicating that the worm first turned into the plus path and later into the minus path. Theoretically there should be a fourth reaction indicated by ∓ but this reaction was never observed. The results of these four sets of experiments are given in Table VI.

An examination of Table V shows that sixty-seven reactions out of seventy (96 per cent) are negative. Out of two hundred and sixty-five reactions given in Table VI, two hundred and fifty-one (95 per cent) are negative. Under these conditions, then, A. fœtida exhibits a well marked and undoubted negative response. But the number of negative reactions includes those in which there was preliminary investigation of the plus path, as well as those in which the worm turned unhesitatingly into the minus path. And these two groups stand in the relation of eighty-nine to one hundred and sixty-two ($33\frac{1}{2}$ per cent to 61 per cent). The worms turned into the plus path one hundred and three times (columns + and ±), although the actual number of positive advances is only fourteen.

In this group of experiments the worms were purposely forced into a "choice" between the directly positive and directly negative positions. When so forced, the species exhibits a negative reaction, and in the majority of cases turns down the negative path like a

moving diagram of an elongated animal illuminated from one side. After watching this reaction, however, it seemed to me that if the worm had not been so forced into one of these two channels, the

TABLE VI.

Ind.	Set 6.			Set 8.			Set 10.			Set 12.			Summary.		
	+	±	−	+	±	−	+	±	−	+	±	−	+	±	−
A	0	0	5	0	1	4	0	5	0	0	3	2	0	9	11
B	0	0	5										0	0	5
C	0	0	5	0	1	4	0	2	3	0	1	4	0	4	16
D	0	4	1	2	2	1	0	0	5	0	2	3	2	8	10
E															
F	0	2	3	1	2	2	0	3	2	0	1	4	1	8	11
G	0	2	3	0	0	5	0	0	5	0	1	4	0	3	17
H	0	2	3	1	3	1	0	3	2	0	1	4	1	9	10
I	0	2	3	0	2	3	0	4	1	0	1	4	0	9	11
J	1	1	3	0	3	2	1	1	3	1	1	3	3	6	11
K	0	0	5	0	1	4	0	2	3	0	1	4	0	4	16
L	1	2	2	0	0	5	0	3	2	0	0	5	1	5	14
M	3	0	2	0	5	0	0	0	5	0	1	4	3	6	11
N	0	3	2	1	3	1	0	3	2	1	1	3	2	10	8
O	0	2	3	1	3	1	0	2	3	0	1	4	1	8	11
												Totals	14	89	162

$+ = 5\%$ $\pm = 33\frac{1}{2}\%$, $- = 61\frac{1}{2}\%$ $= 95\%$

reactions would have been less definite. With this in mind the apparatus was modified so that only the indifferent path remained (Fig. 7).

The worm was introduced as before, and since it crawled through the indifferent path was illuminated from one side for its whole

length. But when it reached the other end of that path it was free to crawl in any direction. The great number of possible directions were grouped under five divisions, the same as those used above in describing the preliminary experiments. Fig. 7 shows the range of each of these signs. If the worm as it left the indifferent path turned at right angles to that path, the reaction was recorded as a simple plus or minus, whether the turn was made as soon as the worm issued from the path (and consequently when the worm continued to crawl against a glass side), or at some little distance from that path. Experience showed that such a sharp turn was always made at or near the end of the indifferent path. With the exception of Set 13, the record was made in each case simply by the use of the appropriate sign, after the worm had entirely crawled out of the indifferent path. The results of Set 13 were recorded differently (in a manner to be described below), although the same signs were used. As in the preceding group, all starts were made from the right except in Set 9. The results of the experiments with this apparatus are given in Table VII.

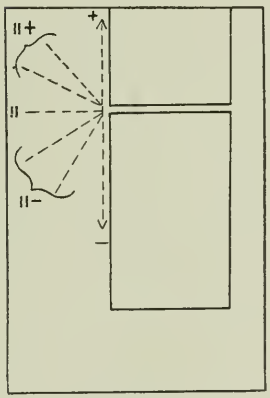

FIGURE 7.

It is to be noticed first that out of three hundred and twenty-five experiments, none are directly plus, and only seven are directly minus. Sixty-eight advances (21 per cent) are continuations of the indifferent path, a high proportion when one considers the tendency of this species to be deflected from a straight course by objects with which it comes in contact.[1] Twenty (6 per cent) are diagonally plus, while a majority of all the reactions are diagonally minus, — two hundred and thirty out of three hundred and twenty-five (71 per cent). Adding the directly negative and diagonally negative, there is a total of two hundred and thirty-seven generally negative reactions, against twenty in a generally positive direction. It thus appears that under these conditions, with the course not determined, A. fœtida has a negative reaction.

It is to be particularly noticed, however, that of the two hundred and thirty-seven negative responses only seven are directly away from

[1] See section on Thigmotaxis.

TABLE VII

Worm.	Set 5.					Set 7.					Set 9.					Set 11.					Set 13.					Summary.				
	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−
A	0	0	1	0	4	0	0	1	0	4	0	0	2	0	3	0	0	1	2	2	0	0	1	0	4	0	0	6	2	17
B	0	0	2	1	2																					0	0	2	1	2
C	0	0	3	0	2	0	0	2	0	3	0	0	0	0	5	0	0	2	0	3	0	0	0	0	5	0	0	7	0	18
D	0	0	2	0	3	0	0	3	0	2	0	0	3	0	2	0	0	0	2	3	0	0	4	0	1	0	0	12	2	11
E																														
F	0	0	2	0	3	0	0	2	0	3	0	0	0	0	5	0	0	2	0	3	0	0	2	0	3	0	0	8	0	17
G	0	0	1	0	4	0	0	0	0	5	0	0	2	0	3	0	0	0	0	5	0	1	1	0	3	0	1	4	0	20
H	0	0	0	0	5	0	0	0	0	5	0	0	1	0	4	0	0	1	1	3	0	0	0	0	5	0	0	2	1	22
I	0	0	0	0	5	0	0	0	0	5	0	0	2	0	3	0	0	1	0	4	0	0	0	3	2	0	0	3	3	19
J	0	0	1	0	4	0	0	2	1	2	0	0	0	0	5	0	0	1	1	3	0	1	0	0	4	0	1	4	2	18
K	0	0	1	0	4	0	0	1	2	2	0	0	1	0	4	0	0	0	0	5	0	0	1	0	4	0	0	4	2	19
L	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	1	1	0	3	0	1	1	0	23
M	0	0	0	1	4	0	1	0	0	4	0	0	0	3	2	0	0	2	0	3	0	1	1	1	2	0	2	3	5	15
N	0	0	1	0	4	0	0	2	0	3	0	0	1	0	4	0	0	3	0	2						0	0	7	0	13
O	0	0	1	1	3	0	1	0	0	4	0	0	2	0	3	0	0	0	0	5	0	1	2	1	1	0	2	5	2	16
Totals	0	0	15	3	52	0	2	13	3	47	0	0	14	3	48	0	0	13	6	46	0	5	13	5	37	0	7	68	20	230

the light, the remaining two hundred and thirty being more or less diagonal. The position, *i.e.*, the angle of these diagonal responses varied so much that in recording Set 13 a new method was adopted. The direction of the worm, as it issued from the indifferent path and

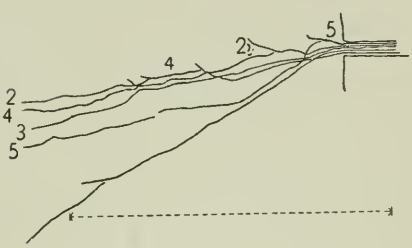

FIGURE 8. — Worm D, Set 13.

Record:

1. ||— 2. || 3. || 4. || 5. ||

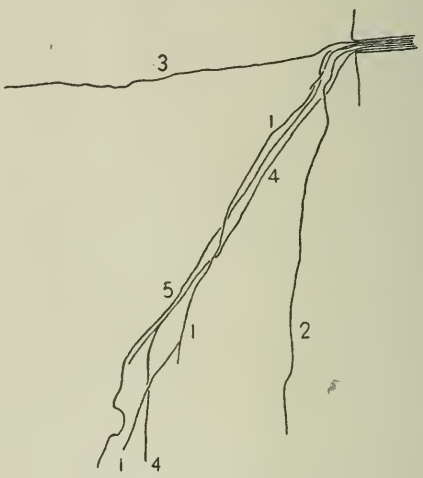

FIGURE 9. — Worm G, Set 13.

Record:

1. ||— 2. — 3. || 4. ||— 5. ||—

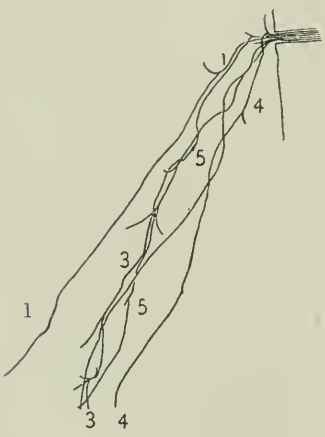

FIGURE 10. — Worm H, Set 13.

Record:

1. ||— 2. ||— 3. ||-- 4. ||— 5. ||—

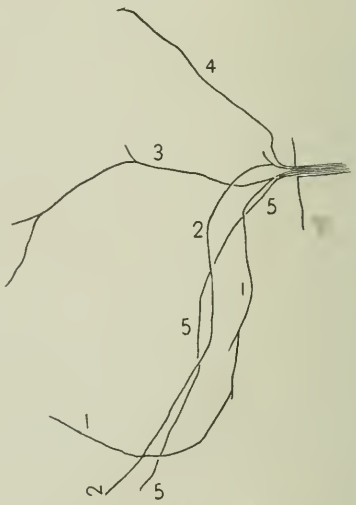

FIGURE 11. — Worm M, Set 13.

Record:

1. — 2. ||— 3. || 4. ||+ 5. ||—

travelled as far as the length of its own body, was traced directly on the plate forming the roof of the apparatus with a brush dipped in India ink. It was then drawn on tracing linen. Figures 8, 9, 10, and 11 are typical examples of the results obtained by this method. The end of the indifferent path is indicated by the straight lines; for the sake of clearness the path is widened in the diagram. Only the long axis of the worm is shown, so that it is possible to include in one diagram the five advances belonging to a set. The twenty experiments shown were made in the same day within one hour, and under the same conditions. They were selected, because they show the extreme differences. In Fig. 8 all five paths lie close together, mainly in the indifferent direction. In Fig. 9 they are more scattered, one indifferent, one directly negative, and three diagonally negative. In Fig. 10 they are close, but at a different angle from those in Fig. 8, while in Fig. 11 they are widely scattered.

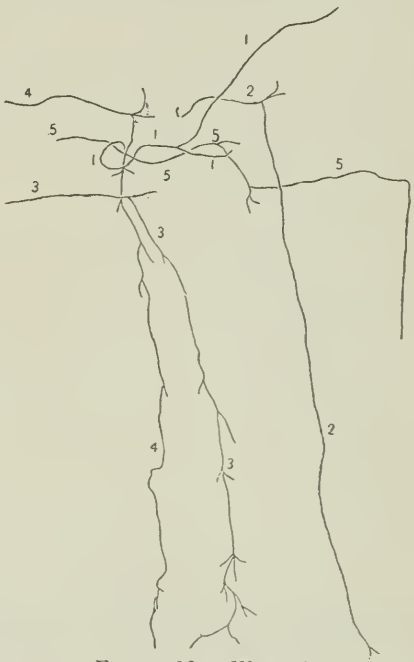

FIGURE 12. — Worm A.

A third series of experiments was made on these fifteen worms, in which the apparatus on the floor of the box consisted only of a glass plate supported at such a height that it just touched the back of the worm. The worm was laid on the filter paper in the indifferent position, the cover quickly and carefully lowered, and the course traced as before. Some of the results are given in Figs. 12, 13, and 14. They show the same variety of response that was obtained in all the preceding experiments. Incidentally these tracings also show the amount of feeling and testing of the surrounding region that precedes most forward crawling.

This great variation can mean only one of two things. Either, under the conditions of the experiments, light has so little directive effect that minor influences such as slight irregularities in the surface of the paper suffice to turn the animals away from an ideal course

leading directly from the light; or Allolobophora fœtida does not respond to light stimuli with the mechanical precision predicated for other earthworms. The filter paper used possessed a slight grain, and to avoid having a surface with constant marks and ridges, the pieces were cut in varying directions across or with the grain. No difference was observed in the results. The illumination was constant through each set of experiments, while the apparatus cut off air currents and provided equal contact on all sides of the body. The conditions therefore were constant, yet the reactions varied considerably. The more carefully the habit of crawling of this species was watched, the more I inclined to the second conclusion, that A. fœtida, while it has a negative reaction to diffuse daylight, does not naturally respond sharply and with mechanical precision. The orientation of the body in relation to light, which theoretically would bring the long axis in a directly minus-plus path (*i. e.*, in a path parallel to the light rays and with the head pointing away from the source of light), is inexact and poorly defined unless the worm is forced to crawl in the directly minus-plus path.

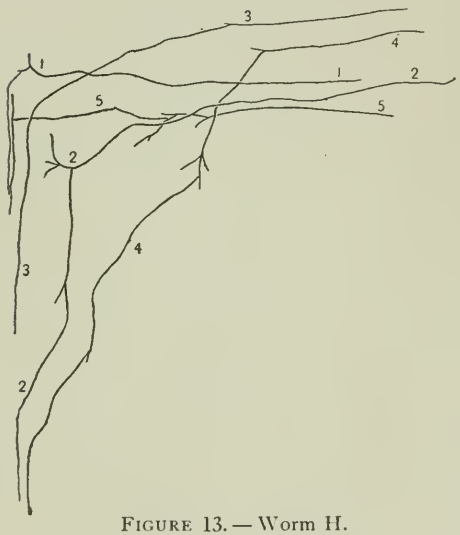

FIGURE 13. — Worm H.

It may be worth while to consider here the factors other than light that could enter into the problem. The grain of the filter paper, mentioned above, was a constant factor through any given set, yet results varied. Therefore the irregularity of response can hardly be due to the grain of the filter paper.

It occurred to me that the previous path may exert some influence, that is, it is possible that an actual trace is left by the worm in crawling, which guides the animal the next time. This I do not think a factor of any importance, for the following reasons. First, there was no indication that the first reaction determined the second, the second the third, and so on, which would necessarily be the case if the pre-

vious path exerted an influence. On the contrary, each successive path varied without relation to the succeeding paths. Second, the filter paper was renewed after each set of five, so that the paths of one set could not have affected those of the following sets. Third, in some previous experiments (not included in the above report), in which fresh paper was used for each single reaction, no difference was observed in the results as compared with those in which the filter paper was less frequently changed.

The influence of the direction from which the worm starts, noted in the preliminary experiments, is also apparent in the results of Series 3. When one considers the marked thigmotaxis of A. fœtida, sixty-eight indifferent movements out of three hundred and twenty-five (21 per cent) seems a large proportion, and is only to be accounted for by the tendency of the animal to continue crawling in the same direction. It is worth noticing that if the worm crawled rapidly through the indifferent path, it was more likely to continue in that direction without turning than when it crawled slowly and deliberately. If the speed at which the worm traverses the indifferent path is an important factor in modifying the effects of light stimuli, then the slower the movement of the worm through the indifferent path, the more decidedly negative should be the response when it leaves that path; while those moving rapidly through the indifferent path ought to show a less negative response. To determine this point exactly would involve the use of apparatus to measure the rates of speed in each experiment, and this is yet to be done.

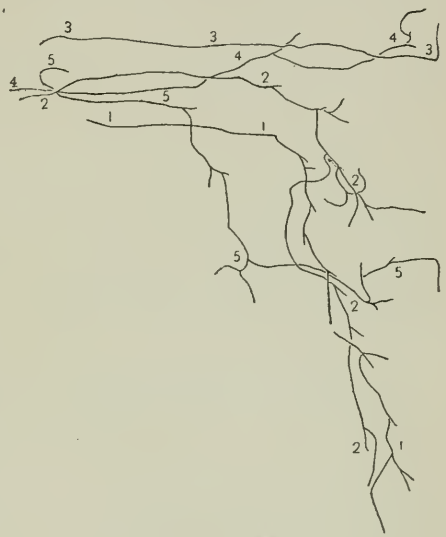

FIGURE 14. — Worm I.

There is some slight indication of consistent individual differences, not bound up with the sexual phase, for an examination of Table V shows worm J made four of the seventeen plus reactions in the whole series. The records of the preliminary experiments also showed this in the terms used to describe the worms. One indi-

vidual was described in each set as "sluggish," the recorder not noticing until the end of the series that the word had been used before, while others were twice described as "lively, irritable" worms. However, the individuality is slight and not sharply defined, although it undoubtedly exists.

After the experiments above recorded were completed, a paper appeared by Prof. G. H. Parker and Mr. L. Arkin in this Journal for April, 1901. The authors find that "The earthworm Allolobophora fœtida (Sav.) when exposed to light of moderate intensity creeps away from the source of light, *i. e.*, is negatively phototactic," and that 26 per cent of head movements were away from the light when the entire animal was illuminated from one side. With the general statement that the animals are negatively phototactic my results are fully in accord. I found, however, that when the worms were free to crawl in any direction after traversing a path in which they were illuminated from one side by diffuse daylight, the negative percentages were higher, and when forced to crawl in one of two definite paths were much higher than in the results quoted from Professor Parker and Mr. Arkin.

Thigmotactic Reactions, and the Modification of this Response by Light.

It is a common observation that earthworms have the habit of lying coiled together in masses. Darwin[1] refers to this habit of Lumbricus in the "Formation of Vegetable Mould" and Hoffmeister[2] says earthworms pass the winter either singly or rolled up with others at the bottoms of their burrows. Allolobophora fœtida has a similar habit. When a number of these worms are kept in a glass dish they can be seen lying against the bottom either coiled together or applied against each other in straight lines. Often the entire mass of worms is found lying in the angle formed by the bottom and sides of the dish. If two or more worms crawling on wet filter paper touch each other, they place themselves side by side. Sometimes they cling so closely in this position that it is difficult to separate them. If a worm in crawling turns so that its head touches its body, the worm turns on itself and crawls along its own body until the head projects

[1] Darwin: Formation of Vegetable Mould by the Action of Earthworms, p. 34.

[2] Hoffmeister: Familie der Regenwürmer, p. 13.

beyond the tail, when it may either continue crawling forward, straightening out the loop, or, turning again on itself, it may bury the head beneath the other folds. If there is an object on the filter paper making an angle with it, the worms crawl into the angle if they touch the object, and if the latter is dark the worms will not leave it for light areas.

Pieces of cylindrical glass rod one quarter of an inch in diameter, of varying lengths in comparison with the length of the worm, were laid in pairs on the filter paper in contact with each other so that a triangular passage was formed between them and the filter paper. Since the rods were transparent, the artificial burrow thus provided was little darker than the paper outside. The worm was laid down near one end of this small passage so that in crawling the head came in contact with the ends of the rods. Such a contact resulted always in a series of investigating head-movements, in the course of which the passage was found. As soon as the head happened to be poked into the narrow chink the worm crept forward into it, often quite rapidly. If the rods were longer than the worm it remained in the passage for some time (varying from eight to twenty minutes), but sooner or later it crawled entirely through and out the opposite end, usually after one or two recoils. If the rods were shorter than the worm the animals entered as before, but either crawled straight through or remained a short time (one to two minutes). If rods are very gently laid on each side of the head while the worm is extended upon the filter paper, so that the head lies within the channel, the worm usually crawls forward, but if the rods are similarly laid against the tail there is no backward crawling into the passage. In those cases where a worm remained in a passage shorter than the body it necessarily happened that a part of the body must lie outside. This uncovered portion was always the tail end. That is, the worms remained quiet for a short time with the tail protruding, but as soon as they crawled far enough forward to thrust out the head they left the passage.

A series of experiments was next made with the apparatus of which a ground plan is shown in Fig. 15. Three thick microscopic glass sides were placed end to end across a square of wet filter paper so that they formed a transparent barrier (*A*, Fig. 15) parallel to the surface of the window by which the apparatus was lighted. A narrow black box (*B*) standing on the slides cast a strip of shadow (*D*) on the filter paper, while between the slides and this shadow was a nar-

row strip of light (*C*). Light from the other sides was cut off by black screens. Experiments were made in diffuse light and in sunlight.

Diffuse Light. — Worms put down in the strip of shadow explored the lighter areas on each side before stretching the head far enough to touch the glass. As soon as the head touched the slides the worms crawled against the glass, thereby lying directly in the light. In this position they remained from one to five minutes, making many exploring head movements and crawling back and forth along the side of the glass. They did not crawl around the free ends of the slides (in which position they would have pointed directly toward the window), but recoiled when the head extended beyond the end. The worms finally left the glass, not by turning the head away from the light and crawling away in a definite direction, but on the contrary this occurred only when the worm in exploring moved the anterior third of the body away from the glass. When this portion of the body was no longer in contact with the glass, the worm crawled away across the light paper, usually in a more or less negative direction. The entire apparatus was now shaded so that there was no difference in illumination between different areas immediately behind the glass, although the remainder of the paper was brightly illuminated. The worms were placed so that in crawling they soon came against the slides. The animals now crawled against the slides as soon as the head came in contact, but instead of crawling away after a short time remained as long as the slides were entirely shaded (ten minutes). During this time the worms explored as before, recoiling each time the head was thrust into the light. When the cover was removed and the apparatus illuminated as in the preceding experi-

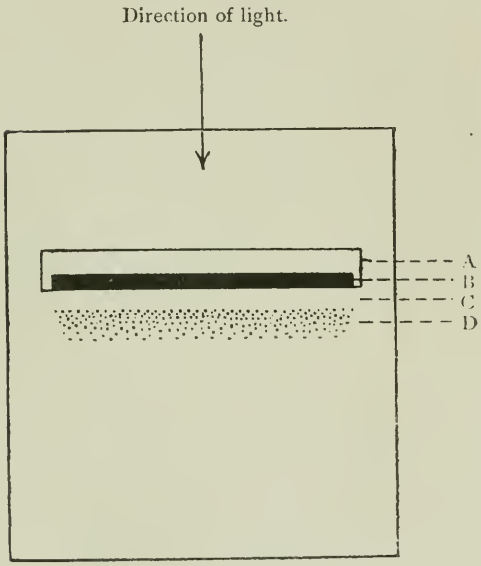

FIGURE 15.

ments the worms crawled back and forth, explored, and soon deserting the strips, moved across the filter paper.

These experiments were repeated on worms from which ten or twelve anterior segments had been cut. When put down on the filter paper with the anterior end in contact with the slides, the animals crawled away tail first. If the barrier was laid across the path of a forward-moving mutilated worm, the animal crawled backward for a short distance after touching the barrier, and sometimes repeated this several times before showing any disposition to apply itself against the barrier. If it did come to lie against the strips there was no testing of the adjacent regions as in normal worms, but on the contrary the worms crawled backward and forward along the glass and finally left it in an entirely indefinite manner.

Sunlight.— When the same apparatus was illuminated by direct sunlight and normal worms placed in the strip of shadow, *D*, they tested the areas on each side of the shadow and crawled quickly over the sunlit paper, whether the head had touched the slides or not. If placed against the slides in the light strip, there was usually a short latent period (ten to twenty seconds), and then the worm crawled away as before. If put on the paper in front of the slides and started crawling toward them the worms tried at first to push through, but failing in that they turned along the glass, crawled to the end and either continued moving in a straight line or turned around the end of the glass.

Beheaded worms exposed to full sunlight crawled at once in the direction in which the anterior end happened to point. If they came against the slides they showed no tendency to remain there.

The next apparatus consisted of a small glass box ($\frac{5}{8}$ in. $\times$ $3\frac{1}{4}$ in. $\times$ $3\frac{1}{4}$ in.) open on one of the four small sides and resting on wet filter paper on one square side. By means of microscopic slides the level of the paper outside was raised flush with the floor of the box. The opening was turned either toward the window, away from the window, or toward one side. The apparatus was illuminated from one side with diffuse light from a sunless window, as above. When a worm crawling over the wet filter paper touched the outside of the box, it turned, applied itself against the box and followed the outline of the latter. Upon reaching the open side the worm crawled around the edge and into the box, keeping always in the angle formed by the bottom and side. It then travelled around the three sides, sometimes doubling on itself and sometimes without interruption of its forward

movement. If the box was now entirely shaded the worm remained in it for some time (half an hour to two hours). If partially shaded it coiled against the side within the shaded portion; if left unshaded it crawled out of the box at one corner. When the head of the worm entered the open side of the box at any point too far away from a corner to have more than the ventral surface in contact with the glass, the worm withdrew from the box and turned in another direction. In general, Allolobophora does not leave wet filter paper for glass, and this experiment shows that it is not contact with glass as a substance that causes entrance into the box, but contact on more than one surface of the worm's body.

The box was next placed on the filter paper in an almost vertical position, with the open end down and resting on one long edge so that a worm could crawl under the open end. The worms were then made to crawl against the inner side of the supporting edge, near one angle. Out of six experiments only one worm entered entirely. It crawled around the three sides, remaining quiet against the top for three minutes, then down a vertical side and out. The others tested the vertical angles and one entered for a third of its length, but all withdrew and crawled away. When the box in this position was covered with black paper two worms entered it and were found against the top. A third did not really enter, but slipped between the box and its paper covering, without vertical crawling. When the uncovered box was supported vertically with the open side up and at the level of the plate on which the filter paper rested, the worms turned down into it as soon as the head ran over the edge of the plate, so as to project over the open side of the box, crawled down one side, around the bottom and finally to the open top after a shorter or longer pause.

By means of eight narrow strips of glass imbedded in paraffin on the oblong bottom of this box, the interior was subdivided into nine narrow spaces. It was thus almost impossible for the worm to explore the open end of the box without finding one or more corners. In addition to the numerous corners and angles thus provided, there were many small splits in the paraffin, while the latter contracted entirely away from the glass in the centre of each long side, leaving a narrow crevice which communicated with each of the nine narrow subdivisions. When the box stood vertically open end up and at the level of the plate, the worms crawled in, down one corner to the bottom and after much exploring wedged themselves into the darker

of the two narrow crevices mentioned above, between the paraffin and the box. If the box was turned over so that the worms came to lie on the lighter side, there was at first no response, but after one or two minutes the worms moved slowly until the head was thrust into shadow, either of its own body or of the paraffin.

When the box with its partitions was placed on the filter paper on one square side, with the opening away from the window, the worms entered as soon as the head touched any partition, and crawled to the back. In all cases the worms crawled as far as possible *under* the paraffin (even though they thereby left a comparatively dark shadow cast by the paraffin for a lighter strip). If the box was turned over, so that the worm was on top of the paraffin and in full light, it slowly changed its position so that it lay *under* the paraffin again. If left uncovered, the worms crawled out of this box after from half an hour to an hour. If covered with black oilcloth and the filter paper kept constantly moist they remained in it for a long time. One individual remained for over eighty hours, apparently moving but little through the chinks and crannies at the back of the box except when the cover was lifted. Then it crawled quickly up and down the angles, but did not leave the box. If a worm is laid on wet filter paper covered darkly with oilcloth and left for some hours, it does not remain on the dark wet filter paper, but is found dried up at some distance. The wet filter paper surely offers a surface more natural than the glass box, even when this is sprinkled inside. The fact that the worms remain in the box so long a time is therefore undoubtedly due to thigmotaxis.

Summary. — Allolobophora fœtida possesses a marked thigmotactic response. In diffuse light the response to contact with a transparent object (such as the glass barrier) overcomes for a time the restlessness and negative reaction to light. In direct sunlight the restlessness is so extreme that it entirely overpowers the thigmotactic response. Sooner or later the thigmotactic reaction is overcome by light, the time during which light must act bearing an inverse relation to its intensity. The smaller the crack, provided the worm is able to enter it, the more marked is the response when the worm pokes its head into one end, and consequently the stronger must be the light stimulus to overpower thigmotaxis. In the dark, thigmotactic response to contact on all sides of the body, such as is offered by a narrow passage, is so strong that the worm remains for days in a somewhat dry and certainly unnatural environment.

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