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THE AMERICAN JOURNAL  
OF  
PHYSIOLOGY.

EDITED FOR

**The American Physiological Society**

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PROCEEDINGS OF THE AMERICAN PHYSIO-  
LOGICAL SOCIETY.

NINETEENTH ANNUAL MEETING.

NEW YORK CITY, DECEMBER 27, 28, and 29, 1906.



PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL  
SOCIETY.

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OBSERVATIONS ON NORMAL GASTRIC PERISTALSIS  
IN THE RABBIT.

By JOHN AUER.

NORMAL gastric peristalsis may be studied in the fed rabbit without any operative interference whatsoever. The animal is merely stretched upon its back and the hair of the epigastrium clipped. A large part of the stomach is now visible, and the peristaltic waves may be studied by inspection or by means of a tambour. By this method a number of observations have been made, some of which have been reported elsewhere.

In the starving rabbit gastric peristalsis is either greatly reduced or entirely absent. If now the stomach be moderately distended with air or water, powerful gastric waves appear at regular intervals.

Ether and chloroform do not have the same effect upon gastric motility. When fully under ether, the peristalsis is regular and powerful, often more regular than before the anæsthesia. Chloroform, on the other hand, greatly reduces the frequency of stomach waves.

Morphine subcutaneously or intramuscularly abolishes stomach movements.

Section of the vagi in the neck stops gastric movements as a rule; there are some exceptions, however.

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FUNCTIONS AND STRUCTURES IN AMCEBA PROTEUS.

By DR. C. F. HODGE AND O. P. DELLINGER.

THE material for this study consisted of living amœba, specimens killed in various ways and stained whole by different stains, and serial sections, also hardened and stained by different methods.

Contraction is effected by a meshwork of fibrillæ, united into heavy trabeculæ with wide intertrabecular spaces in the interior (endosarc) and into very fine trabeculæ over the exterior (ectosarc). This mechanism is all that can be demonstrated to account for all movements, locomotion, ingestion, egestion, contraction of vacuole and internal circulation. Movements are co-ordinated, but no differentiation of conducting fibrillæ has been clearly demonstrated. This material, when supplied with necessary nutrient substances, must possess the function of growth.

The function of digestion is mediated in all animals by gland cells, characterized by zymogen granules. The only structure in amœba which is definitely granular is the nucleus, and sections show these granules apparently passing out of the nucleus into the food vacuoles. This accounts for all functions of the animal, reproduction being undifferentiated from growth and respiration and excretion and circulation being effected by movements of the whole body and supplemented by a similar action of the contractile vacuole. Except as noted above, sections reveal no differences between ectosarc and endosarc.

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## THE INFLUENCE OF MECHANICAL ENERGY IN PHLORHIZIN DIABETES.

By GRAHAM LUSK.

MECHANICAL effort in a fasting dog made diabetic with phlorhizin only slightly increases proteid metabolism, but leads to a small increase in the output of sugar. This extra sugar may be derived from the residue of glycogen present in the animal.

---

## THE SPARING ACTION OF GELATIN.

By J. R. MURLIN (by invitation).

REILLY, Nolan, and Lusk showed several years ago that in diabetes gelatin may yield as much as 60 per cent of its weight as dextrose. An attempt has been made to determine to what extent this purely carbonaceous part of the gelatin molecule may account for the great

sparing of the body's proteid when gelatin is fed. Experiments on dogs show that a diet limited to a quantity of carbohydrates equal to 12 per cent of the body's heat requirement does not spare proteid, but entails a loss of nitrogen; whereas 20 per cent of the body's caloric requirement in gelatin alone spares 30 per cent or more of the starvation nitrogen.

The same negative effect has been obtained in a student weighing 46 kilograms. After securing nitrogen equilibrium on a mixed diet containing 41 calories per kilo, in which nitrogen corresponding to the quantity eliminated in starvation was taken two-thirds in gelatin and one-third in meat, the gelatin was replaced by 60 per cent of its weight of dextrose. The sparing effect of the gelatin was entirely lost.

The conclusion is that the sparing action of gelatin must be accounted for entirely by its nitrogenous constituents.

---

#### SOME PHYSICAL REACTIONS OF PHYSA.

BY JEAN DAWSON (by invitation).

*Reactions of the snail to oxygen and to carbon dioxide.*—In the lake and river systems studied, the conditions making up the habitats of *Physa* vary greatly, and there is a correspondingly great variation in the number of snails found in them. A comparison of the most favorable habitats, wherever found, show strikingly similar conditions. They contain water that has the highest percentage of oxygen and the lowest percentage of carbon dioxide. A series of laboratory experiments corroborates the observations made in the field, *Physa* reacting positively to oxygen and negatively to carbon dioxide.

*Physa's reactions to chemical stimuli.*—Only a small portion of the snail's body is sensitive to chemical stimuli, and the snail responds negatively or positively according to the nature of the stimulating substance. The physiological state of the snail, due to the amount of the food taken, affects its reactions to both chemical and mechanical stimuli. A positive reaction, induced by the application of a chemical, inhibits the reaction to a second chemical which would normally produce an immediate negative response if applied to a fresh snail and *vice versa*. *Physa's* food is solid, and if one of these snails come in contact with food while in motion, the animal receives both a me-

chanical and a chemical stimulus; the resulting reaction depends largely upon the momentum of the snail and the character of the food substance.

---

THE FAILURE OF REGENERATION OF THE SUPERIOR CERVICAL GANGLION TWENTY-SIX MONTHS AFTER ITS REMOVAL. A DEMONSTRATION.

By S. J. MELTZER.

TWENTY-SIX months after the removal of the superior cervical ganglion a subcutaneous or intramuscular injection of adrenalin still causes a long lasting dilatation of the pupil on the corresponding side.

---

THE EFFECT OF SECTION OF ONE VAGUS UPON THE SECONDARY PERISTALSIS OF THE ŒSOPHAGUS.

By S. J. MELTZER AND J. AUER.

THE prevailing opinion is that section of one vagus has no effect upon the peristalsis of the œsophagus. In a series of experiments upon dogs it was found that section of one vagus reduces considerably or abolishes the secondary peristalsis in the thoracic part of the œsophagus. By secondary peristalsis it is intended to designate the peristaltic movement of the œsophagus which is not preceded by an act of deglutition and which depends upon a chain of reflexes, as was previously described by Meltzer. Besides the impairment of the secondary peristalsis, the cardia becomes relaxed soon after section of one vagus.

---

PERISTALSIS OF THE RABBIT'S CŒCUM.

By J. AUER AND S. J. MELTZER.

THE literature contains no definite statements with regard to the movements of the rabbit's cœcum. When the abdomen is opened, practically no movements can be observed. However, the cœcum

possesses well-marked frequent movements which can easily be seen through the normal skin and of which graphic records can easily be obtained. They are also well seen through the abdominal muscles after removal of the skin. But they disappear completely immediately on opening the abdominal cavity. The movements are arrested by stimulation of the vagi. This effect is best seen after administration of ergol.

---

## ARTIFICIAL REGULATION OF THE HEART RATE.

By VANDELL HENDERSON.

At the meeting of the Society in December, 1905, the writer stated that in an animal under artificial respiration the heart rate varies with the rate and depth of the respiration (*i. e.*, with the extent of the pulmonary ventilation). Excessive pulmonary ventilation induces extreme tachycardia. He further suggested that a diminution in the CO<sub>2</sub> content of the blood resulting from the excessive respiration is the cause of this tachycardia.

In a series of experiments upon dogs, the blood gases have been determined (by means of a Hill pump). The analytical results and pulse tracings show a constant inverse relation between the CO<sub>2</sub> content of the blood and the heart rate in animals under artificial respiration. This relation holds true also under natural respiration, except when the animal is hyperpnœic. In this case the respiratory excitement induces a rapid pulse even when the CO<sub>2</sub> content of the blood is high.

When the thorax of a dog has been opened, the heart rate can be regulated at the will of the operator by the following method. The bellows is connected with the tracheal cannula by a piece of large rubber tubing (15 mm. interior diameter and 50 to 250 cm. long according to the size of the animal). At both ends of this tube are adjustable escape vents. If the vent near the tracheal cannula be open and that near the bellows closed, a rather rapid and full movement of the bellows induces a rapid acceleration of the heart rate. If the tracheal vent be then closed and the vent near the bellows be opened, the heart rate is gradually slowed. In one experiment between 10 A. M. and 5 P. M. the heart rate was four times raised above 200 per minute, and as often slowed below 50. The animal was under morphin and ether.

In experiments in which, after the opening of the thorax, the animals were maintained under natural respiration of compressed air (Sauerbruch-Brauer method), the increase and diminution of the respiratory "dead space" by means of the large tube with two vents (used in these cases to connect the tracheal cannula with a gasometer into which air was pumped at a constant pressure of 10 to 13 cm. of water), results identical with those above described were obtained. In experiments in which the thorax was not opened vigorous artificial respiration also induced tachycardia, as did also the hyperpnœa resulting from the stimulation of an afferent nerve. Under ether it was thereafter difficult to regain a slow pulse rate, owing apparently to the difficulty of diminishing the hyperpnœa. Under deep chloroform anæsthesia, on the contrary, it was found that increasing the respiratory "dead space" by attaching a tube to the trachea slowed the heart rate to 50 or fewer beats per minute.

In experiments on men it was found that voluntary forced respiration continued vigorously for a couple of minutes induces a heart rate of 130 or more beats per minute.

These observations seem to indicate that the maintenance of the normal heart rate is in large degree dependent on the uniformity of the CO<sub>2</sub> content of the arterial blood. This view harmonizes with the conclusion of the researches of Haldane and Priestley, that the respiratory activity is adjusted primarily to maintain a uniform CO<sub>2</sub> content in the blood.

---

#### PRODUCTION OF ARTIFICIAL PARTHENOGENESIS IN ASTERIAS THROUGH MOMENTARY ELEVATION OF TEMPERATURE.

By RALPH S. LILLIE.

EXPOSURE of the eggs of *Asterias forbsii* during maturation to temperatures ranging from 34° to 38°, for periods varying from thirty seconds to two minutes, is followed by typical membrane formation and by cleavage, which in favorable cases is regular and leads to apparently normal development. Bipinnariæ indistinguishable from those reared from normally fertilized eggs have been obtained by this method. The most favorable results are gained by transfer of eggs during the early maturation period (*i. e.*, within half an hour after removal) to sea water at 35°, 36°, 37°, and 38° for periods varying from fifteen seconds to eighty seconds; the higher the temperature



of the warm sea water, the shorter the optimum period of immersion; the eggs are then transferred to a large volume of sea water at the normal summer temperature ( $20^{\circ}$ – $22^{\circ}$ ). Fertilization, membrane formation, and cleavage follow; development is slower than normal, and a smaller proportion of eggs reach the blastula and later stages. Membrane formation is especially readily induced by this treatment, and is often shown by eggs with germinal vesicle intact. If the conditions of temperature or time of exposure are unfavorably adjusted, amoeboid movements of the egg protoplasm and irregular cleavage or fragmentation occur (independently of nuclear division) in a large proportion of eggs; the protoplasm of such eggs soon undergoes a coagulative change, followed by disintegration.

---

#### THE ACTION OF BLOOD SERUM AND TISSUE EXTRACTS ON THE COAGULATION OF THE BLOOD.

By LEO LOEB (by invitation).

CERTAIN analogies exist between the action of salts in the coagulation of lobster blood, in the precipitation of casein and paracasein, and in the digestion of proteid by pancreatic juice. In each case the optimal amount of calcium can be split in a fraction which can be substituted by Mg or Na and presumably other kations, and in another fraction which can neither be substituted by Na nor (with the exception of the precipitation of casein and paracasein) by Mg.

Analogies exist likewise in the influence exerted by tissue extracts on the coagulation of blood, on the coagulation of milk, and on the pancreatic digestion of proteid (action of exterokinase).

It has been suggested that in each case different substances combine to form the active ferment. In the case of the coagulation of invertebrate blood it can be shown that tissue extract (tissue coagulin) and serum (thrombin) act both independently of each other on the fibrinogen. In vertebrate blood complicating factors arise; substances are found in the blood serum which accelerate and inhibit the action of tissue coagulins. But no fact is known which makes it likely that the relations between thrombin, tissue coagulin, and salts differ essentially in the clotting of vertebrate and invertebrate blood.

THE FUNCTIONS OF THE EAR OF THE DANCING MOUSE.

BY ROBERT M. YERKES.

BOTH the static and the acoustic functions of the ear of the dancer differ markedly from those of the common mouse.

Orientation and equilibration are fairly good. There is no evidence of turning dizziness. The whirling movement which is characteristic of the race appears as soon as the young mouse is strong enough to stand. It is somewhat more pronounced in the female than in the male, and occurs chiefly toward evening. With respect to this movement there are three well-defined groups of dancers: those which almost always whirl toward the right, those which whirl toward the left, and those which whirl now one way now the other. At present I have no satisfactory evidence of the inheritance of the tendency to whirl in a certain way.

Direct and indirect methods of testing acoustic sensitiveness have given negative results in the case of the adult, but the young dancer responds to certain sounds for from two to five days during the third week of life. This period of sensitiveness to sounds is preceded by a marked change in behavior.

---

THE CAUSE OF THE TREPPE.

BY FREDERIC S. LEE.

THE *treppe* is usually ascribed to increased irritability caused by activity. The cause of the increased irritability has remained obscure. In studying the depressing action on muscle of its fatigue substances the author often observed augmentation of activity instead of depression. A more careful investigation of this phenomenon shows that it may be produced by all of the three recognized fatigue substances,—namely, carbon dioxide, monopotassium phosphate, and paralactic acid. When a muscle is irrigated with an indifferent fluid containing one of these substances in small quantity, and compared with its mate, irrigated only by the indifferent fluid, a fatigue record being made from both, more intense contractions frequently occur in the poisoned muscle at the beginning of the experiment, and may last until exhaustion sets in. When a fatigue record is being made from a muscle with the circulation intact, intravenous

injection of a fatigue substance causes augmentation of contraction. The author concludes that the *treppe* is due to the augmenting action of fatigue substances in small quantities,—the same substances which in larger quantities cause depression or fatigue.

An excellent mode of demonstrating the augmenting action of  $\text{CO}_2$  in the cat is to record the contractions of the *Tibialis anticus* in the living animal, and while the record is being made, to clamp the trachea. A marked *treppe* follows.

If two corresponding muscles be compared, one with the circulation intact, and the other with the arteries ligated, the latter muscle performs more intense contractions and exhibits a more rapidly developing *treppe*, owing to the accumulation of fatigue substances.

The chemical theory of the *treppe* is able to explain several other known phenomena. The author has experimented on both frogs and cats. The augmenting action of the fatigue substances is observed even when curare is employed.

---

#### THE FORMATION OF FAT IN ANIMALS FATTENED FOR SLAUGHTER.

BY GEORGE T. KEMP AND L. D. HALL.

THIS research was undertaken to determine, microscopically, how fat was deposited in the "marbling" of beef, and was extended to include a study of the relation of fat to the muscle cell, both histologically and chemically.

The histological reagents used to stain the fat were osmic acid, Sudan III, and Scharlach R. We agree with the majority of observers in giving preference to the last. The beef was frozen, and cut with a Bardeen freezing microtome. Some tissues were fresh, and others were fixed in formaldehyde (and sodium chloride) before freezing.

Even in the fattest tissues no fat was found within the sarcolemma. It appeared to be confined exclusively to the connective tissue, as far as microscopical observations were concerned.

Some specimens of very lean meat yielded a much larger percentage of fat, by extraction, than could be accounted for by the fat which showed under the microscope.

The theory has been put forward, that the toughness of meat is partially produced by a thickening of the sarcolemma. We found nothing to support this theory.

- The following communications were also presented :
- THE MINIMAL PROTEID REQUIREMENT OF SOME HIGH PROTEID ANIMALS.  
By R. H. CHITTENDEN.
- THE RATE OF LOSS OF WEIGHT OF NORMAL MAN. By W. P. LOMBARD.
- THE EFFECT OF MUSCULAR ACTIVITY ON KREATININ EXCRETION ; WITH  
PRELIMINARY OBSERVATIONS ON THE EXCRETION OF KREATININ IN  
HEALTH AND DISEASE. By P. A. SHAFFER (by invitation).
- THE OCCURRENCE AND FORMATION OF ALKYLAMINES AND ALKVEUREAS. By  
O. FOLIN.
- THE FORMATION OF SUGAR FROM AMINO-ACIDS. By W. SALANT.
- THE EFFECTS OF COCAINE ON THE LIVER. By G. B. WALLACE AND J. S.  
DIAMOND.
- ON THE ELIMINATION OF RADIUM IN NORMAL AND NEPHRECTOMIZED  
ANIMALS. By W. SALANT AND G. M. MEYER.
- THE RELATION OF INORGANIC SALTS TO LECITHIN AND KEPHALIN. By W.  
KOCH.
- CONTRIBUTIONS TO THE PHYSIOLOGY OF THE PHOSPHATES. By C. L. ALS-  
BERG, L. J. HENDERSON, H. B. WEBSTER, AND R. FITZ.
- CONCERNING GLYCOLYSIS. By C. L. ALSBERG AND G. W. HALL.
- SOME NEW OBSERVATIONS ON THE ACTION OF LIPASE. By A. S. LOEVEN-  
HART, G. PEIRCE, AND C. G. SOUDER.
- NUCLEINS OF CODFISH ROE. By J. R. MANDEL AND P. A. LEVENE (read  
by title).
- GLUCOTHIONIC ACID IN PUS. By J. A. MANDEL AND P. A. LEVENE.
- PRELIMINARY REPORT ON THE ENZYMES OF UNFERTILIZED AND FERTILIZED  
EGGS. By E. P. LYON AND O. P. TERRY.
- EXPERIMENTS ON RESUSCITATION. By G. N. STEWART (read by title).
- ON THE SO-CALLED "LIGATURE OF STANNIUS IN THE MAMMALIAN HEART."  
By J. ERLANGER AND J. R. BLACKMAN.
- ON THE MECHANISM OF THE SO-CALLED REFRACTORY PERIOD OF THE  
HEART. By A. J. CARLSON.
- ON THE RELATION OF THE NORMAL RHYTHM TO THE SODIUM CHLORIDE  
RHYTHM OF THE HEART. By A. J. CARLSON (read by title).
- VASOMOTOR REFLEXES. By W. T. PORTER.
- THE INFLUENCE OF THE DIGITALIS SERIES UPON THE VELOCITY OF THE  
BLOOD STREAM. By C. W. EDMUNDS.
- METHODS OF STUDYING FATIGUE. By F. S. LEE (a demonstration).
- DEMONSTRATION OF THE ADIABATIC CALORIMETER OF RICHARDS, HENDER-  
SON, AND FREVERT. By C. L. ALSBERG.

- ON THE ALLEGED ADAPTATION OF THE SALIVARY GLANDS TO DIET. By  
F. P. UNDERHILL AND L. B. MENDEL.
- ADAPTATION OF SALIVA TO DIET. By C. H. NEILSON (by invitation).
- THE EFFECT OF PHOSPHORUS STARVATION ON ASPERGILLUS NIGER. By  
W. KOCH AND H. S. REED (read by title).
- NEW CHEMICAL FACTS ABOUT TENDON AND COMPOUND PROTEINS. By  
W. J. GIES (read by title).
- A FURTHER STUDY OF PEPTOLYSIS. By W. J. GIES AND W. N. BERG  
(read by title).
- SOME OBSERVATIONS ON THE OESOPHAGUS AFTER BILATERAL VAGOTOMY. By  
W. B. CANNON.
- CONCERNING THE PHARMACOLOGICAL ACTION OF SALICYLIC ACID. By L. B.  
STOOKEY AND M. MORRIS (read by title).
- NUCLEIN METABOLISM EXPERIMENT ON A DOG WITH ECK'S FISTULA. By  
P. A. LEVENE AND J. E. SWEET.
- PROTEIN ANALYSIS. By P. A. LEVENE, W. A. BEATTY, D. R. MACLAURIN,  
AND C. H. RULLER.
- PRESERVATION OF BLOOD VESSELS IN COLD STORAGE. By A. CARREL (by  
invitation).



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NO. I.

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OBSERVATIONS ON THE EXCRETION OF CARBON DIOXIDE GAS AND THE RECTAL TEMPERATURE OF RATS KEPT IN A WARM ATMOSPHERE WHICH WAS EITHER VERY MOIST OR VERY DRY.<sup>1</sup>

By J. J. R. MACLEOD.

(WITH THE COLLABORATION OF J. D. KNOX.)

[From the Physiological Laboratory, Western Reserve University, Cleveland, Ohio.]

IN a recent paper<sup>2</sup> Haldane calls attention to the small amount of work that has been done on the influence of a warm humid atmosphere on man. That such an atmosphere, in comparison with a dry one at the same temperature, has a most hurtful influence is a well-known fact, and it is believed that this is owing to interference with heat loss from the body: the evaporation of sweat being reduced to a minimum in such an atmosphere. The exact degree of humidity and temperature necessary to bring about such a condition and the influence of other factors, such as the movement of the air, etc., have been little investigated in this connection.

Haldane made observations on the rectal and mouth temperatures, and on the pulse, respirations, and general sensations of men in moist and dry atmospheres at high temperatures. He found, briefly stated, that in still air during rest the rectal temperature of men lightly clothed or nearly naked rises whenever the wet bulb thermometer

<sup>1</sup> The following investigation was started over three years ago at the suggestion of LEONARD HILL, F. R. S., but had to be discontinued on account of pressure of other work. During the past summer the research was resumed and extended so as to include observations made during muscular work. The expenses of the research were partly defrayed by a grant from the Royal Society of London.

<sup>2</sup> HALDANE, J. S.: The journal of hygiene, 1905, v, p. 494.

reaches 88° F. and it continues to rise for some time (two and three-quarters hours) after coming out of the hot atmosphere. The higher the temperature of the air (as read on wet bulb thermometer), the greater is the rise in rectal temperature. In moving air (fifty-one metres per minute) the rise in rectal temperature is not noticed till the wet bulb thermometer reaches 93° F.

In still air even moderate muscular work causes a rise in rectal temperature when the wet bulb thermometer is at 78° F. In moving air muscular work can be performed at 85° F. without abnormal rise of rectal temperature.

Haldane noticed that the uncomfortable symptoms produced by warm air depend only to a certain extent on the rise in body temperature; they are also, in part at least, directly due to the high external temperature (wet bulb). He further points out that it is the temperature registered by the wet bulb thermometer which is of importance in these observations, and not that of the dry bulb. This fact is easily explained: of the factors which control the body temperature in man, by influencing the heat loss, the evaporation of sweat is undoubtedly the most important at high temperatures. In the above observations of Haldane's we can accordingly account for the rise in rectal temperature as being due to the inefficient evaporation of sweat from the surface of the body in the moist atmosphere. In warm atmospheres, so long as the reading on the wet bulb thermometer stands well below that of the skin, evaporation of sweat will proceed freely, and the temperature of the air, when dry, may rise even to 250° F. without immediately influencing the body temperature. On the other hand, whenever the reading on the wet bulb thermometer nears that of the skin, evaporation of sweat is interfered with, the body is not cooled down as it should be, and the rectal temperature rises in consequence.

In small animals the relative amount of body heat lost by evaporation is, in comparison with man, much less than that lost by radiation, conduction, and convection. Thus, in a man (70 kg.), of the total heat loss 71 per cent is by conduction, radiation, and convection, and 22.9 per cent by evaporation; whereas in a guinea pig (0.55 kg.) the heat loss by radiation, conduction, and convection is 93.5 per cent and by evaporation 6.5 per cent.<sup>1</sup> These figures relate presumably to ordinary temperatures; at higher temperatures the percentage loss of heat by evaporation will be much greater. In the case of the

<sup>1</sup> HILL: Recent advances in physiology (Longmans, Green & Co., New York), 1906, p. 267.



rat the proportion of heat lost by evaporation will be still lower than that in the guinea pig. The absence of sweat glands in the skin of the rat further shows that the heat regulation by this means does not occur, and although in hot atmospheres the quickened respirations will drain away heat for the evaporation of moisture in the expired air, yet it is probable that conduction, etc. from the skin is a much more important mechanism.

On account of these considerations it seemed to us of interest to investigate the rectal temperature and the excretion of carbon dioxide gas of small animals kept in dry and moist atmospheres at high temperatures. In the following observations rats were chosen for this purpose and were kept in as warm an atmosphere as was consistent with safety. In one series of observations they were kept at rest, in another they were made to do muscular work; and the atmosphere was either as dry as possible or almost saturated with moisture. The amount of carbon dioxide expired by the rat while in the chamber was ascertained and, after removal, the rectal temperature was taken. Since the temperature of the chamber—just below  $36^{\circ}$  C.—was very near that of the rat's body—about  $38.3^{\circ}$  C.—there could not be much heat loss from the latter by the processes of radiation, conduction, and convection; on the other hand, in order to saturate the expired air with moisture, an amount of body heat sufficient to prevent the body temperature from rising might yet be drained away. The experiments here reported were conducted partly to show whether this source of heat loss is of much importance in the case of small animals; if it is of importance, we should expect to find that the body temperature and consequently the excretion of carbon dioxide rise higher when evaporation is more or less prevented by saturating the inspired air with moisture than when evaporation is encouraged as in dry air at the same temperature.

In observations on mice recorded by Leonard Hill and myself,<sup>1</sup> we found that the greater heat conductivity of wet as compared with dry air made it impossible for many of the animals to withstand a moist atmosphere below  $24^{\circ}$  C.; for a time, in such an atmosphere, the increased heat loss was made good by increased heat production, but after a time this failed, the rectal temperature fell, and, if not removed to a warm atmosphere, the mouse died. In several preliminary experiments at temperatures a little above  $24^{\circ}$  C. we did not find moist air to have any deleterious influence. We thought it of interest to see whether at higher temperatures the moisture would influence the

<sup>1</sup> HILL and MACLEOD: *Journal of physiology*, 1903, xxix, p. 508.

body temperature and excretion of carbon dioxide in the opposite direction to that observed at lower temperatures.

#### THE METHOD OF EXPERIMENT.

Tame white rats were employed. The respiration chamber consisted of a large-sized desiccator containing in the case of the dry-air experiments sulphuric acid and in the case of the moist-air experiments water. The inlet tube entered at the side of the desiccator, and was connected either with a tower of sulphuric acid and pumice stone or with one containing a sponge soaked in water. The respi-

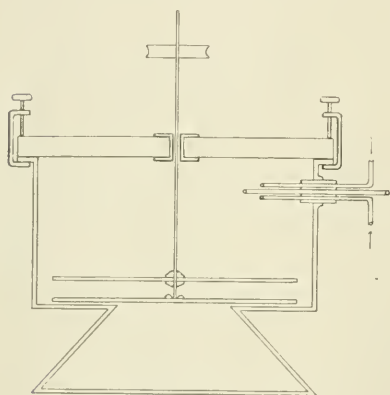


FIGURE 1.

ration chamber and towers were placed in a zinc tank containing water kept at a constant temperature by means of a flame regulated by a thermo-regulator. The ingoing air, previously to passing through these towers, was freed of carbonic acid by passing it through towers containing soda lime. It was also passed through a worm tube submerged in the water bath so as to warm it. The outlet tube, leaving the desiccator at the same opening as the inlet

tube, led to a large Woulfe bottle containing pumice stone and sulphuric acid and then to a calcium chloride tube. Beyond this were attached the weighed absorption tubes filled with soda lime and calcium chloride. Great care was taken to see that these were absorbing properly. A metre was connected at the beginning of the inlet tube and an aspirator at the end of the outlet tube.

For the work experiments (see Fig. 1) a lid of hard wood was tightly clamped on to the desiccator in place of the glass cover. This lid was one inch thick, so as to prevent warping, and through the centre of it was passed a brass tube carrying an accurately fitting brass shaft twelve inches long and three-eighths of an inch in diameter. At about half an inch from its lower end this shaft was attached to the centre of a circular platform which formed the floor of the wider portion of the desiccator, the end of the shaft being prolonged beyond the platform and ending in a point which rested on a hollow brass disc. The brass disc was held in position by means of wooden stays.

The upper end of the shaft carried a pulley connected by a belt with gear wheels and a motor so that the platform was made to revolve at the rate of about twenty revolutions per minute. A piece of sheet zinc was suspended from the lid so as to make a partition on one side of the chamber. On coming against this the rat was compelled to work. The rate of ventilation in the different experiments varied between 50 and 400 c.c. per minute. When a slow rate of ventilation was employed, the chamber was flushed out with a quick stream of air before weighing the absorption tubes, so as to remove all traces of carbonic acid gas from the chamber. In the case of the rest experiments the rat was kept in a flat porcelain dish covered with wire gauze, and this was weighed with the rat at the beginning and end of the experiment. In the work experiments such a scheme could not be employed, and moreover the rat's body often got smeared with the vaseline used to keep the lid air tight, so that it was useless to record the body weight.

#### CONSIDERATION OF RESULTS

**The rectal temperature of the rats.**—We have noted, in recording the rectal temperature of the rats, that great care has to be taken that the thermometer is well inserted in the rectum, as, on the diet of bread and water on which the rats were kept, the large amount of *fæces* may quite markedly affect the reading on the thermometer unless it is well inserted. The normal temperature of the rats used in the experiments varied from 99.5° to 101.4° F., the average being 100.2° F. This is practically the same as that given by Pembrey.<sup>1</sup>

The rectal temperature of rats kept at rest in atmospheres between 30° and 33° C. for considerable periods of time did not appear to show any difference according to whether the atmosphere was dry or moist. Below 35° C. the rats could be kept for any length of time without being killed, and the rectal temperature did not rise above 103° or 104° F. By referring to Tables II and III evidence on this point will be obtained. At higher temperatures, namely, at 37° C., the rectal temperature quickly rose, and the rats could not be kept at such a temperature for much more than half an hour without being killed by hyperpyrexia. After being in such an atmosphere for half an hour, the rise in rectal temperature was practically the same whether the

<sup>1</sup> PEMBREY: *art.* The chemistry of respiration; SCHÄFER'S *Physiology*; YOUNG, J. PENTLAND: *Edinburgh*, 1898, i, p. 790.

air was moist or dry, as will be seen from the following table of results (Table I).<sup>1</sup> In these observations the work apparatus was not employed, although in practically all of them the rats were moving about actively while in the chamber.

TABLE I.

Nature of atmosphere.	Temperature of chamber.	Rectal temperature (Fahr.).		Remarks.
		Before.	After.	
Dry	37	101.0	104.4	In all these observations the rats were kept for half an hour in the chamber, after which they were removed and the rectal temperature taken.
Dry	37-39	....	105.3	
Dry	35-37	....	103.0	
Dry	36-38	99.8	105.2	
Dry	36-39	100.0	106.0	
Dry	36-37	....	104.4	
Wet	36-38	100.8	106.2	
Wet	36-38	99.5	106.2	
Wet	35-37	....	105.0	
Wet	36-37	99.6	105.8	
Wet	36-37	99.6	106.5	

**The excretion of carbon dioxide.** — The results relating to the excretion of this are given in Tables II, III, IV, and V. In the experiments of which Tables II and III give the results the rats were placed in a porcelain basin covered with wire gauze, and this was then placed in the respiration chamber. In connection with these it will be noticed that the general average of the results is the same in the dry as in the moist air. In certain of the preliminary experiments which we performed there appeared to be a distinctly greater excretion in the moist as compared with the dry air, but when the average of a large number of the observations is taken, and especially when the same rat is employed for both the wet and dry air observations, it is evident that no such differences exist. Such differences as are occasionally noticed (*e. g.* in the case of rats 1 and 2)

<sup>1</sup> There seems, from these results, to be a greater rise in the moist than in the dry air, but we believe this is accidental.

TABLE II.

EXCRETION OF CARBON DIOXIDE IN GRAMS PER KILO BODY WEIGHT AND PER MINUTE AT 30° C.

A. IN DRY AIR.						
Number of rat and its weight in grams.	Duration of observation in minutes.	Rate of ventilation in c.c. per minute.	Rectal temp. of rat on removal.	Loss in weight of rat per minute and kilo.	CO <sub>2</sub> per minute and kg.	Averages of CO <sub>2</sub> excretion.
I. 204	30	....	....	0.069	0.034	0.0325
	30	....	100.8	0.080	0.034	
	32	180	101.8	0.065	0.032	
	60	60	104.4	0.031	0.030	
II. 177	30	....	100.8	0.067	0.032	0.0313
	100	290	....	0.038	0.031	
	120	150	100.6	0.039	0.031	
III. 174	30	....	....	0.025?	0.032	0.0315
	30	130	101.2	0.061	0.031	
IV. 139	30	....	....	0.102	0.026	0.029
	30	....	....	0.059	0.032	
Average of all observations . . .						0.0310
B. IN MOIST AIR.						
I. 209	130	110	100.8	0.039	0.031	0.0306
	200	170	102.6	0.037	0.030	
	180	160	101.8	0.031	0.031	
II. 173	60	130	103.0	0.058	0.028	0.0280
	170	90	....	0.028	0.028	
	200	120	101.6	0.026	0.028	
	220	180	100.7	0.026	0.029	
	182	70	....	0.027	0.027	
III. 166	165	130	....	0.027	0.029	0.0290
IV. 133	200	90	103.6	0.033	0.034	0.0345
	250	150	101.0	0.040	0.035	
	200	180	101.3	0.033	0.032	
	185	160	100.8	0.048	0.037	
Average of all observations . . .						0.0305

TABLE III.

OBSERVATIONS ON THE RECTAL TEMPERATURE AND CARBON DIOXIDE EXCRETION (PER KILO BODY WEIGHT AND PER MINUTE) OF RATS KEPT IN A CHAMBER AT 33° C.

A. IN DRY AIR.						
Number of rat and its weight in grams.	Duration of observation in minutes.	Rate of ventilation in c. c. per minute.	Rectal temp. of rat on removal.	Loss of weight of rat per minute and kilo.	CO <sub>2</sub> per minute and kilo.	Averages of CO <sub>2</sub> excretion.
I. 208	40	210	100.6	0.066	....	0.036
	60	360	103.8	0.064	0.036	
	120	260	102.6	0.050	0.033	
	165	300	103.2	0.075	0.038	
II. 183	120	170	100.6	0.056	0.030	0.032
	120	220	102.2	0.045	0.030	
	100	290	105.0	0.054	0.035	
	120	210	103.6	0.062	0.033	
	200	220	....	0.059	0.032	
III. 160	30	160	100.8	0.072	....	0.033
	200	410	101.8	0.066	0.033	
IV. 133	60	180	102.2	0.072	0.037	0.035
	120	220	101.6	0.078	0.039	
	150	330	102.0	0.047	0.031	
	200	180	101.2	0.060	0.032	
Average of all observations . . .						0.034
B. IN MOIST AIR.						
I.	135	60	102.8	0.076	0.038	0.036
	143	30	102.2	0.065	0.035	
	90	160	102.3	0.056	0.036	
II.	180	140	101.8	0.033	0.033	0.034
	60	210	102.4	0.024	0.035	
III.	130	40	102.6	0.086	0.038	0.0375
	160	10	101.7	0.079	0.037	
IV.	50	200	101.2	0.061	0.042	0.043
	210	250	103.4	0.063	0.043	
	80	270	102.2	0.063	0.046	
Average of all observations . . .						0.0370

are probably to be accounted for by differences in the extent to which digestion was proceeding at the time of the observations, for, although all the rats were on the same diet (bread and water), yet some of them must have been recently feeding when removed from the cage, others not for some time.

The influence of body weight on the excretion of carbon dioxide is not so distinct as when the observations are made at lower temperatures.<sup>1</sup> It has been found, namely, that as the body weight decreases the excretion of carbon dioxide per unit of time and body weight becomes greater. This is due to the greater surface in relation to body weight in the smaller animals, so that there is a greater loss of heat from the surface of the body by radiation, conduction, and convection, and consequently a more active metabolism to make good the increased heat loss. Now, at the temperatures worked at in the observations here described, the heat loss by these means is reduced to a minimum, so that to maintain the body temperature scarcely any more active a tissue metabolism will be required by the small than by the large rats.

It will further be noticed that at 33° C. the carbon dioxide excretion is higher than at 30° C. For all animals there is an external temperature at which the metabolism is at a minimum and above or below which it becomes increased. In the case of the dog Page found this temperature to be 25° C. So far as we are aware, it is not known for small animals such as the rat. This increased metabolism at high temperatures, acting along with the diminished heat loss, accounts for the rapid rise in rectal temperature which we have seen to occur when the temperature of the chamber is above 35° C.<sup>2</sup>

In the observations of which Tables IV and V give the results the rats were placed in the work apparatus already described. The temperature of the chamber was not kept so constant as in the rest experiments, and was as a rule somewhat higher than in these. In Table IV, *A*, the results of the dry-air experiments are given, and in IV, *B*, those for moist air. In Table V it will be noticed that in each obser-

<sup>1</sup> *Vide* PEMBREY : *Loc. cit.*

<sup>2</sup> It will be noticed (Tables II and III) that the loss of body weight is often less in the wet than in the dry air experiments. In the former the urine and faeces passed during the experiment will not evaporate, and since the inspired air is already saturated, or nearly so, with water, there will be little loss of water through the lungs. In the latter, on the other hand, the urine and faeces passed during the experiment will dry out and the loss of water through the lungs will not be interfered with.

TABLE IV.

OBSERVATIONS ON THE RECTAL TEMPERATURE AND CARBON DIOXIDE EXCRETION (PER KILO BODY WEIGHT AND PER MINUTE) OF RATS DOING MUSCULAR WORK IN A CHAMBER AT TEMPERATURES BETWEEN 33° AND 36° C.

A. DRY AIR.							
Weight of rat.	Duration of observation (min.).	Temperature of chamber.	Rectal temperature of rat on removal.	CO <sub>2</sub> per minute and kilo.	Rate of ventilation in c.c. per minute.	Averages for CO <sub>2</sub> .	Remarks.
103.8	30	33	....	0.059	260	....	
	30	....	....	0.061	....	....	
	30	....	101.4	0.067	300	0.062	In chamber 2 hrs.
172.9(B)	40	34-35	....	0.067	....	....	
	50	34	....	0.059	....	0.063	In chamber 2½ hrs.
176.4	60	34.5	....	0.061	300	....	
	80	34.5	....	0.067	150	....	
	30	35	....	0.100	250	....	
	60	....	....	....	....	....	
	20	32.5	....	0.120	....	....	
	30	34	....	0.082	300	0.086	In chamber 5½ hrs.
110.0	30	33-35	....	0.076	....	....	
	60	35	100.4	0.073	....	0.0745	In chamber 2¼ hrs.
115.2	30	34	....	0.064	100	....	
	50	....	....	0.066	150	....	
	60	35	....	0.077	260	....	
	30	36	106.2	0.076	....	0.071	In chamber 3 hrs.
120.0	70	34	....	0.084	200	....	
	30	....	100.8	0.069	....	0.076	In chamber 2 hrs.
115.0	40	35	....	0.073	300	....	
	40	35	....	0.074	220	....	
	40	35.5	101.4	0.091	300	0.079	In chamber 3 hrs.
124.3	70	35-36	....	0.072	400	....	
	40	....	....	....	....	....	
	40	35	....	0.075	400	....	
	30	35.5	....	0.062	250	....	
	40	36	104.0	0.080	....	0.072	In chamber 3½ hrs.
Grand average . . .						0.073	



TABLE IV (continued).

B. MOIST AIR.							
Weight of rat.	Duration of observation (min.).	Temperature of chamber.	Rectal temperature of rat on removal.	CO <sub>2</sub> per minute and kilo.	Rate of ventilation in c.c. per minute.	Averages for CO <sub>2</sub> .	Remarks.
156.6 (R)	70	31.5	. . .	0.063	350	....	.
	24	....	....	0.083	....	....	
	30	....	....	0.054	....	0.067	Dura'n of ex. 2½ hrs.
157.9 (R)	30	31.5	....	0.046	....	....	
	30	....	....	0.0687	....	....	
	20	....	....	0.044	....	....	
	10	....	100.8	0.069	....	0.057	Dura'n of ex. 2 hrs.
180 S (B)	60	30.5	....	0.063	....	....	Chamber not perfectly air-tight.
	60	32.5	....	0.090	....	....	
	60	....	....	0.109	....	....	
	60	....	102.5	0.089	....	0.088	Dura'n of ex. 5 hrs.
123.8 (L)	40	32	....	0.088	125	....	
	40	32	....	0.082	225	....	
	30	....	101.2	0.081	66	0.083	Dura'n of ex. 2 hrs.
118.9 (L)	30	35	....	0.071	100	....	
	30	35	....	0.086	50	....	
	60	35	....	0.054	....	....	
	60	35	....	0.116	50	....	
	30	35.2	....	0.071	250	....	
	30	35.5	103.5	0.094	....	0.082	Dura'n of ex. 4 hrs.
153.6 (N)	30	36-37	....	0.071	330	....	20 m. after removal temp. was 102.
	30	36-37	106.2	0.087	300	0.079	Rat was dead next morning.
160.4 (R)	30	36-37	....	0.112	fast stream	....	After about 30 min. rat was seen to be exhausted. Died a few hours after removal.
123.0 (L)	50	35	....	0.079	300	....	
	30	35	....	0.068	....	....	
	40	35	102.0	0.063	....	0.070	
Grand average . . . .						0.075	

vation the air was changed from dry to wet while the experiment was in progress. This was accomplished by changing the ingoing air current from passing through a sulphuric acid tower to one containing sponge soaked in water and running water at the same temperature as that of the chamber down the inlet tube into the latter.

TABLE V.

OBSERVATIONS ON THE CARBON DIOXIDE EXCRETION OF RATS DOING WORK IN A CHAMBER HEATED TO ABOUT 33° C. DURING THE FIRST HALF OF THE OBSERVATION THE AIR OF THE CHAMBER WAS DRY, DURING THE SECOND HALF MOIST.

Weight of rat in grams.	Nature of air.	Duration of observation.	Temperature of chamber.	CO <sub>2</sub> per minute and kilo.	Averages for CO <sub>2</sub> .	Remarks.
125.5	Dry	30	33	0.064	0.0625	In dry air 90 min.
	Dry	30	....	0.061		Interval of 15 min.
	Wet	30	35	0.057	0.0620	In wet air 120 min.
	Wet	30	....	0.064		
Wet	30	....	0.071			
108.2	Dry	30	33	0.060	0.0525	In dry air 90 min.
	Dry	30	....	0.045		Interval of 15 min.
	Wet	30	34	0.054	0.049	In wet air 90 min.
	Wet	30	....	0.045		
131.1	Dry	30	34	0.058	0.0625	In dry air 90 min.
	Dry	30	....	0.067		Interval 15 min.
	Wet	40	35	0.057	0.055	In moist air 90 min.
	Wet	30	....	0.053		

At the start in these experiments the chamber did not of course contain sulphuric acid but had been thoroughly dried before placing the rat in it. It will be seen that, so far as the carbonic acid excretion is concerned, there is no difference between the wet and the dry air experiments. In general the highest results both in wet and in dry air are obtained when the temperature of the chamber is high, but the relationship of body weight to the excretion is, as in the rest experiments, not evident. As a general average the excretion of carbonic acid during work is twice as great as during rest. It is surprising that the increased tissue combustion, which this points to, did not cause a much more rapid rise in rectal temperature.

CONCLUSION.

The deleterious influence of a hot humid atmosphere as contrasted with a dry one at the same temperature is not evident in the case of small animals such as rats.<sup>1</sup> This is because such animals do not depend, to any extent at least, on evaporation of moisture in regulating the heat loss from their bodies.

<sup>1</sup> Such animals, therefore, will not be able to withstand very hot, dry atmospheres as well as man can.

# THE RELATION OF THE ACTIVITY OF THE EXCISED MAMMALIAN HEART TO PRESSURE IN THE CORONARY VESSELS AND TO ITS NUTRITION.

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## INTRODUCTION.

THE experiments reported in this paper were begun primarily to test the relative efficiency of various inorganic salt solutions on the isolated mammalian heart under similar conditions of temperature and pressure, with a view to determining the most suitable fluid and the optimum conditions for restoring the mammalian heart to efficiency *in situ*. The results of Porter<sup>1</sup> and his pupils, of Langendorff,<sup>2</sup> and other workers, as well as observations of our own on mammalian hearts after restoration, both isolated and *in situ*, had led to the belief that the activity of the isolated mammalian heart bore a close relation to the blood pressure in the coronary arteries. This question was re-investigated by direct experiments on the excised heart.

<sup>1</sup> PORTER: This journal, 1898, i, p. 511.

<sup>2</sup> LANGENDORFF: Archiv für die gesammte Physiologie, 1895, lxi, p. 291.

Porter<sup>1</sup> and Langendorff<sup>2</sup> have recently given reviews of the literature. Only a few papers will be referred to in connection with various special points as they arise in the paper. Two preliminary notes<sup>3</sup> have appeared.

#### METHODS.

1. **The regulation of the pressure.**— A standpipe two metres high furnished the pressure for injection. It was provided with an inlet tube connected with the water faucet and an outflow tube at the bottom, and overflow pipes at intervals of 20 cm. along the side, the first being 60 cm. above the outflow pipe. The outflow tube led from the bottom of the standpipe to a large glass bottle filled with air. Air pressure was transmitted through tubing from this large bottle to the bottle containing the fluid used for perfusion. The height of the column of water in the standpipe was regulated by opening or closing the orifice at the lower end of the overflow pipes, which were continued downward from the point where they left the standpipe to terminate at a uniform level below the opening of the outflow tube. Opening all the overflow pipes leaving the standpipe above a certain level, or closing all below this level, gave a column of water in the standpipe of any desired height, and permitted rapid changes of pressure, either from low to high or conversely. By making the inflow from the faucet larger than any possible outflow through the pressure bottle, the height of the column could be made constant to within a few millimetres of water. If necessary, the height of the water column could be increased 60 or 70 cm. by lowering the pressure bottle below the lower end of the standpipe. The pressure actually used for perfusion was, in most cases, recorded on a drum by a mercury manometer connected with the perfusion cannula in the coronary artery. The pressure used varied from 40 mm. to 240 mm. of mercury.

2. **The regulation of the temperature.**— A tank holding about five gallons was elevated above the table sufficiently to allow water to flow from an opening in the bottom of it into the top of the jacket of a large condenser. The outflow was from the bottom of the jacket. The tank was filled with warm water from the laboratory supply. If necessary, the water in the tank was heated by burners

<sup>1</sup> PORTER: American text-book of physiology, 1900, i, p. 179.

<sup>2</sup> LANGENDORFF: *Ergebnisse der Physiologie*, 1905, abth. 2, p. 764.

<sup>3</sup> GUTHRIE and PIKE: *Science*, 1906, N. S., xxiv, p. 52; *Biophysikalisches Centralblatt*, 1906, ii, p. 151.

placed beneath it. Thermometers were placed in the outlet tube from the tank and in the condenser. The relatively large volume of the water in the tank prevented any great or rapid changes in temperature. The fluid used for perfusion was led from the bottle containing it through the worm of the condenser and then through the glass tubes with rubber connections to the heart to be perfused. The bulb of a third thermometer was placed in the fluid at the mouth of the injection cannula.

3. **The preparation of the heart.** — The animals were etherized and bled until respiration ceased. The heart was then rapidly excised and the pericardium removed, the blood being defibrinated in the meantime. If the heart was sufficiently large, as in dogs, cats, and rabbits, a cannula was usually introduced into the anterior coronary artery (following Porter's method), although sometimes into the aorta. In the case of very small hearts, *e. g.*, of guinea pigs and kittens, the cannula was introduced into the aorta. In a few experiments the cannula was tied into the coronary sinus and the perfusion made through the coronary veins (after the method of Porter and Pratt<sup>1</sup>).

The heart was suspended by the base and connected with a writing lever by a thread usually attached near the apices of the ventricles. In some experiments auricular tracings also were taken by a second lever. The fluid used was applied to the exterior of the heart before perfusion was begun. During the perfusion the heart was usually kept sufficiently wet by fluid escaping from the coronary vessels, but, when necessary, its outer surface was moistened with it. The hearts of guinea pigs, rabbits, cats, and dogs were used, but most of the experiments were done on cats' hearts. A few turtle hearts were used for comparison.

4. **The preparation of the fluids.** — (*a*) Of the various current salt solutions, four were mainly used. The formulas are given below.

HOWELL AND GREENE'S SOLUTIONS.

	Per cent.	Gm. per litre.
I. <sup>2</sup> NaCl . . . .	0.700	7.00
CaCl <sub>2</sub> . . . .	0.026	0.26
KCl. . . . .	0.030	0.30
II. NaCl . . . .	0.700	7.00
CaCl <sub>2</sub> . . . .	0.066	0.66
KCl. . . . .	0.040	0.40

<sup>1</sup> PRATT: This journal, 1898, i, p. 86.

<sup>2</sup> GREENE: American journal of physiology, 1898, ii, p. 106.

LOCKE'S<sup>1</sup> SOLUTION.

	Per cent.	Gm. per litre.
I. NaCl . . . .	0.900	9.000
Dextrose . . . .	0.100	1.000
CaCl <sub>2</sub> . . . .	0.020	0.200
KCl . . . .	0.020	0.200

II. The same solution, with the addition of 0.1 gm. of sodium bicarbonate per litre.

Several of Ringer's solutions and 0.9 per cent sodium chloride were used for comparison. As a rule, no special technique was employed for oxygenating the solutions other than shaking them thoroughly and letting them stand in contact with the air. Locke's and similar solutions (also blood dilutions) were in some cases oxygenated by oxygen at atmospheric pressure and the heart was kept in an atmosphere of oxygen. Only slightly better results were obtained than by the usual technique.

(b) Blood and serum dilutions, and the proteid containing fluids. — The defibrinated blood of the animal, diluted with three to ten volumes of 0.9 per cent sodium chloride solution, was more frequently employed than any other fluid.

Washed red blood corpuscles in 0.9 per cent sodium chloride, and blood serum as free from corpuscles as possible, each diluted with several volumes of 0.9 per cent sodium chloride, were also used. The relative proportion of corpuscles in the washed corpuscle suspension, and the relative proportion of serum in the serum mixture, were the same as in the defibrinated blood diluted 1 to 5.

A hæmoglobin solution, obtained by laking the red corpuscles by drying<sup>2</sup> them at room temperature and extracting with 0.9 per cent sodium chloride solution, was employed in one experiment.

A solution containing the inorganic salts of the blood serum was made by diluting 250 c.c. of ox serum to one litre with distilled water, boiling and filtering. The filtrate was evaporated to dryness, the residue made up to 250 gm. with distilled water, extracted for twenty-four hours, and again filtered. This second filtrate was used for perfusion.

Egg-white was dissolved in 0.9 per cent sodium chloride solution and used in a few experiments, but with unsatisfactory results.

<sup>1</sup> LOCKE: Centralblatt für Physiologie, 1901, xiv, p. 672.

<sup>2</sup> GUTHRIE: American journal of physiology, 1903, vii, p. 241.

Another fluid was made from cow's milk. The milk was allowed to stand on ice for some hours, after which the portion below the cream was siphoned off, strongly acidified with hydrochloric acid to precipitate the casein, and filtered. The filtrate was then rendered slightly alkaline with sodium carbonate. Before using, the alkaline filtrate was diluted by adding to it about three volumes of 0.9 per cent sodium chloride solution in order to bring down the concentration of the inorganic constituents other than sodium and chlorine, as the former exist in milk in greater concentration than in blood serum. The dilution also increases the proportion of sodium and chlorine, which exist in a much lower concentration in milk than in serum. To determine the effect of heating on the action of milk prepared in this way, a part of the filtrate was heated to 85° or 100° C. on a water bath for an hour or more and again filtered. This second filtrate, diluted as above with 0.9 per cent sodium chloride solution, was then used for perfusion. The milk used was obtained from a very large dairy, and was therefore of average and nearly constant composition.

It is not difficult to see, from the analyses of milk given by König and Söldner,<sup>1</sup> that the inorganic constituents, with the exception of magnesium oxide and phosphorus pentoxide, would be present in our fluid in about the same proportion found in the artificial salt solutions. Sugar would be present in a considerably greater amount than in Locke's solution, and of a different kind, namely, lactose.

Washed hydrogen gas, prepared from metallic zinc and sulphuric acid, and commercial paraffin oil were the only fluids employed which were free from inorganic salts.

#### THE NUTRITION OF THE EXCISED MAMMALIAN HEART.

1. **The action of the salt solutions.** — All the salt solutions employed produced beats more or less rhythmical in character, which terminated in a comparatively short time, apparently either through the production of rigor in the ventricles or from exhaustion of the heart. Delirium cordis is relatively easy to produce by means of these solutions. The beat of the heart is, in general, more rapid and far more irregular than with fluids containing serum proteid. The external application of these solutions to the heart almost invariably produced contractions, if the heart had not been excised too long (Fig. 1).

<sup>1</sup> Cited by HAMMARSTEN: Text-book of physiological chemistry, fourth English edition, 1904, pp. 448 *et seq.*



Locke's solution, containing 0.01 per cent of sodium bicarbonate, has given better results than any of the others employed. The relation of the hypotonicity<sup>1</sup> of Howell and Greene's solutions to their stimulating effect, as well as their composition, must be considered in forming an opinion of their action on the mammalian heart, and the

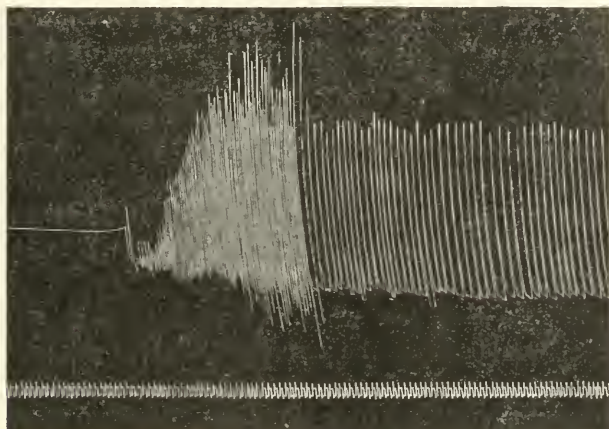


FIGURE 1.—Cat's heart. The injection cannula was full of Locke's solution when connected with the tube from the milk reservoir. The first beats are due to the Locke's solution. Up strokes indicate systole, down strokes diastole. Time trace in seconds.

relative isotonicity of Locke's solution may be one reason why it conserves the beat of the mammalian heart longer than the other salt solutions used.

2. **The effect of blood and serum dilutions.**—Defibrinated blood, diluted with three to ten volumes of 0.9 per cent sodium chloride solution, conserved the beat of the heart for long periods of time, usually longer, in fact, than we cared to continue the experiment. The ventricles did not go into rigor, and delirium cordis never resulted at ordinary injection pressures.

Serum, freed from corpuscles and diluted with 0.9 per cent sodium chloride, gave results as good as were obtained by the use of dilutions of defibrinated blood.

Red blood corpuscles, washed free from serum and mixed with several volumes of 0.9 per cent sodium chloride, did not give as good results as dilutions of defibrinated blood or of serum alone (Fig. 2).

The suspension of laked corpuscles did not give as good results as

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 351.

the other fluids, and caused much trouble by clotting during perfusion. The fluids most frequently used were defibrinated blood and serum dilutions.

All the defibrinated blood or serum mixtures produced rhythmical and co-ordinated beats, slower, as a rule, than resulted from perfusion with the salt solutions, and they continued for a much longer time.

The aqueous serum extract, although doubtless containing some proteid, did not sustain the activity of the excised heart as long as the unheated serum. The action of the heart, as regards regularity and co-ordination of the beat, resembled more closely that following perfusion with Locke's solution than that produced by perfusion with the blood dilutions. Greene<sup>1</sup> has used a similar aqueous extract of the serum salts of the terrapin on strips of terrapin's ventricle. The action was more like that of diluted serum than that of Ringer's solution. Undiluted serum produced no beats.

3. **The effect of milk and whey.**—Von Ott<sup>2</sup> first showed that milk and whey would sustain the activity of the frog's heart. Ringer,<sup>3</sup> using 1 to 3 c.c. of milk to each 100 c.c. of saline solution, kept a frog's heart beating regularly for a long time. Bufalini and Torsellini<sup>4</sup> found that milk and whey, even when dialyzed, neutralized, and made up to the normal sodium content, were toxic to the turtle's heart. The original paper is not accessible to us, and we are unable to learn whether or not the milk was diluted. As will be pointed out below, undiluted milk will not long sustain the beats of the mammalian heart.

Howell and Cooke<sup>5</sup> used an aqueous extract of dried milk, which contained the inorganic salts and the sugar, and were thereby able to sustain contractions of the frog's heart for considerable periods.

Milk containing caseinogen is unsuitable for perfusion of the mammalian heart, for the reason that the caseinogen coagulates in the coronary vessels and seriously interferes with the perfusion.<sup>6</sup> It

<sup>1</sup> GREENE: American journal of physiology, 1898, ii, p. 111.

<sup>2</sup> KRONECKER: Archiv für Physiologie, 1881, p. 569; V. OTT, *Ibid*, 1883, p. 1.

<sup>3</sup> RINGER: Journal of physiology, 1885, vi, p. 364.

<sup>4</sup> BUFALINI and TORSSELLINI: Bolletino dalla Societa tra i cultori di scienze mediche, Anno IV, No. 5, Sienna, 1886: cited by FINN, Zeitschrift für Biologie, 1905, xxix, p. 329.

<sup>5</sup> HOWELL and COOKE: Journal of physiology, 1893, xiv, p. 209.

<sup>6</sup> EDMUNDS: Journal of physiology, 1896, xix, pp. 466-476, has found milk-curdling ferments in testis, liver, kidney, brain, mesenteric lymph glands, thyroid gland, small intestine, ovary, and in a blood clot. Judging from the behavior of milk containing caseinogen, the presence of such a ferment in mammalian heart-tissue seems probable.

has been found, also, that if the undiluted whey fluid, after precipitation of the casein and neutralization of the hydrochloric acid, is injected, the activity of the heart is not long maintained. The fluid prepared as previously described gives results more nearly approximating those of blood and serum dilutions than any other fluid employed. The beats were strong, rhythmical, well co-ordinated, and maintained as long as we desired to continue the experiment (Fig. 1). The left ventricle did not go into rigor, even after being for twenty-

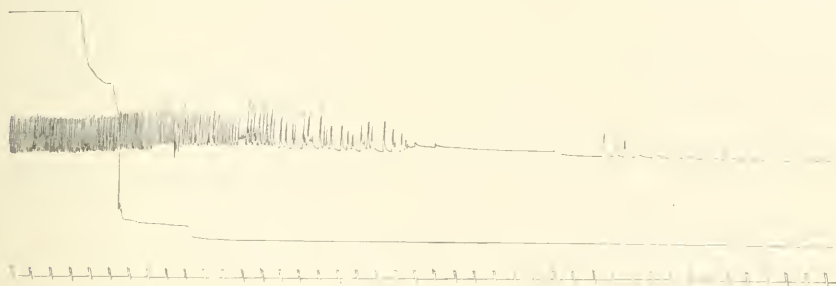


FIGURE 2. — Two-thirds the original size. Kitten's heart. Perfusion with suspension of washed corpuscles in 0.9 per cent NaCl. Note the secondary series of beats following complete stoppage of the heart after the pressure has fallen to the base line. Time trace in seconds.

four hours and more in contact with the solution. If the fluid is heated to  $85^{\circ}$  to  $100^{\circ}$  C. and filtered, the filtrate does not maintain the activity of the heart as long as the unheated fluid, and rigor of the left ventricle is more likely to come on. The external application of the milk preparation to the heart did not cause contractions.

4. **The effect of hydrogen gas and paraffin oil.** — The injection of hydrogen gas<sup>1</sup> into the coronary arteries was sufficient to cause rhythmical beats, not very strong nor long maintained, but fairly well co-ordinated and regular. The injection of paraffin oil into the coronary arteries, in accordance with Sollmann's<sup>2</sup> statement, produced rhythmical contractions. We did not, however, first perfuse the heart with Locke's solution, as Sollmann did. There could be, therefore, in our experiments no introduction of inorganic salts into the coronary arteries except such as were already present in the tissues.

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

<sup>2</sup> SOLLMANN: *This journal*, 1906, xv, p. 121.

THE RELATION OF THE ACTIVITY OF THE EXCISED HEART TO  
PRESSURE IN THE CORONARY VESSELS.

Tschirjew<sup>1</sup> found that increase of intra-cardiac pressure up to a certain limit produced in most cases a more rapid rate of the isolated frog's heart. Ludwig and Luchsinger<sup>2</sup> found that the pulse rate of the isolated frog's heart increased with pressure. This was more particularly true of the heart deprived of its sinus and of the ganglion-free apex. Sewall and Donaldson,<sup>3</sup> working with the isolated frog's heart, failed to get any noticeable variation in rate with change of intra-cardiac pressure. Stewart<sup>4</sup> states that in the isolated frog's heart *in situ* the rate is not generally affected by change of pressure.

Martin<sup>5</sup> found that variations in the blood pressure did not affect the rate of the dog's heart after it was severed from all connection with the body except the connection with the lungs. The cause of Martin's failure to obtain a change in the rate with a change in pressure will later be considered in detail.

Magrath and Kennedy,<sup>6</sup> working with the isolated heart of the cat *in situ* and using defibrinated blood for perfusion, were unable to get any considerable change in the rate with change in pressure in the coronary arteries. The pressures which they used in their experiments (50 to 90 mm. mercury) were, in general, lower than those which we used, and lower than the normal blood pressure of a vigorous cat. As the normal cat's heart is capable of maintaining a blood pressure of 150 to 200 mm. of mercury, it is possible that their range of pressure was not great enough to give the change in rate.

With Locke's solution and other salt solutions, the heart becomes arrhythmical, and, as stated above, goes into delirium cordis at comparatively low pressures, and the beat soon becomes unco-ordinated, so that change in rate with change in pressure is not so noticeable. With albuminous fluids such as blood and its dilutions and the milk preparation, and also with paraffin oil, the change of rate with change of pressure at constant temperature is striking.

<sup>1</sup> TSCHIRJEW: Archiv für Anatomie und Physiologie, 1877, p. 180.

<sup>2</sup> LUDWIG and LUCHSINGER: Centralblatt für die medicinische Wissenschaften, 1879, p. 404.

<sup>3</sup> SEWALL and DONALDSON: Journal of physiology, 1882, iii, p. 357.

<sup>4</sup> STEWART: Journal of physiology, 1892, xiii, p. 140.

<sup>5</sup> MARTIN: Collected physiological papers, 1895, p. 25; cited by HOWELL: Text-book, 1905, p. 528.

<sup>6</sup> MAGRATH and KENNEDY: Journal of experimental medicine, 1897, ii, p. 13.

Starting with a low pressure, the beat has at first a slow rhythm with a comparatively small amplitude. As the pressure is gradually increased, the rate becomes higher, and the strength of the contraction increases until a certain pressure, which we shall call the optimum pressure, is reached, at which there exists the maximum rate without a decrease in amplitude. If the pressure is increased still more, the rate becomes higher, but the amplitude is decreased. As the optimum pressure of injection is exceeded, the diastole of the heart becomes less and less complete, until there is produced a tracing bearing a striking resemblance to the genesis of tetanus in skeletal muscle. Delirium cordis may result if a very high pressure is used.

The relation between pressure of injection and rate of beat may best be shown numerically. This relation is as follows: —

Pressure in mm. of mercury.	Rate of beat per minute.
90	90
140	138
156	162
175	204
104	96

Variation in pressure, 94.4 per cent. Variation in rate, 126.6 per cent.

With Locke's solution, delirium cordis may be induced at a pressure often lower than the normal blood pressure of the animal. Working with blood dilutions, we have induced a very high rate at high pressures in the hearts of adult cats and dogs, but have only occasionally succeeded in inducing delirium cordis. With kittens' hearts we have been able to produce delirium cordis even with the blood dilutions. If the pressure, after it has passed the optimum, be reduced, the rhythm becomes slower and the amplitude greater until the optimum is reached, below which, as the pressure falls still lower, there is a concomitant decrease in amplitude along with the change in pressure. The rate and the amplitude at corresponding pressures when rising and falling agree very closely. Extreme pressures sometimes rupture the vessels so that the heart does not recover very well when the pressure is lowered.

At low pressure, when the heart is first beginning to beat, there is a gradual increase in amplitude until a maximum for that pressure is reached, when there appears in the tracing a second beat, each one occurring between two of the stronger beats, barely rising above the base line at first, but gradually becoming stronger until it equals in

amplitude the original beat. On lowering the pressure, the second beat appears in inverse order, becoming gradually weaker until it ceases, after which the remaining beat diminishes in amplitude until it also finally ceases. This double beat is shown in the tracings of Magrath and Kennedy,<sup>1</sup> and has also been observed by Cushny;<sup>2</sup> and Mathews,<sup>3</sup> following the administration of substances of the digitalis series and of aconitin respectively. Dr. Lingle<sup>4</sup> has observed it in strips of the turtle's heart.

When the pressure of the injected fluid is reduced to about zero, the beat of the heart entirely stops for some minutes, following which there sometimes occurs a series of peculiar beats (Fig. 2). Finally these beats cease, and no more appear until the pressure of the injected fluid is again raised to a suitable level. This secondary group of heart beats bears a striking analogy to the "secondary" group of respirations of the Cheyne-Stokes type following cerebral anæmia.<sup>5</sup>

The optimum pressure for the heart varies with the temperature and nature of the fluid, with the individuality of the heart, and also at different times during the experiment with the same heart. A higher pressure is generally needed to produce the same effect two hours after perfusion is begun than at the start. With the blood or serum dilutions or milk the optimum pressure for hearts in good condition is not far from the normal blood pressure of the animal, and the rhythm at this optimum pressure is very nearly the normal cardiac rhythm of the animal. On the other hand, the optimum pressure for the salt solutions is less than the normal blood pressure. If the perfusion is begun before the heart has ceased to beat, a very low pressure is the optimum, and the change in rate with change in pressure is not so apparent.

A sudden and great fall from high to low pressure may be followed in the excised heart (*a*) by a sudden slowing or even stoppage of the heart followed by the same rhythm previously observed for that pressure, or (*b*) by a very rapid rhythm which soon decreases to that previously observed for that pressure.

<sup>1</sup> MAGRATH and KENNEDY: *Loc. cit.*

<sup>2</sup> CUSHNY: *Journal of experimental medicine*, 1897, ii, p. 233.

<sup>3</sup> MATHEWS: *Ibid.*, p. 593.

<sup>4</sup> Personally communicated.

<sup>5</sup> STEWART, GUTHRIE, BURNS, and PIKE: *Journal of experimental medicine*, 1906, viii, p. 300.

The first result bears a striking resemblance to partial or complete vagus inhibition in the intact heart, and the second is much like the acceleration of the heart *in situ* which follows a sudden fall in blood pressure in the intact animal. Stewart<sup>1</sup> has also observed the stand-still in diastole of the isolated frog's heart when the intra-cardiac pressure is suddenly lowered.

The change in rate is due to change in pressure alone, and not to a concomitant change in temperature. An increase of pressure produces an increase in rate even where the temperature of the perfused fluid is falling.

PERFUSION OF THE EXCISED HEART THROUGH THE CORONARY VEINS.

The cannula was inserted into the sinus, and the fluid escaped through the coronary arteries or from the capillaries when the ventricles were freely incised. When blood and its dilutions were perfused, rhythmical beats of the heart occurred. The character of the beat differs somewhat from that produced by perfusion through the coronary arteries. The amplitude is less, and the rate greater than would be produced by the same pressure in the coronary artery. The relation between the rate and the pressure in the vein in a typical experiment is as follows :

Pressure in mm. of mercury.	Beats per minute.
70	39
76	57
92	66
102	78
116	120
128	126
204	144

Variation in pressure, 191.4 per cent. Variation in rate, 270 per cent.

From the above facts we conclude (*a*) that the presence of a suitable fluid somewhere in the coronary circulation at a suitable pressure is sufficient to cause rhythmical beats of a heart in proper condition. (*b*) The fact that the pressure in the coronary vein necessary to produce beats must be high enough to permit the passage of the fluid into the capillaries is strong evidence that it is the presence or pressure of the blood in the capillaries, and not its passage along or pressure in the coronary artery or vein, which is the essential thing to produce rhythmical beats of the heart.

<sup>1</sup> STEWART: *Loc. cit.*, pp. 143, 153.

## THE EFFECT OF TEMPERATURE.

No attempt was made to study exhaustively the effect of temperature on the heart, but some of the phenomena incidentally observed in the course of the experiments are given here.

The effect of temperature on the excised mammalian heart is probably more marked than on the frog's heart. The heart of the mammal may not work at all at a given pressure if the temperature is low, but as the temperature is raised, it rather suddenly begins to beat. As the temperature is raised still more, the pressure remaining constant, the beat becomes faster until the point of heat standstill is reached. The most favorable temperatures for the excised mammalian heart appear to lie between  $33^{\circ}$  and  $37.5^{\circ}$  C. Heat standstill in diastole of the heart occurs at about  $38.5^{\circ}$  to  $40^{\circ}$  C. when the milk preparation is used.

These limits are considerably narrower than those found by Langendorff and Nawrocki,<sup>1</sup> who obtained beats of the cat's heart at temperatures of  $46^{\circ}$  C. and above, and as low as  $20^{\circ}$  C. when blood dilutions were used for perfusion. The effect of the general nutrition of the excised heart upon its resistance to change in temperature and to heat standstill may be one further cause of the difference in result. It is not probable that the nutrition of the heart is as good when the milk preparation, particularly after heating, is used for perfusion as when blood dilutions are used, and because of this poorer nutrition, it is possible that the heart may succumb the easier to the higher temperatures. Although our observations on Locke's fluid are too few to warrant drawing conclusions, there are some indications that it causes heat standstill at lower temperatures than blood.

Robertson,<sup>2</sup> from a study of the relation between temperature and the rate of heart beat in a crustacean, concludes that a chemical reaction is involved in the rhythmically contracting heart.

Gaskell<sup>3</sup> has supposed that, in the perfectly quiet frog's heart, stimulation of the augmentor nerves cannot elicit beats, and that when the heart is apparently roused to activity, slight beats of the sinus or auricles have been going on with blocking of conduction at the auriculo-ventricular groove. Whether this is correct or not for

<sup>1</sup> LANGENDORFF: *Archiv für die gesammte Physiologie*, 1897, lxvi, p. 355

<sup>2</sup> ROBERTSON: *Biological bulletin*, 1906, x, p. 242.

<sup>3</sup> GASKELL: SCHAEFER'S *Text-book*, 1900, ii, p. 217; see also HERING: *Archiv für die gesammte Physiologie*, 1906, cxv, p. 354.



the frog's heart, we can state that it does not hold for the cat's heart in heat standstill at the minimum temperature necessary to produce the standstill. There are no beats of the auricles of the cat's heart in this condition, but the whole heart is completely stopped. Furthermore, we have many times caused contractions of the completely quiescent cat's or dog's heart by electrical or mechanical stimulation of the *nervi accelerantes*.

Recovery from heat standstill, when milk or serum is used for perfusion, is entirely possible if the temperature be lowered, and the



FIGURE 3.—One-half the original size. Heat-standstill of cat's heart. Milk solution used for perfusion had previously been heated to 85° C. Temperature at mouth of perfusion cannula was 39° C. when heart stopped. Temperature 36.5° C. when heart again started to beat. Pressure remained constant. Time trace in seconds.

heart may beat strongly and regularly afterward for an hour or more (Fig. 3). Heat rigor of the ventricles does not occur in heat standstill if the temperature is not permitted to go above 40° C. when the proteid fluids are used for perfusion. We confirm Herlitzka<sup>1</sup> on this point.

Although Sollmann<sup>2</sup> neither looked for nor noted the connection between pressure in the coronary arteries and the rate of the heart beat, an inspection of his protocols shows this effect of pressure, but the accompanying changes of temperature obscure it to some extent.

#### MISCELLANEOUS RESULTS, AND DISCUSSION.

The difference between the reaction to pressure of the intact heart *in situ* and of the excised heart is striking. It is well known that in the normal animal with the cardiac nerves intact a high blood pressure is associated with a slow cardiac rhythm.<sup>3</sup> Nawrocki found that after section of both vagi and cervical sympathetics, changes in blood

<sup>1</sup> HERLITZKA: *Zeitschrift für allgemeine Physiologie*, 1905, v, p. 265.

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

<sup>3</sup> See AUBERT: HERMANN'S *Handbuch der Physiologie*, iv, 1 t, p. 397; and COLSON: *Archives de biologie*, 1890, x, p. 431, for the earlier literature. See also Herlitzka: *Archiv für die gesammte Physiologie*, 1903, cvii, p. 557.

pressure had no effect on the rate of the heart *in situ*. Tschirjew found that in most cases great and sudden changes in blood pressure affected the heart even after section of all the cardiac nerves. A fall of blood pressure caused an increase in pulse rate, and a rise of blood pressure caused a slowing of the heart. MacWilliam<sup>1</sup> states that the heart is insensitive to changes of blood pressure after complete section of the extrinsic cardiac nerves. We have produced great and rapid changes in pressure by occluding by means of a ligature and releasing the thoracic aorta of cats, and have found that after complete section of the extrinsic cardiac nerves there is either no change in the pulse rate, or an increase in the rate with a fall in pressure and a decrease in rate with a rise in pressure. Even when all the extrinsic nerves are cut, the heart *in situ* does not follow the law of the excised heart as regards pressure changes. The conclusion seems inevitable that there is a local controlling mechanism normally present in the heart *in situ* which is inactive in the excised heart under the conditions of our experiments. The nature of this controlling mechanism is unknown to us. From the well-known lower resistance of nervous tissue to injurious influences, *c. g.*, asphyxia, and the greater difficulty in keeping it active in excised organs, one is inclined to regard this controlling mechanism as nervous in nature. On this view, the greater stability of the rhythm of the heart *in situ* in the face of changes of pressure would be due to an intrinsic nervous mechanism which Kaiser<sup>2</sup> postulates.

It is obvious, also, from a consideration of the response of the excised heart to pressure changes, that the all or none law fails to hold under the conditions of our experiments.

We have much evidence, obtained from a study of the heart *in situ*, that the loss of function of this mechanism, whatever it may be, in the excised heart, is closely connected with the temporary stoppage of the circulation. The rate of the heart *in situ*, when started by perfusion of a suitable fluid after comparatively long periods of stoppage, varies directly as the pressure in the aorta. The slow rate of the intact heart with high pressure is not due to the inability of the heart muscle to contract as rapidly against a high pressure as against a low pressure.<sup>3</sup>

<sup>1</sup> MACWILLIAM: British medical journal. 1904. ii. p. 739.

<sup>2</sup> KAISER: Zeitschrift für Biologie. 1893, xxix, p. 203; *Ibid.*, 1894, xxx, p. 279. See also HENDERSON: This journal. 1906, xvi, pp. 359-362.

<sup>3</sup> One of us (P) has found in a single experiment on a dog that, after section of all the extrinsic cardiac nerves and the intravenous injection of atropine in sufficient

If the conditions of the experiment are such that the activity of this controlling mechanism is conserved, increase in pressure of the perfused fluid will not cause an increase in the rate of the excised heart. These conditions may have been fulfilled in Martin's<sup>1</sup> experiments, and also in Magrath and Kennedy's<sup>2</sup> work. On this view, we can also understand why the isolated frog's heart *in situ* should fail to respond to changes of intra-cardiac pressure by a change in rate.

In some instances, when the excised heart has been beating slowly, the coronary arteries, or the tissues immediately surrounding them, have been observed to pulsate, and the beat to spread from the vessels over the ventricles. There is a peculiar shortening of the vessels, all drawing up toward the common point of origin of the larger vessels. If two strips of kitten's ventricle, the first one taken from the left ventricle so as to include longitudinal portions of the larger branches of the anterior coronary artery, and the second cut from the anterior margin of the right ventricle, but including longitudinal portions of the smaller vessels only, be suspended under a tension of a few grams' weight, attached to a muscle lever and irrigated with 0.9 per cent sodium chloride solution, the deportment of the two strips is very different. The first strip soon goes into tonus, the curve rising rather abruptly from the base line to a height which soon becomes uniform. Small, more or less rhythmical contractions may appear superposed on the tonus curve, but are not constant. The second strip shows no tonus phenomena at all, but soon begins to beat more or less irregularly (Fig. 4). Howell<sup>3</sup> has obtained rhythmical contractions similar to those exhibited by the second strip of ventricle from a strip of vena cava taken from the terrapin.

A longitudinal strip of a cat's thoracic or abdominal aorta, suspended under the same conditions as the ventricular strips, shows the same deportment as the first ventricular strip containing the longitudinal portions of the coronary artery. Irrigation with Howell and Greene's or Locke's solutions will produce the same tonus effect on the strip of aorta as irrigation with sodium chloride solution.

amounts to throw out the inhibitory function of the vagus, the heart *in situ* follows the law of the excised heart, showing a higher rate during high than during low blood pressure.

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

<sup>2</sup> MAGRATH and KENNEDY: *Loc. cit.*

<sup>3</sup> HOWELL: *American journal of physiology*, 1898, ii, p. 57.

The area at the anterior margin of the right ventricle manifests a peculiar deportment in certain other points. This area is apparently very sensitive to many different influences. It oftens remains contracted when other parts of the ventricles are relaxed, presenting the appearance of tonus. Contractions, more or less rhythmical in character, may be seen here when the rest of the heart is quiet. Occasionally, also, stimulation of the accelerators will cause contraction in this area when the rest of the heart fails to respond. On opening the thorax after asphyxiation of the animal, we have often noticed fibrillations in this area while no movement was visible in other parts of the heart.

Martius<sup>1</sup> showed that the frog's heart, after exhaustion from saline solution, could be revived by perfusion with saline solution containing 3 to 5 mg. of sodium carbonate in 100 c.c. Martius was of the opinion that after exhaustion from the second fluid the heart could be revived only by perfusion with a fluid containing albumin. Howell and Cooke<sup>2</sup> state that such a heart may be revived by perfusion with a saturated solution of calcium phosphate in physiological salt solution, to each 100 c.c. of which were added 3 c.c. of a one per cent solution of potassium chloride (Ringer's solution). Howell states that the beats of a heart perfused with Ringer's solution were not so normal as those of a heart perfused with serum. Hearts perfused with a solution of inorganic salts of serum, minus the extractives, showed a tendency to form Luciani's<sup>3</sup> groups, *i. e.*, alternate periods of contraction and rest. Göthlin,<sup>4</sup> working with frog's hearts, found that the addition of serum proteid to a solution of the inorganic salts of the blood (Serumsalzflüssigkeit) would restore the activity of the heart or prolong it for several hours. Similar results have been obtained by other observers, among them being White.<sup>5</sup> White answers Howell's contention by saying that the hearts Howell worked with were not fully exhausted in Martius' sense of the term, and reaffirms the statement of Martius that frogs' hearts which have ceased to beat on perfusion with Ringer's solution, and which do not respond to strong electrical stimulation, can be revived only by perfusion with a fluid containing proteid. In a later paper<sup>6</sup> Howell reaffirms his former position.

<sup>1</sup> MARTIUS: *Archiv für Physiologie*, 1882, p. 543.

<sup>2</sup> HOWELL and COOKE: *Journal of physiology*, 1893, xiv, p. 200.

<sup>3</sup> *Vide* HERMANN: *Handbuch der Physiologie*, iv, 1 t., p. 363.

<sup>4</sup> GÖTHLIN: *Skandinavisches Archiv für Physiologie*, 1902, xii, p. 1.

<sup>5</sup> WHITE: *Journal of physiology*, 1896, xix, p. 344.

<sup>6</sup> HOWELL: *American journal of physiology*, 1898, ii, p. 50.

Baglioni<sup>1</sup> has shown that the inorganic salts are incapable of maintaining the activity of the Selachian heart, but that the addition of two per cent of urea to the fluid used would give a solution capable of maintaining cardiac activity for a long time.

The mammalian heart is more sensitive to the action of salt solutions than the frog's or turtle's heart, and the irregularities are more noticeable than in the frog's heart. Group formation is particularly likely to occur with Locke's solution. If a heart which is exhausted

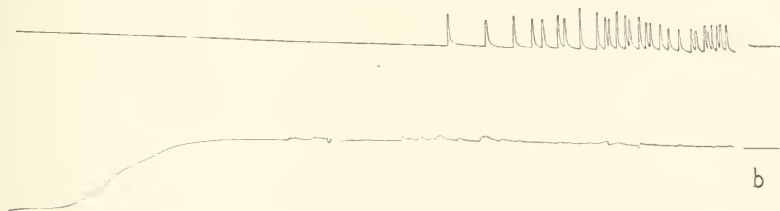


FIGURE 4. — One-half the original size. Two strips of kitten's ventricle suspended and irrigated with 0.9 per cent NaCl. The lower strip was cut so as to include a considerable portion of the anterior coronary artery. The upper strip was cut from the sensitive area at the anterior margin of the right ventricle. The relative conditions of the two strips some six hours later are shown at *b*.

from perfusion with Locke's solution, and which shows Luciani's groups, is perfused with blood or serum fluids, or with milk prepared as above, the groups may continue until the heart finally stops, or, in case of restoration of the heart, until replaced by a regular, rhythmical beat. If the perfusion with Locke's fluid is stopped for a time by removing the pressure, a more rhythmical beat may result on resuming the perfusion, but the groups soon reappear. When the mammalian heart is nearly exhausted and perfusion is begun with the milk dilution, one group of beats may follow at a given pressure, but on the cessation of the beats of this group the heart will not contract again until the pressure is raised, when another single group may follow, more or less tardily, the rise in pressure. The upper limit of pressure is soon reached, and no more groups follow a still further increase in pressure. There is, then, a certain limit following perfusion with salt solutions beyond which the mammalian heart is not readily revived by perfusion with serum or milk or other albumin-containing fluid, and this limit is apparently much lower than in the frog's heart.

<sup>1</sup> BAGLIONI: *Zeitschrift für Allgemeine Physiologie*, 1906, vi, p. 71.

These groups are not, as Gaskell<sup>1</sup> contends, due to a blocking of conduction from auricle to ventricle. The auricle of the cat's heart is sufficiently large to watch with a lens with a considerable degree of accuracy. There were no contractions of the auricles during the period intervening between any two successive groups of ventricular beats. Asynchrony of auricles and ventricles is very common in excised mammalian hearts during perfusion with artificial solutions, but Luciani's groups may occur independently of this asynchrony.

The difference in the rhythm and character of the beat produced by perfusion with Locke's solution, as compared with the beat produced by perfusion with such a fluid as the milk preparation, may be shown by leaving the injecting cannula full of Locke's solution and connecting with the reservoir containing the milk preparation. When the pressure is turned on, the first fluid passing through the heart is Locke's solution, and it is immediately followed by the milk. The pressure of injection being the same for both fluids, the contrast is somewhat striking (Fig. 1). The slower, more regular beat under the influence of the milk is sharply marked off from the rapid, irregular rhythm due to the Locke's solution. When the perfusion is started with the cannula full of milk, the first beats are as regular as any of the others.

It may be questioned whether hydrogen gas, as used by Magnus<sup>2</sup> and in our own experiments, is an indifferent substance for the heart. Hydrogen is slightly soluble in water and the other fluids of the body, and it may be objected that the hydrogen molecules in solution may have some chemical action, but it is improbable that this would account for the effect obtained.

Again, cottonseed oil, which undergoes metabolism in the tissues, may conceivably be acted upon by the ferments in the heart tissue in such a manner as to yield energy. While this is improbable, Sollmann's<sup>3</sup> argument for a physical factor in the cause of the heart beat would thus be weakened. When the heart is previously flushed out with Locke's solution, as in Sollmann's experiments, it may be objected that enough of the inorganic constituents remain in the coronary system to cause the heart to beat when perfused later with paraffin oil, though this is improbable. There is small reason for believing that paraffin oil itself is acted upon by the tissues, or acts

<sup>1</sup> GASKELL: SCHAEFER'S Text-book, 1900, ii, p. 227.

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

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

upon them, except in a purely physical or mechanical way. If no inorganic salts exist therein except such as are normally present in the tissues before perfusion, the evidence seems very strong that a purely mechanical stimulus, applied within the coronary vessels, will cause rhythmical beats of the excised heart. Howell and Cooke<sup>1</sup> state that "if one examines carefully the various experiments which have been made from time to time with the purpose of showing that blood does not act as a chemical stimulus to the heart, the objection may be justly made that they do not definitely exclude such stimulation."

On examining into the phenomena presented in our experiments, two general explanations seem open to us.

As it is impossible to get rid of all the lymph lying in the tissue spaces of the mammalian heart during the time of an experiment, we may first consider the relation of this lymph to the cause of the beat. When paraffin oil is used for perfusion, it is conceivable, and indeed probable, that the pressure of the lymph upon the muscle cells and nervous apparatus of the heart is increased. This increase of pressure may, in itself, so modify the nutrition of these cells that they again become capable of functioning. It is possible, also, that the lymph surrounding any particular cell may be driven away by the pressure, and a fresh supply come in to take its place. The source of such lymph, however, is problematical. The lymph might therefore be regarded as the direct cause of the heart beat, acting independently of the nature of the fluid exerting the pressure in the coronary arteries. Since the inorganic salts of the blood are present in lymph also, we cannot, on the above view, absolutely exclude them as a possible cause of the heart beat. Since the proteids are likewise present in lymph, it is equally impossible to exclude them from a similar rôle. A crucial experiment to determine whether the inorganic salts of the blood are the direct cause of the heart beat is therefore still lacking, but it appears to us that mere intra-vascular pressure can no longer be excluded as an indirect cause of the heart-beat under the conditions of our experiments.

As a second possibility, we may consider the intra-vascular pressure to act as the direct cause of the heart beat. When the cannula is inserted directly into the coronary artery, the pressure at the mouth of this artery, and probably in its larger branches also, is the same as

<sup>1</sup> HOWELL and COOKE : *Journal of physiology*, 1893, xiv, p. 218.

that in the cannula. Ceradini<sup>1</sup> showed that, during the ventricular systole, the semilunar valves do not, as was previously supposed, close the openings of the coronary arteries. Rebatel<sup>2</sup> and Porter<sup>3</sup> have made further studies of the coronary circulation. Porter has shown that, in the living animal, the pressure curve in the larger branches of the coronary arteries does not differ materially from the pressure curve in the carotid. The pressure conditions at the mouth of the coronary artery are, therefore, essentially alike in the intact and in the excised heart during perfusion. We may, then, assume that the whole coronary circulation is essentially the same in the two cases. Rebatel has measured the velocity of the flow through the coronary arteries and finds that there is a sudden decrease during systole. According to Porter, we may consider the intra-mural capillaries as empty at the close of the systole. During diastole these capillaries are opened, and the pressure is, for a time, very low. The capillaries do not long remain empty, for the blood from the larger branches of the coronaries rushes in, and the pressure rapidly rises. As above stated, we regard the circulation in the capillaries as the one necessary for the production of rhythmical beats, and the circulation in the arteries and veins as important only because it is a necessary condition for the circulation in the capillaries. There would come then, sometime during the diastole, a mechanical stimulus to contraction in the form of an increase of pressure in, and velocity of flow through, the intra-mural capillaries. This stimulus would have a certain threshold value which would in general increase as the experiment proceeded. (It has already been stated that, in general, a higher injection pressure is necessary to produce the same effect as the experiment proceeds.) As the pressure for injection is increased, it is not difficult to see that the intra-mural capillaries would be filled more rapidly, and that the pressure therein would reach the threshold value more quickly after the beginning of diastole, thus causing a contraction sooner than it would occur with a low injection pressure. The chief variation in the cardiac cycle is the variation in the length of diastole, and when the pulse rate is high the diastolic period is very short.<sup>4</sup>

<sup>1</sup> CERADINI: *Il Meccanismo delle valvole semilunari del cuore*, Gazzetta Medica Lombarda, 1871; Opere, ii, p. 437; Milan, 1906.

<sup>2</sup> REBATEL: *Recherches experimentales sur la circulation dans les arteres coronaires*, Paris, 1872.

<sup>3</sup> PORTER: *This journal*, 1898, i, p. 145.

<sup>4</sup> HENDERSON: *Loc. cit.*



The high injection pressure therefore, by causing the stimulus to reach its threshold value earlier in diastole, gives us a primal condition for a high rate, — the shortened diastolic period. As the pressure is increased still more, the impulse to contraction would come before the completion of diastole, and the amplitude of the beat be diminished because of the consequent incomplete relaxation of the ventricular walls. Finally, at extremely high pressures for injection, the stimulus to contraction would come almost at the instant diastole began. The relaxation of the ventricle would be very incomplete and the amplitude of the beat greatly decreased, but the rate would be very high. The tracing from a heart under these conditions bears a strong resemblance to the genesis of tetanus or even to incomplete tetanus.

At the highest pressure the amplitude of the systole, as well as the length of the diastole, is apparently greatly decreased. It becomes a question, therefore, as to whether the ventricles are able to contract fully and completely empty the intra-mural vessels when extreme pressures are used for injection. And furthermore, it is but a short step from this condition to delirium cordis. Indeed, the heart may be said to have been in delirium at the time of the highest pressure.

It is therefore not necessary, for purposes of explaining the origin of the beat and the regulation of the rate in the excised heart, to postulate any action of inorganic salts.

Since blood, although containing the inorganic salts, produces no contraction of the heart when applied externally, but does produce a rhythmical beat when perfused, at a suitable pressure, through the coronary vessels, it appears to us that the pressure alone may be a factor in producing the beat of the normal heart.

The pressure is, in all probability, not the only factor in the production of the normal heart beat, since the excised mammalian heart may continue to beat without perfusion for a time after removal from the body. Czermak and von Piotrowski<sup>1</sup> observed that the excised hearts of rabbits beat for periods varying from three and one-fourth minutes to thirty-six minutes after removal. The mean period for sixty hearts was eleven minutes and forty-six seconds. The pressure in the coronary arteries, if any existed, must have been extremely low, but that they were completely empty is not probable.

It should, however, be borne in mind that in the intact mammalian

<sup>1</sup> CZERMAK and VON PIOTROWSKI: Sitzungsberichte der Kaiserlichen Academie der Wissenschaften zu Wien, 1857. xxv, p. 431.

heart the stimulus necessary to be applied within the intra-mural capillaries to cause contraction has, in all probability, a vastly lower threshold value than in the excised heart after stoppage. The mere entrance of the blood into the intra-mural capillaries may be a sufficient stimulus to cause a contraction. At the moment of excision we must suppose this threshold value of the stimulus to be unchanged. There is still some blood in the coronary arteries, and this may find its way into the capillaries during diastole and afford a sufficient stimulus to contraction. But the heart tissue, either from the lack of oxygen or impaired nutrition, possibly from both, gradually loses its irritability until the threshold value of the stimulus necessary to cause contraction rises above that which the blood in the capillaries is able to exert, and the heart stops. Certain it is that an extremely low pressure for injection will give as great activity of an excised heart which has barely ceased to beat as a much higher pressure will evoke in the same heart some hours later.

If pressure is not the only factor concerned in the production of the heart beat, can we find a further important factor in the inorganic salts of the perfusion fluid? There is good indirect evidence to the contrary, and this is supported by observations on the effect of such solutions on the other tissues. As examples of such action, we may take the following instances.

Of the many attempts to produce artificial parthenogenesis of the eggs of the sea urchin and other lower forms of animal life, not one has as yet resulted in the development of a single individual to sexual maturity, and the rôle of the inorganic salts is still far from clear.

Attempts to maintain the activity of the reflex nervous centres of animals by perfusion with the salt solutions have resulted in failure. We have reviewed the literature on this subject in a previous paper,<sup>1</sup> to which the reader is referred for a fuller discussion.

As already stated, such solutions are not capable of maintaining the activity of the Selachian heart for more than a short time. In view of the abnormalities and irregularities appearing in the tracing of the excised mammalian heart under their influence when in aqueous solution, and of their general incompetence in maintaining other physiological functions, it is highly improbable that the inorganic salts of the blood are the direct cause of the normal heart beat.

In our opinion, there exists in the literature a certain amount of confusion between the actual cause of the heart beat and the mainte-

<sup>1</sup> GUTHRIE, PIKE, and STEWART: This journal, 1906, xvii, p. 344.

nance of a suitable condition which permits of beats, *i. e.*, the maintenance of normal nutrition of the organ. A similar confusion exists between the stimuli which elicit physiological actions of many other tissues and the factors on which their nutrition depends. That a chemical reaction is involved in the rhythmical contraction of the heart muscle<sup>1</sup> is scarcely to be questioned. That this reaction is the direct cause of the beat is not so evident. It is our opinion that the actions of the inorganic salts upon the heart need extend no further than their action in other tissues, in which they doubtless assist in the nutritive and general metabolic processes.

There are weighty reasons for considering that fluids act upon the heart in two ways: (1) Certain fluids, such as blood and its dilutions, and milk, properly prepared, are nutritive or physiological in their effects; (2) Certain other fluids are purely artificial or stimulating in their effects. We do not consider the action of fluids of this second class to be truly physiological.

The train of events following perfusion of the excised mammalian heart with the inorganic salt solutions is, we believe, not a true picture of the events occurring normally in the intact heart.

#### CONCLUSIONS.

1. Increase in pressure in the coronary arteries, up to a certain limit, causes a concomitant increase in rate and amplitude of beat in the excised mammalian heart. This increase in rate occurs with constant or even with falling temperature.

2. Increase in temperature, up to a certain limit, causes an increase in the rate of the excised mammalian heart. This increase occurs with constant pressure. The optimum temperature is from 35° to 37.5° C.

3. Heat standstill occurs at 39° to 40° C. The heart may recover from heat standstill.

4. Defibrinated blood and serum, diluted with 0.9 per cent sodium chloride solution, are the best of the fluids used for perfusing the excised mammalian heart. Serum, free from corpuscles, gives better results than corpuscles free from serum, each being diluted to the same degree. Milk whey, prepared by precipitating the casein with hydrochloric acid, and diluting it with about three volumes of 0.9 per cent sodium chloride solution, gives results closely approximating those obtained by perfusion with blood and serum dilutions.

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

5. The inorganic salt solutions do not maintain the activity of the excised mammalian heart as long as the albuminous fluids. Luciani's groups appear in the tracing, and the heart soon stops, presumably from exhaustion.

6. Aqueous extracts of serum salts, and milk whey after heating, produce results more like those caused by the inorganic salts than by the albuminous fluids.

7. The heart, after stoppage from the inorganic salt solutions, may often be restored by perfusion with the albuminous fluids.

8. Since blood produces no contractions until perfused through the coronary vessels at a suitable pressure, it is possible that there is a purely physical or mechanical element in the cause of the normal heart beat.

9. In a slowly beating excised heart, the coronary arteries or the tissues about them may sometimes be seen to contract and the beat to spread from the coronary vessels over the heart.

A strip of cat's thoracic or abdominal aorta, when suspended, behaves much like the first strip of ventricle.

10. A circulation in the capillaries of the coronary vessels is sufficient to produce beats of the excised heart, and pressure on the walls of the larger arteries or veins is not an absolutely essential condition.

We wish to acknowledge our obligation to our colleagues in the department of physiology, and particularly to Prof. G. N. Stewart, for many helpful suggestions during the progress of the work.

## AN APPARENT PHARMACOLOGICAL "ACTION AT A DISTANCE" BY METALS AND METALLOIDS.

BY A. P. MATHEWS.

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A SHORT note by Herbst,<sup>1</sup> in 1904, called attention to the fact that if a piece of silver or copper was placed in a dish with sea-urchin eggs some of the eggs put out fertilization membranes. A very minute quantity of the silver was sufficient to produce the result. Thus, if a dish which had had a coin in it was washed with only ordinary precautions and then a new lot of eggs put in it, some of the latter put out fertilization membranes. Silver was more efficient in producing membranes than copper. Minute traces of silver salts, the chloride or nitrate, produced the same effect as the coins. Herbst left it an open question whether this action of the metal was due to the ions of the metal or not.

This observation appeared in so many ways interesting that I took the matter up for the purpose of finding out what other metals would produce the same result, and how the metal acted. I have accordingly tested the action of several metals and metalloids upon the mature eggs of the starfish, *Asterias Forbesii*.

The method consisted in placing a piece of the carefully cleaned metal or coin in about 50 c.c. of sea water in a glass dish and then introducing a large number of eggs which had been in sea water maturing for about one hour, so that the eggs fell thickly all about the piece of metal. The eggs were then left quite undisturbed. The metals used were iron, silver, copper, lead, zinc, tin, platinum, gold, and mercury, and the metalloids were bromine and iodine. As I had no pure silver, a silver coin, a ten-cent piece or a quarter, was used instead.

<sup>1</sup> HERBST: *Mitteilungen aus dem Zoologischen Station zu Neapel*, 1904, xvi, pp. 445-457.

The result was as follows: with iron, zinc, lead, tin, gold, and platinum I got no fertilization membranes. The iron dissolved a good deal and killed the eggs near it. The lead, tin, zinc, nickel, gold, and platinum seemed not to affect the eggs at all, since they matured and lived, for several hours at least, when lying almost in contact with the metal. Eggs matured and lived in a tin vessel when the eggs were lying against the tin. Copper, silver, and mercury and the metalloids bromine and iodine caused the eggs to put out fertilization membranes. Of the metals, copper was the most powerful, silver came a little after it, and mercury was efficient only when the eggs were extremely sensitive. Iodine is as powerful apparently as copper, and bromine is not so good as iodine. We may, therefore, add mercury to the list of efficient metals, as given by Herbst, and iodine and bromine.

In working with the metalloids a slightly different procedure was adopted. A minute piece of iodine or a small drop of bromine was put in the midst of a lot of eggs. The eggs near the metalloid took up some of it and became strongly colored. A few of these eggs were then removed with a very fine pipette, washed in a little sea water, picked up again with the pipette, and allowed to fall among a new lot of eggs in fresh sea water. Each of the eggs which had the metalloid thus served as a minute source of the metalloid, and acted upon the other eggs in its immediate neighborhood. This method was adopted owing to the solubility of these metalloids when introduced in substance. So much of the iodine or bromine went into solution as to kill the eggs near by unless this method was used.

The time required after metal and eggs were brought together before the action began was in many cases very short, an effect being visible within a minute after the eggs were introduced. In other cases, however, a longer interval of five or ten minutes elapsed before the membranes began to appear. The time appeared to depend upon the sensitiveness of the eggs.

In no case were the whole or a majority of the eggs in the dish affected, as reported by Herbst. On the contrary, if the eggs were left quite undisturbed only those within a short distance of the metal were affected. Eggs a millimetre or more from the metal were very seldom acted upon. This difference from Herbst's results may be due to the starfish eggs being less sensitive, or to the fact that in his experiments the eggs were not left undisturbed, so that many eggs in succession came within the sphere of action of the wire.

The actual phenomena of the changes in the egg are interesting.

If the eggs are left entirely quiet, it will be seen that *the change in the egg is strictly polar*. Each egg near the wire puts out a fertilization membrane on the side toward the wire or piece of metal, and on the side farthest away from the metalloids. The picture thus obtained is very striking and is represented in the diagram in Fig. 1.

The membranes may ultimately extend clear around the egg, although this is more often not the case; but even when this happens, the membrane is always wider on the side toward the metal, or on the side away from the metalloid. When I first saw the eggs thus so strikingly oriented, I thought it a beautiful demonstration of the action of the metals in throwing off their ions and thus bombarding the eggs on the sides turned toward them, but I afterwards had to modify this interpretation.

The second interesting observation is that the change does not stop ordinarily with the extrusion of a membrane. A further change takes place in the protoplasm of the egg, and this change also is strictly polar. Thus the protoplasm on the side of the egg turned toward the metal, or away from the metalloid, begins to liquefy and swell, so that the egg becomes balloon-shaped; and it coagulates on the side turned *away* from the metal and *toward* the metalloid. Still later one may get secondary coagulation in the previously expanded portion in the side turned toward the metal.

What, then, is the means by which the metals produce these effects? When I started the investigation I felt confident that this would prove to be an action due to the ions of the metal. Herbst's statement that silver salts produced the same result seemed to show this. To settle the matter, I tried the effects of solutions of the salts of the metals, but to my astonishment, I got entirely negative results, so far as the extrusion of fertilization membranes was concerned. All of these metal salts if used in concentrations great enough to produce any effect at all, produced always a coagulation. There was never the slightest indication of liquefaction or of membrane formation. As

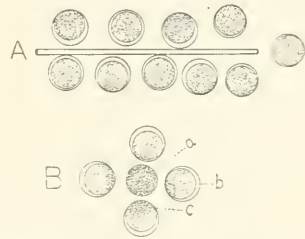


FIGURE 1.—*A*, copper wire surrounded by starfish eggs. Fertilization membranes are out on the side of the egg toward the wire. Slight coagulation in some eggs at a point farthest from wire, and liquefaction toward wire. *B*, starfish egg containing iodine at *a*, surrounded by other eggs extruding fertilization membranes, *b*, on the side farthest from *a*, and coagulating, *c*, on the side nearest *a*.

there was danger of overlooking some favorable concentration of the salts, I used a large number of different dilutions as follows :

1. Lead Salts,  $\text{PbCl}_2$ :  $\frac{m}{10,000}$ ,  $\frac{m}{20,000}$ ,  $\frac{m}{40,000}$ ,  $\frac{m}{80,000}$ ,  $\frac{m}{160,000}$ ,  $\frac{m}{320,000}$ ,  
 $\frac{m}{1,000,000}$ .
2. Copper Salts,  $\text{CuCl}_2$ :  $\frac{m}{200}$ ,  $\frac{m}{400}$ ,  $\frac{m}{500}$ ,  $\frac{m}{666}$ ,  $\frac{m}{1,333}$ ,  $\frac{m}{2,000}$ ,  $\frac{m}{4,000}$ ,  $\frac{m}{6,666}$ ,  
 $\frac{m}{10,000}$ ,  $\frac{m}{20,000}$ ,  $\frac{m}{40,000}$ ,  $\frac{m}{50,000}$ ,  $\frac{m}{66,666}$ ,  $\frac{m}{80,000}$ ,  $\frac{m}{100,000}$ ,  $\frac{m}{200,000}$ ,  $\frac{m}{400,000}$ .  
 $\frac{m}{160,000}$ ,  $\frac{m}{500,000}$ ,  $\frac{m}{666,666}$ ,  $\frac{m}{800,000}$ ,  $\frac{m}{1,000,000}$ ,  $\frac{m}{1,200,000}$ ,  $\frac{m}{2,000,000}$ .  
 $\frac{m}{3,200,000}$ ,  $\frac{m}{4,000,000}$ ,  $\frac{m}{5,000,000}$ ,  $\frac{m}{6,666,666}$ ,  $\frac{m}{10,000,000}$ ,  $\frac{m}{20,000,000}$ ,  
 $\frac{m}{40,000,000}$ .
3. Silver Salts,  $\text{AgCl}$ : Saturated solution in sea water and many dilutions from this.  $\text{AgNO}_3$ : in sea water,  $\frac{n}{2,000,000}$ ,  $\frac{n}{1,000,000}$ ,  $\frac{n}{500,000}$ .

As the above experiments gave a negative result, solutions of other salts were tried. These also resulted negatively. Thus in  $\text{CaCl}_2$ ,  $\frac{3}{8} m$ , maturation proceeds normally and some eggs segment into four cells, but no membranes appear.  $\text{MgCl}_2$ ,  $m \frac{3}{8}$ , permitted maturation, but no membranes were formed. In sea water containing manganese chloride,  $\frac{m}{200}$ ,  $\frac{m}{100}$ , and  $\frac{m}{500}$ , there were no membranes formed. The same result was obtained with hydrochloric acid. The eggs formed membranes when transferred from the acid to the sea water, but not while they were in the acid containing sea water. Potassium iodide, sodium sulphate, sodium citrate, and sodium hydrate were ineffective. Of the hydrate I added 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 c.c. respectively to 100 c.c. of sea water in different glasses. The eggs dissolved in the last glass, but no good membranes appeared. The dissolution of the peripheral layer of protoplasm looks somewhat like a process of membrane formation, but I do not think it identical with it.

*From these experiments it seems to be clear that the metals are not acting directly by their ions.* The ions of the metals in all cases caused coagulation, but the metals themselves always cause liquefaction first in the egg protoplasm nearest the metal. It is only after many minutes that a secondary coagulation may ensue, and this is apparently due to the action of the metallic ions, as is indicated by the light blue color of the coagulum in the case of the eggs near the copper wire. Furthermore, eggs which had been in the more dilute solutions of cupric salts were transferred to a dish containing a copper wire, when those eggs near the wire immediately put out their mem-



branes. Eggs from the stronger solutions put out no membranes either in the solution or when brought near a wire. Against the theory of the action of the ions the curious fact may also be recalled that, with the metalloids, the membrane is put out very quickly, but on the side farthest from the metalloid. For these reasons I feel compelled to relinquish the theory I started with, that the action is due to the ions thrown off from the metal.

The question arises, What, then, is the cause of the action? Two possibilities suggest themselves: first, that the metals throw off not their ions, but minute particles of the metals themselves, which hit the eggs and thus cause an action; or possibly there is an electrostatic field about the metal in water and this produces the effect.

In favor of the first supposition it may be said that certainly iodine and bromine do dissolve in this way. But the probability that silver, copper, and mercury are thus dissolving and that these particles come off with sufficient speed and in sufficient numbers to produce this action, appears to me exceedingly remote. The amount of silver thus dissolved in a metallic state in sea water must be almost infinitesimal, otherwise silver would give us solutions of metallic silver in water. Furthermore, it is improbable that these small particles would differ in the manner of action from the larger particles from which they are derived. This hypothesis appears hence very improbable.

In favor of the second hypothesis, that the metals are acting by means of an electrostatic field about them, there is this evidence: first, such a field is known to exist. When a metal is placed in water free from its salts, it throws off into the water positively charged ions in virtue of its solution tension. By this means the metal becomes electronegative, the water electropositive. By this means the further solution of the metal is prevented. Now this electrostatic field can have any great intensity only in the immediate neighborhood of the metal.

This would accord with the fact that only the eggs close to the wire, though not in contact with it, are affected. Eggs more than a fraction of a millimetre away are not affected at all. In the second place, platinum and gold, which have almost no electrostatic field about them, are ineffective. These metals have nearly as great a tendency to throw off negative as positive ions and remain hence almost uncharged. However iron and zinc, lead and tin, should be, on this theory, more efficient, unless some secondary cause comes into

play, since their solution tension is greater. In the case of zinc, lead, and tin the quick coating of the metal by a non-conducting sheath of oxychloride may interfere with their action. The ions of these metals certainly do not get into solution, since, although these ions are very poisonous, the eggs will develop for some time in contact with the metal without harm, thus showing that no ions get into the solution and consequently the electrostatic field is nil. As regards iron and the metals of higher solution tension, these metals throw their ions into the solution in sufficient numbers to poison the eggs. As these metals are able to discharge the hydrogen ion from the water, particularly in the presence of oxygen, it may be that their lack of action is due to this.

The hypothesis that the action is of an electrostatic kind is supported also by the polar nature of the change and by the fact that the membrane comes out on the side *toward* the electropositive metal and *away* from the electronegative metalloid, and coagulation takes place in each case at the opposite poles. This fact clearly points toward an electrical action of some kind. Furthermore electrical stimulation of the eggs will cause a closely similar polar change. I passed a series of make and break induction shocks with the secondary coil pushed up over the primary, through a lot of eggs. They put out fertilization membranes and ultimately liquefied at the two poles of the egg towards the electrodes. The membrane comes out most readily on the cathode side with make shocks, and on the same side, but far better, with break shocks. The membranes come out hence on the break of the current on the cathode side. I then diluted the sea water one-half with isosmotic glucose solution, introduced a fresh lot of eggs, and sent through them a constant current of about twenty-five volts. The membranes came out on passing the current on the anode side, and liquefaction took place here as reported by R. Lillie. This shows, therefore, that the passage of a constant current brings out the membrane on the anode side while the current passes, and on the cathode side on breaking the current.

It may be, therefore, that in introducing the egg into the electrostatic field about the metal the effect is the same as if a current of high intensity had been passed through it. The ions in the egg are separated, the positive ions being driven away from the metal, the negative ions toward it. By this means exactly the same separation of ions in the egg could be produced as is produced by electrical stimulation. The current would appear to pass through the egg from

the side toward the wire, *i. e.*, the anode side, and here liquefaction and membrane formation occur. Similarly for the metalloid the current would pass in the opposite direction, and here liquefaction and membrane formation take place on the side away from the metalloid.

The extrusion of the fertilization membrane when the eggs are transferred from acid containing sea water to normal sea water might be explained in the same way. The rapidly moving hydrogen ion striving to get into solution leaves the surface membrane of the egg electronegative, with the result that the membranes are thrown out and slight liquefaction takes place at the periphery.

I believe the electrostatic explanation of the action of the metals accords best with the phenomena observed. We have, if this be the true explanation, a real “action at a distance.” That is, the metal produces an action on the egg, although neither the metal itself nor any particles from it come in contact with the egg. The metal in this case would be exerting its action by means of the effect it produces upon the ions in the eggs lying in its immediate neighborhood, thus leading to a separation of these ions. It may be that these eggs would furnish a convenient means of demonstrating the separation of ions by electrostatic action.

Possibly the pharmacological action, the so-called oligodynamic action, of finely divided metals and colloidal solutions may involve this same principle and have a similar explanation, entirely independent of the action of the ions of the metal. Also, it occurs to me, that these observations may be of interest in connection with the formation of the cell asters. The phenomena in this case indicate some kind of an electrostatic action on the part of the astro-centres as pointed out by Hartog and R. S. Lillie. Possibly an investigation of these phenomena shown by metals when placed in water may lead to a better understanding of the method by which such an electrostatic action might be produced within the protoplasm itself and in the astral centres.

I hope to extend these observations further, and I publish them in an incomplete form in the hope that others also may examine these interesting phenomena.

#### SUMMARY.

1. Confirming Herbst, it is found that metallic silver and copper cause mature Echinoderm eggs to put out fertilization membranes.
2. The action takes place readily in the eggs of *Asterias Forbesii*. Only the eggs near the metal are affected.

3. Besides silver and copper, mercury, iodine, and bromine were found to produce membranes. Iron, nickel, lead, tin, platinum, and gold were ineffective. Hydrogen was doubtful. Mercury acts only when the eggs are very sensitive.

4. The change in the egg is strictly polar. Membranes appear first and liquefaction takes place later, on the side of the egg toward the metal and on the side of the egg away from the metalloid. Slight coagulation occurs on the side of the egg *away from* the metal, and marked coagulation on the side of the egg *toward* the metalloid.

5. Salts of the metals produced only coagulation when in concentrations strong enough to cause any action. The action of the metal is not due, hence, in this case to the action of ions of the metal.

6. The polar nature of the change, the quickness with which it occurs, and the identity of the changes with the changes caused by electrical stimulation indicate that the cause of the change is electrical.

7. The most probable explanation appears to be that the metal is producing its action by an electrostatic field about it, thus causing a separation of the ions in the egg, positive ions being driven away from the wire and negative ions being attracted toward it.

8. If this interpretation is correct, the metals are producing their action without any particle of the metal coming in contact with the egg, and in this sense the metals are acting at a distance from themselves.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE  
MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.  
E. L. MARK, DIRECTOR. No. 186.

## THE REACTIONS OF CYCLOPS TO LIGHT AND TO GRAVITY.

By C. O. ESTERLY.

IT is the purpose of this paper to report a series of experiments which appear to throw new light upon the diurnal movements of certain plankton crustaceans. Most investigators of this subject have held that the response of the organisms to light determines in a large degree the time of leaving or entering the upper strata of the water. As evidence that this is true in certain cases may be cited the experiments of Groom and Loeb ('90) on barnacle larvæ, of Loeb ('93<sup>a</sup>, '93<sup>b</sup>) on *Temora longicornis* and *Polygordius* larvæ, and of Parker (:02) upon *Labidocera æstiva*. All of these investigators found that an important factor in causing the upward movement of the organisms is a positive response to light of low intensity. Conversely, a negative response to strong light is important in causing the downward movement. The results of my experiments go to show that, in the species investigated, a phototropic response has comparatively little to do in bringing about an upward or downward migration, though exposure to light has an important part.

The observations were made on the movements of fresh-water copepods. Some of the animals were collected in October in ponds near Cambridge, Mass., and kept in aquaria for several months. Material was also obtained from the permanent fresh-water tanks in the aquarium room of the zoölogical laboratories in the Museum of Comparative Zoölogy. The animals used were all females, and belonged to the species *Cyclops albidus* Jurine. The work was carried on under the direction of Prof. G. H. Parker, and the writer is greatly indebted to him for valuable advice and criticism.

It was found that a suitable artificial light could be obtained from a glowing Nernst filament; this was used, accordingly, throughout the experiments.

In experiments to determine the nature of the response to light, the animals were confined in rectangular glass vessels with parallel sides; the vessels were 3 cm. wide, 7 cm. long, and 7 cm. high, inside measurement. The aquarium was always placed with its length in the direction of the rays of light, and the wall away from the light was coated on the inside with paraffin and lampblack to check reflection as much as possible. The vessel as a whole was protected from reflections from the walls of the room by black screens. The Nernst filament was covered by a hood of black sheet iron, and the light reached the animals through an aperture in the hood a little wider than the filament was long. Care was taken that as far as practicable the rays of light should fall perpendicularly upon the wall of the aquarium, and the work was always done in a dark room.

The candle power of the light was determined by means of a Lummer-Brodhun photometer, and from this the intensity was reckoned in candle metres. The experiments, with the exception of one, were carried on in lights of five intensities: 8, 420, 825, 1700, and 2200 candle metres. These figures represent the intensity at the wall of the aquarium nearest the light. The lights used most were the first one and last two of those named, the others being employed to check results. One set of animals was generally subjected to three lights of different intensities on one day, and on the following days to the same three but in another order. After an experiment the animals were always allowed to rest for twenty-four hours, under conditions as nearly normal as possible before being used again.

The vessel containing the animals for an experiment was placed in the desired position, covered so as to exclude all light and left undisturbed for from six to eighteen hours before beginning the experiment. As soon as possible after exposing the animals to the light the number in one half of the aquarium was recorded (those in the half toward the light being designated as positive, those in the other half as negative), and records were made thereafter at half-minute intervals for from half an hour to an hour. Then the intensity of the light was changed by moving the aquarium toward or away from the filament, and the same method of recording continued. It will be seen that in this way at the end of the third day of experimenting records were obtained of the distribution of the animals in

each of three intensities of light following darkness, and also after previous exposure to light.

It was found necessary to use a small number of animals at one time in order to count accurately and quickly. The number usually taken was five, and the whole number of times that animals were found in one half of the aquarium was calculated from records made as above. The percentage this number bore to the whole number of records was taken as evidence whether a given set of animals was positive or negative.

**Reactions to light after protracted retention in darkness.** — It may be said at once that, in lights of 420 candle metres and below, the animals experimented with were neutral when exposed to the light after they had been in darkness for some time. The results of such experiments show that 50.9 per cent of the total number of records made under the conditions named and in the 420 candle-metre light represent animals in the negative half of the aquarium. Usually the animals under experimentation move from one end of the aquarium to the other frequently, the movement being general and not confined to one or two individuals. Even if at a certain moment more animals are positive than negative or *vice versa*, it is possible with a large number of records extending over a considerable time to determine whether as a whole the set is positive or negative. The lights of lower intensity than 420 candle metres that were used were 8 and 0.55 candle metres. The latter was used on only one occasion, and special care was taken to prevent any but the most direct rays reaching the animals. The number of negative and positive animals was recorded every fifteen minutes for a period of six hours, and after each reading was taken, the water was agitated to redistribute the animals. The results for the entire period show that exactly as many animals were negative as positive, though the number at different times in the negative half varied from one to five. In this case six individuals were under observation.

During observation in the 8 candle-metre light an aquarium was used in which both ends were clear, so that it could be turned end for end. The aquarium was left in position and in the dark for several hours before observations were commenced. When brought from darkness into the light, the animals were not counted for the first five minutes; then records were made every half minute for ten minutes, when the vessel was turned end for end as carefully as possible and after five minutes counting was resumed. Two sets

of records were made for each of the two positions, and the same animals were used on three different days. The results of a number of trials made in this way show that as a whole 47.1 per cent of the animals experimented with were negative. Here, then, there is some evidence of a positive response, but it is probably of slight significance, as indicated by comparison with the results obtained by the use of the lights of 420 and 0.55 candle-metre intensity.

TABLE I.

THE AVERAGE NEGATIVITY IN PER CENT OF CYCLOPS IN VARYING INTENSITIES OF LIGHT FOLLOWING PERIODS IN DARKNESS.

Light in C.M.	Number of animals tested.	Percentage of animals found in the negative half of the aquarium.
0.55	6	50.0
8.0	11	47.1
420.0	21	50.9
825.0	19	66.6
1700.0	22	83.8
2200.0	15	84.7

The conclusion to be drawn from these results is that in lights of low intensity *following darkness* the animals are neutral. When lights of greater intensity than 420 candle metres are used following darkness, the reaction is invariably negative, the negativity increasing as the intensity of the light is increased. Table I shows the averages of a number of trials with the different intensities following darkness.

This table shows that there is no clearly negative reaction until an intensity of 420 candle metres has been passed. In all trials in the 825, 1700, or 2200 candle-metre lights the negativity of the animals was clear and convincing.

**Reactions to light after exposure to light.** — If the animals are tested after they have been exposed for some time to light of any intensity, their reactions are invariably negative. It does not appear that there is an increase in the negativity with an increase in the intensity of the light, as there was when the animals were brought from darkness into light. Table II shows the average per cent of animals that were found in the half of the aquarium away from a light of given intensity (indi-



cated in the upper part of the table) after exposure to light of another intensity (in column at left).

The figures presented in this table make plain that the intensity of the previous stimulation by light does not affect the response to a given light. The animals are negative to all lights whether previously subjected to light of high or low intensity within the limits employed. The variation in the figures representing the percentages does not

TABLE II.

THE AVERAGE NEGATIVITY IN PER CENT OF CYCLOPS IN LIGHTS OF VARIOUS INTENSITIES AFTER PREVIOUS EXPOSURE TO LIGHT OF THE SAME QUALITY, BUT OF A DIFFERENT INTENSITY.

Intensities of lights to which cyclops had been previously subjected.	Intensities of lights in which records were taken.				
	8 C.M.	420 C.M.	825 C.M.	1700 C.M.	2200 C.M.
8 C.M.	....	85.7	85	65.7	80.0
420 "	61.4	....	....	87.5	....
825 "	79.9	....	....	70.0	....
1700 "	80.0	91.1	....	....	77.2
2200 "	71.1	..	....	81.8	....

seem significant; for the present purpose it is sufficient to note that without exception the females of *Cyclops albidus* are negative to light of any intensity if tested after exposure to light of the same quality.

**Reactions to gravity.** — The species used in these tests was *Cyclops albidus*, and the animals were all females. It can be said at the outset that in by far the majority of cases the animals were positively geotropic. The author had many times tried the experiment of liberating individuals one by one at the top of a tall column of water in a cylinder of large calibre, and almost without exception they passed at once to the bottom. As a rule they remained at the bottom, but certain ones made short excursions toward the top and then dropped down again. It was rare to find the animals anywhere except in a section two or three centimetres deep at the bottom of the vessel. If the cylinder with a number of animals at the bottom was inverted, most of them passed at once to the bottom. It cannot be said that every animal of any set was positively geotropic, but the great major-

ity were. The most usual inhibition of the response occurred when an animal came in contact with the side of the vessel after once starting down; it might cling to the glass for some time, but even such individuals have often been seen to reach the bottom after a few minutes.

We may conclude that in daylight and under ordinary conditions the response of the females of *Cyclops albidus* to gravity is positive. But if the vessel containing the animals was covered for a short time so as to exclude all light, and the distribution noted at the end of this time, it was seen that there had been an upward migration to a greater extent than ever occurred if light and dark periods did not alternate. To test this carefully a tall glass cylinder was marked off into five sections each about 10 centimetres in height, and the sections were numbered from the top down. Thus the position of the animals could be noted rapidly when the jar was uncovered. A single laboratory record will show the result of keeping the animals in the dark for short periods and then exposing them to diffuse daylight long enough to note their vertical distribution.

April 7, 1905. — 10.15 A. M. Cylinder with six positively geotropic animals was covered so as to exclude light.

10.20 A. M. Covering removed; one animal in Section 1, moved to bottom at once; five in Section 5. Covering replaced.

10.25 A. M. Covering removed; one in Section 2, and went down at once; five in Section 5. Covering replaced.

10.30 A. M. Covering removed; one in Section 2; three in Section 1, and all moved down at once in the light; two in Section 5. Covering replaced.

10.40 A. M. Covering removed; one in Section 3; two in Section 2; two in Section 1, but one of these does not move down; one in Section 5. Covering replaced.

10.50 A. M. Covering removed; one in Section 3; two in Section 2; two in Section 1; one in Section 5; all pass to bottom. Covering replaced.

11.00 A. M. Covering removed; two in Section 3; two in Section 2, these two reach the bottom in thirty seconds; two in Section 5.

This experiment, which is typical of all, proves that in the absence of light the animals, which are otherwise positively geotropic, tend to become negatively geotropic, and that in daylight this response is reversed. The same is true when the animals are exposed from below

to illumination of such intensity that if the rays were to strike horizontally the phototropic reaction would be negative. These experiments were carried out with a Nernst light and in an illumination of 1000 candle metres. The animals were placed in the graduated tube, and an apparatus was arranged so that light would enter the tube only from below and could be cut off by a tightly fitting shutter; the whole experiment was carried on in the dark room, extraneous light being carefully guarded against. A heat screen was interposed between the light and the bottom of the cylinder, and care was taken that the water in the screen was cool. The animals were nearly always put into the cylinder at night and not tested until the following morning; then they were exposed to the light for five-minute periods, alternating with five minutes in darkness until records had been made for five periods or more in each state.

Table III shows the distribution of the animals through the cylinder. Half of the records were made after a period in darkness and as soon as the light entered the vessel; the other half were made at the end of a period in the light immediately before the shutter was closed. The figures in the table are the sums of the numbers of animals found in the different sections of the cylinder for the entire number of trials on one day. It will be seen that the table contains records of six separate tests, each of which consisted of at least five five-minute periods in darkness and a like number in light.

Inspection of the table shows that in the six experiments animals appeared in the uppermost section more than three times as often immediately after periods in complete darkness as they did at the end of like periods of exposure to illumination from below, and likewise more than twice as often in the second section after darkness as after exposure to light. It will be seen that during both light and dark periods more of the animals which leave the lower section are found in sections 1 and 2 than in any other. Fifty animals were tested with the Nernst light, and only one set was used twice in succession.

**Discussion of results.**— It has been seen that the phototropic response of these animals is negative; they are neutral to lights of low intensity if tested after retention in darkness. It cannot be claimed that the response to light was modified by handling or other means, because when tested the animals had been undisturbed and in the dark for hours. In the cases where the aquarium was turned end for end during an experiment there was of course some mechanical stim-

ulation, but it was certainly very slight and may be regarded as not affecting these results, especially since no record was made of the distribution until after a period of quiescence. If it were possible that in nature light could affect the organisms apart from gravity, we

TABLE III.

THE COMPARATIVE VERTICAL DISTRIBUTION OF THE FEMALES OF *CYCLOPS ALBIDUS* THROUGH A CYLINDER OF WATER AFTER ALTERNATING FIVE-MINUTE PERIODS IN DARKNESS AND IN LIGHT.

IN THE DARK.							
Number of section of cylinder.	The numbers of animals in each of the sections of the cylinder in each of six experiments.						Totals.
	Number of the experiment.						
	1	2	3	4	5	6	
1	11	3	11	6	12	10	53
2	7	9	7	4	4	3	34
3	3	3	5	4	3	1	19
4	0	2	3	0	2	0	7
5	27	25	22	36	29	35	174
IN THE LIGHT.							
1	1	0	1	1	10	4	17
2	0	2	3	2	3	4	14
3	0	0	4	0	3	1	8
4	0	4	2	3	0	0	9
5	47	30	38	44	34	42	235

should be led to say that light alone has very little to do in causing vertical migration. A light of comparatively low intensity, such as would be experienced, for example, in early evening, would not bring the animals to the surface, since they are at most only slightly positive to weak lights *after having been in darkness*, and we have seen that they are negative to all intensities of light if previously exposed to light. It seems improbable that phototropic responses are even the main factors in causing diurnal migrations.

It has been shown that the female copepods used in these tests have a strong positive geotropism. In this respect they differ from the females of *Labidocera aëstiva*, which have been shown by Parker (:02) to be negatively geotropic. It is noticeable that the females of *Cyclops albidus* are not negative to a light from below of such intensity that, if it operated apart from gravity, the animals would move away from it. Thus the positive geotropism is seen to be stronger than the negative phototropism. However, *in darkness* this geotropic response is changed and the animals ascend through the water for a considerable distance; that is, they are negatively geotropic. When exposed to light, such negative animals move down at once; there is at that time a return to the condition of positive geotropism. This occurs, as has been shown, in diffuse daylight and in the light from a Nernst lamp. In the former case the light could have no directive effect, though that would be possible in the latter case. We are led to conclude, then, that light has at least a kinetic effect upon the negatively geotropic animals in that it induces locomotion. Carpenter (:05) has shown that light has a kinetic effect upon the pomace-fly, *Drosophila*. It seems probable, however, that in the case of *Cyclops* light does more than induce locomotion, since an animal which is not at the top of the column of water might be expected to move up as readily as down, if the effect of illumination were only kinetic. It may be that light produces a chemical change in the organism by virtue of which it responds positively to gravity. The case seems to be strictly analogous to those reported by Loeb (:04) in which heliotropic reactions were controlled by chemicals. The effect of light upon *Cyclops* is best seen when negatively geotropic animals are suddenly illuminated from below. In many cases the animals give a leap as soon as the light strikes them, and swim rapidly to the bottom. The speed of this movement is considerable, since they often pass through 40 cm. of water in from thirty to sixty seconds. Some animals may sink passively, but whatever the mode of progress, the response begins when the light reaches them, except in the very few cases of animals that remain attached to the surface film or to the sides of the vessel.

It has been said that the animals were left in the cylinder in darkness for hours before testing them with the Nernst light. Without doubt there is an upward migration when the jar is covered, and it would be desirable to know when the animals go down. They are always at the bottom when exposed to the light early in the morn-

ing, but I have not determined whether they begin to move down a few minutes or several hours after they are put into darkness. If there is a physiological effect due to light (or darkness) which does not wear off for several hours, it has an important bearing in explaining the cases when, in nature, animals do not reach the surface for several hours after sunset, and leave in the morning before sunrise when there is no more light than at midnight. (On this subject see Juday, :04.)

Another unsettled point is whether or not, after exposure to light, absolute darkness is necessary for the upward migration. It is probable that weak light would have the same influence, and that we could reproduce in the laboratory the effects of twilight or dawn in causing upward or downward migration.

No attempt has been made to test the effect of temperature, along the lines of the theory of Ostwald (:03), in causing daily migrations, but it seems very probable that light and gravity are the predominant factors in bringing about the present phenomenon.

#### SUMMARY.

1. The females of *Cyclops albidus* are neutral to artificial lights of low intensity, and negative to that of high intensities if subjected to the light after confinement in darkness.
2. After exposure for some time to light of any intensity, they are negative to light of low as well as of high intensity.
3. When the negative reaction appears after the animals have been in darkness, the negativity increases with the intensity of the light (Table I).
4. Ordinarily the females of *Cyclops albidus* are positively geotropic, but after having been in the light such animals tend to become negatively geotropic in darkness.
5. Negatively geotropic animals become positive even if exposed to such intense illumination from below that if the light were acting alone they would be negative to it.

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## THE CAUSE OF THE PHARMACOLOGICAL ACTION OF AMMONIUM SALTS.

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IT is now well established that the pharmacological action of most inorganic salts is due to the ions of the salt; that the kind of action depends on the character of the charge of the ion, whether positive or negative; and that the degree of action is determined by the available energy in the ion and chiefly by its potential energy. The potential energy depends upon the electrical affinity, or potential, of the ion.<sup>1</sup> To these general rules ammonium salts are in some respects exceptional, and it is the purpose of this paper to examine the cause of action of ammonium salts to see whether these salts are in reality exceptional as they appear to be.

In a previous paper I have endeavored to show that among inorganic salts several apparent exceptions to the principles quoted above were in reality to be explained in harmony with those principles, these exceptions being due to the fact that more than one kind of dissociation occurs in these cases. The actions of the chlorates, iodates, and nitrites, for example, which cannot be explained on the basis of their dissociating only into metal and chlorate, bromate, iodate, or nitrite ions, are readily explained if we assume that some molecules dissociate also into other kinds of ions, as their chemical behavior indicates.<sup>2</sup> Furthermore the character of the dissociation in these cases depends on the reaction of the solution, and in accord with this their action was found to be much greater in acid than in alkaline tissues. Their action can, moreover, be counteracted by alkalis.

In the second place, it was pointed out for many anesthetics that whatever the cause of the activity of these compounds might be,

<sup>1</sup> See MATHEWS: Biological studies by the pupils of WM. T. SEDGWICK, 1906, p. 81. Boston.

<sup>2</sup> MATHEWS: American journal of physiology, 1904, xi, p. 237.



there was a close parallelism between their chemical activity and their pharmacological activity, and it was suggested that the latter property must depend on the former. If, as Nef thinks, chemical activity depends upon previous dissociation of the compound, these facts clearly indicated that the pharmacological action of the anesthetics and other drugs was also due to the dissociation products of these substances.

Ammonium salts dissociate principally into  $\text{NH}_4$  ions and the acid ion with which the ammonia is combined. They dissociate, also, somewhat hydrolytically, so that there is in all such solutions a small amount of the free acid and ammonium hydrate. Furthermore, ammonium hydrate dissociates into water and ammonia gas. The amount of the dissociation into ammonium hydrate and ammonia gas is variable, being least in the chloride and sulphate and larger in the case of the carbonate.

Pharmacologically ammonium salts occupy an anomalous position in many particulars of their action, for while in their action on *Fundulus heteroclitus* eggs, upon muscle and the motor nerve, they closely resemble potassium salts, in their action on certain nerve centres they differ entirely from them. Ammonium chloride injected intravenously produces in sufficient doses tetanic convulsions resembling those of strychnine; increases blood pressure; stimulates the vagus centre, and is in fact a strong stimulant of the central nervous system. It has also a strong depressant action on the conduction of nerve impulses from the nerve to the muscle. Ammonium hydrate, also, acts as a powerful stimulant on mucous surfaces and rapidly annihilates nerve irritability without stimulation.

If the two ions  $\text{NH}_4^+$  and  $\text{Cl}^-$  be examined, we get no clew to the cause of these actions. The chlorine ion is found in the tissues normally, and the slight increase in amount due to the injected chloride cannot cause these results. The ammonium ion has a velocity very close to that of potassium, and its potential energy is not far from that of potassium, although it is slightly different. From both the kinetic energy and potential energy content the ammonium ion ought to act much like potassium. As a matter of fact, many of the actions of ammonium chloride are like those of potassium chloride, as is well known. These actions may conveniently be ascribed to the  $\text{NH}_4^+$  ions. Potassium chloride, however, produces no tetanic stimulation of the central nervous system, but always depression. The stimulant action

of ammonium compounds cannot be due then, I think, to the ammonium ion.

The stimulant action is, however, readily explicable by a reference to the other dissociation, that is, the ammonium hydrate formed by hydrolytic dissociation. Ammonium hydrate, while it so rapidly destroys the conductivity of motor nerves, acts as a powerful stimulant to many sensory nerve ends. Furthermore, it is well known that ammonium carbonate is a far more powerful stimulant of the central nervous system than ammonium chloride, and in the former the amount of ammonium hydrate is far greater than in the latter.

That certainly some of the action of ammonium carbonate is probably due to the ammonium hydrate in it, is shown by the following observation. I found that the addition of a little ammonium carbonate to sea water, containing immature eggs of *Asterias Forbesii*, caused an almost instantaneous solution of the nucleolus of the germinal vesicle, before any change could be perceived in the rest of the egg. In this particular ammonium carbonate seemed peculiar. In no other salt solution, except ammonium hydrate, could I see a similar result. Neither ammonium chloride, ammonium sulphate, nor sodium carbonate would produce any similar effect. The nucleolus never disappeared as it did in ammonium carbonate. I found, however, that if a slide which had on its under surface a hanging drop containing starfish eggs was placed over sea water containing ammonia, the nucleoli disappeared as if by magic, and later the whole egg dissolved. This experiment shows that this particular pharmacological action is due to the ammonium hydrate. This observation is of interest in that it is, I believe, the first time an agent has been found which has such a specific action on one of the nuclear structures.

The question comes, therefore, to the determination of what it is in the ammonium hydrate which gives it its action. Both the ammonium and hydroxyl ions may, I think, be disregarded, since the fact that neither ammonium chloride nor sodium hydrate produces any similar action shows that these ions are not causing the effect.

Further evidence that the action of ammonium hydrate is due neither to the ammonium nor the hydroxyl ions was obtained by a study of the action of the hydrate on muscle. If a frog's gastrocnemius is suspended in the ordinary way, attached to a lever so that the contraction registers on a drum, and then 2 c.c. of a  $\frac{1}{3}$  solution of ammonium chloride is allowed to run over the surface of the

muscle from top to tendon, no shortening occurs. If, however, ammonium hydrate of  $\frac{m}{250}$  strength is applied, a short contraction followed by a slow relaxation takes place. An  $\frac{m}{280}$  solution of ammonium hydrate is generally too weak to cause any contraction. The contraction of the muscle is not due either to the ammonium ion or to the hydrate ion, for neither ammonium chloride nor sodium hy-

EXPERIMENT 1.

Solution.	Time.	Minimum stimulus. Secondary coil, cms. from primary.	Solution.	Time.	Minimum stimulus. Secondary coil: cms. from primary.
NH <sub>4</sub> Cl $\frac{m}{7}$	11.01	86.4	NH <sub>4</sub> Cl $\frac{m}{7}$ + NH <sub>4</sub> OH $\frac{m}{280}$ .	11.01	86.4
"	Nerve in solution at 11.02.		"	Nerve in solution at 11.02.	
"	11.05	85.9	"	11.06	73.8
"	11.10	74.8	"	11.11	62.0
"	11.15	74.0	"	11.16	45.0
"	11.20	63.0	"	11.18	Non-irritable in one-half of nerve.
"	11.26	Non-irritable at tip.			

drate of  $\frac{m}{250}$  strength produces the contraction, although the number of ammonium ions is vastly greater in the first, and the hydroxyl ions in the second, than in ammonium hydrate.

Furthermore, the following experiment shows that the action runs *pari passu* with the number of undissociated molecules of NH<sub>4</sub>OH present. To an  $\frac{m}{8}$  solution of ammonium chloride ammonium hydrate was added sufficient to make an  $\frac{m}{280}$  solution. The number of undissociated NH<sub>4</sub>OH molecules in such a mixture is greater than in the  $\frac{m}{280}$  ammonium hydrate by itself. When such a solution is applied to the muscle, a marked shortening results, although ammonium hydrate of the same strength is ineffective, or nearly so. There can, therefore, be no doubt that an increase in the number of *undissociated* ammonium hydrate molecules means in this case an increased action.

Similar experiments have been tried upon the motor nerve of the frog, with closely similar results, as shown in Experiments I-III. The addition of ammonium hydrate to ammonium chloride greatly in-

creases the toxic action of the chloride upon the nerve, whereas sodium hydrate, in concentrations containing more hydroxyl ions than this strength of ammonia, does not increase the toxicity of sodium hydrate.

## EXPERIMENT 2.

EFFECTS OF ADDITION OF  $\text{NH}_4\text{OH}$  TO  $\text{NH}_4\text{Cl}$  ON TOXICITY. SCIATIC OF FROG.

Solution.	Time.	Minimum stimulus.	Solution.	Time.	Minimum stimulus.
$\text{NH}_4\text{Cl} \frac{n}{8}$	1.50	87	$\text{NH}_4\text{Cl} \frac{n}{8} + \text{NH}_4\text{OH} \frac{n}{280}$	1.50	87.0
"	In solution at 1.51.		"	1.52	Nerve immersed.
"	1.55	87	"	1.55	86.0
"	1.59	80	"	1.60	72.8
"	2.05	72	"	2.06	62.0
"	2.18	64	"	2.16	Non-irritable
"	2.30	Non-irritable at tip.			

## EXPERIMENT 3.

Solution.	Time.	Minimum stimulus.	Solution.	Time.	Minimum stimulus.
$\text{NaCl} \frac{n}{8}$	11.37	82.5	$\text{NaCl} \frac{n}{8} + \text{NaOH} \frac{n}{500}$	11.37	79
"	11.38	Nerve immersed.	"	11.39	Nerve immersed.
"	11.53	80.5	"	11.55	75
"	12.00	76.0	"	12.00	78
"	1.30	Spontaneous contractions.	"	1.30	Contractions.
"	1.31	76.0	"	1.31	78
"	2.40	83.0	"	2.45	90

The question which has not been solved is whether the toxic action is due to the undissociated ammonium hydrate molecules, or to the  $\text{NH}_3$  formed from it. I think this question may be answered provisionally, although it is impossible to determine it directly, so far as I can see.

Ammonium hydrate is constantly dissociating into water and  $\text{NH}_3$ . When this dissociation takes place, there is an instant when the bonds on the nitrogen atom which formerly bound the water are free or open, — there is a moment, in other words, when the  $\text{NH}_3$  and the water exist in a nascent form, before the two valencies set free saturate themselves. The chemical reactions of ammonia ( $\text{NH}_3$ ) are due either to the saturation of these two free valencies by the substances added, as for example hydrochloric acid, or to the replacement of one of the hydrogen atoms. Ammonium hydrate, on the other hand, enters into combination, only by means of its  $\text{NH}_4$  and hydroxyl ions, which are in this case ruled out.

It is, therefore, probable that the action of the ammonium hydrate is due to the dissociated  $\text{NH}_3$  present, and particularly to the action of this substance in the moment of its origin, when the valencies of the nitrogen atom are open or dissociated.

These experiments show that the pharmacological actions of ammonium compounds are due, in part, to the ammonium and acid ions present, but that certain pharmacological effects and presumably the characteristic stimulating action of ammonium salts run parallel with the amount of undissociated ammonium hydrate present in the solution and formed by hydrolytic dissociation. The action of the ammonium hydrate is in its turn to be ascribed probably, as is the chemical action, to the  $\text{NH}_3$  formed by dissociation of the hydrate, and probably to the  $\text{NH}_3$  in nascent state when the valencies of the nitrogen are open. The action of ammonium compounds is not, therefore, contrary to the principles of pharmacological action quoted at the beginning of the paper, but necessitate the recognition, in addition thereto, of the pharmacological action of dissociated particles which are non-ionic, or rather twin-ionic, such as  $\begin{matrix} + \\ \diagdown \\ \_ \\ \diagup \end{matrix} \text{NH}_3$  particles. The well-known greater activity of the free alkaloids as contrasted with their salts is, in my opinion, to be explained in the same way, the alkaloid splitting off water and having thus free nascent bonds in its nitrogen.

## THE RHYTHM OF THE TURTLE'S SINUS VENOSUS IN ISOTONIC SOLUTIONS OF NON-ELECTROLYTES.

By H. E. EGGERS.

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

A CONSIDERABLE portion of the more recent investigations of the heart rhythm has been devoted to attempts to illustrate the mechanisms of the normal rhythm by the production of artificial rhythms in non-automatic tissues by various means. From the fact that non-electrolytes (sugar, urea, glycerine, etc.) in isotonic solutions do not produce rhythmic contractions in non-automatic tissues, and the further fact that such rhythms are produced by some of the inorganic salts of the blood, some physiologists have been led to look for the immediate stimulus to the heart rhythm in the inorganic salts of the blood (or rather of the heart tissues). Carlson<sup>1</sup> has recently pointed out that this view rests on very meagre evidence. It is to be hoped that the study of artificial rhythms will ultimately throw some light on the mechanisms of the normal heart rhythm, but direct evidence can be obtained only by studying the normal automatism. The fact that sodium chloride will produce a transient rhythm in non-automatic muscle is no more a proof that this salt bears this same relation to the normal heart rhythm than the fact that a muscle fibre can be made to contract by direct action of the electric current is a proof that the nervous impulse is an electrical current. While the non-electrolytes do not produce rhythms in non-automatic tissue, it is common knowledge that the automatic parts of the heart continue in activity for some time in isotonic solutions of such non-electrolytes as the sugars. It is usually assumed, however, in this case that the non-electrolyte is neutral, and that the rhythm is maintained by the electrolytes within the automatic cells or within the tissue spaces. But Carlson has shown, for the automatic as well as for the non-automatic tissues of the *Limulus* heart, that neither sugar, urea, nor

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

glycerine can be considered as neutral substances or as acting by means of the osmotic pressure factor alone. In isotonic solutions these electrolytes have a purely depressant action on the heart muscle and a primary stimulating action on the heart ganglion, while the duration of the ganglionic rhythm in isotonic solutions of these substances depends on the condition of the ganglion as well as on the nature of the non-electrolyte. Miss Denis,<sup>1</sup> working in this laboratory, has recently shown that the relative rapidity with which these non-electrolytes stop the ganglionic rhythm bears a direct relation to the rate of diffusion of the blood salts into solutions of the non-electrolytes and *vice versa*; but the fact that the duration of the rhythm depends on the condition of the ganglion goes to show that the direct action of the non-electrolyte on the automatic cells is a factor in the ultimate cessation of the rhythm.

At Carlson's suggestion his experiments on the heart of *Limulus* were repeated on the sinus venosus of the turtle in order to determine whether the conclusions based on the *Limulus* heart are applicable to the heart of vertebrates. The sinus venosus was chosen rather than the ventricle or auricles for the following reasons. The sinus is the automatic part of the heart *par excellence*. The walls of this part of the heart are the thinnest; hence when immersed in any solution the cells throughout the entire sinus will sooner be surrounded by the same conditions than would be the case with the auricles or the ventricles.

In all experiments the rhythm of the sinus was recorded by the ordinary graphic method, the sinus being suspended within a glass cylinder. This rendered large sinuses particularly desirable. The material was accordingly taken from large specimens of the common snapping turtle (*Chelydra serpentina*).

The sinus was removed with as little handling as possible, immediately after the death of the animal, and when not used at once, was kept in serum until used. The sinus was of course freed as completely as possible from auricular tissue. The great size of the organ in this turtle made it advisable to separate the right and left halves, and this was accordingly done, the behavior of the two being recorded separately. In general the two halves behaved similarly, sometimes the one, sometimes the other, beating the longer, depending apparently on the degree of injury each had sustained in removal.

The solutions used were quarter normal, this making them practi-

<sup>1</sup> DENIS: This journal, 1906, xvii, p. 35.

cally isotonic with the serum of the animal. The solutions were chemically pure, for the most part of Schuchardt's preparation. The water used was redistilled in glass.

Work was done with solutions of cane sugar, dextrose, lævulose, urea, and glycerine. The action of the different sugars is practically the same both quantitatively and qualitatively. Urea and glycerine

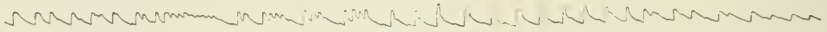


FIGURE 1.— Four-fifths the original size. Tracing from the turtle's sinus immersed in an isotonic solution of lævulose, showing fibrillary contractions.

act with much greater rapidity than do the sugars. The primary action of glycerine, moreover, appears to be different from that of the other non-electrolytes.

In considering the action of these solutions on the rhythm of the sinus tissue, the effects on the rate and on the amplitude of the beats will be taken up separately. That to some extent the two are interdependent is of course obvious; but differences of such a character were obtained as could not, it is believed, be explained on this basis.



FIGURE 2.— One-third the original size. Tracing from the turtle's sinus immersed in an isotonic solution of lævulose, showing the typical periods of rapid rhythm alternating with periods of quiescence.

1. **Effect on rate of beat.**— Urea and the sugars augment the rate of the sinus rhythm. The rhythm is at first perfectly regular, but finally becomes irregular and apparently assumes the character of fibrillar contraction (Fig. 1). In other words, the sinus goes into a state of delirium. That this irregular rhythm actually partakes of the character of delirium cordis could in some instances be seen by direct observation. This irregular rhythm is followed by temporary or permanent cessation of the automatic activity. In some of the preparations exhibiting the temporary cessation of the rhythm the alternating periods of activity and quiescence were continued for a considerable period before the final stoppage of the rhythm (Fig. 2).

In the case of glycerine no primary augmentation of rate, aside from delirium, was observed. The rapid onset of delirium in the glycerine solution is illustrated in Fig. 3.

2. **Effect on the intensity of the beat.**— All the sugars used produced a primary augmentation of the amplitude of the contraction



(Fig. 4, *B*). This augmentation appeared very soon after immersion of the sinus in the solution. It is of rather brief duration, and in these respects different from the stimulating action on the rate, which appears later and is of longer duration. The two overlapped, however. The primary augmentation of the strength of the beat is followed by a gradual depression, progressing till the final cessation of the rhythm. Needless to say, the appearance of the delirium was attended by marked decrease in intensity of the contraction.



FIGURE 3.—About two-thirds the original size. Tracing from the turtle's sinus immersed in an isotonic solution of glycerine. *x*, application of the glycerine solution, showing rapid onset of delirium cordis in the glycerine solution.

Urea apparently does not increase the strength of the contraction. The application of the solution resulted, when the sinus was originally beating strongly, in an almost immediate tonus; any increase of amplitude was of too brief duration to be apparent after even the short interval necessary to readjust the recording lever to the drum.

No augmentation of the strength of the beats was observed with the glycerine solution.

The primary stimulating action of one of the sugars is strikingly shown in Fig. 5, *A*. In this case the sinus had been excised twenty-four hours previously, and had at that time been placed in the lævulose solution till the rhythm ceased. It was then placed in serum on ice, being warmed to room temperature before using. It will be observed that the inactive (save for tonus rhythm) sinus, after immersion in the lævulose solution a second time, resumed for a time a perfectly regular rhythm.

3. **The duration of the sinus rhythm in the different solutions.**—When comparing the rate of action of the sugar, urea, and glycerine solutions, some differences between the *Limulus* heart ganglion and the turtle's sinus come to light. The sinus maintains its activity the longest in the sugar solutions, just as Carlson found to be the case with the automatic heart ganglion of *Limulus*. But glycerine stops the sinus rhythm about as soon as urea, while the *Limulus* heart

ganglion is brought to a standstill much quicker by the urea than by the glycerine solutions. Hence for the turtle's sinus the duration of automatism in these solutions does not bear a direct relation to the rate of diffusion of the blood salts into the solutions or the rate of diffusion of the electrolytes themselves in distilled water, or in  $\frac{1}{8}$  sodium chloride.

There is considerable individual variation in the length of time that the sinuses from different specimens continued in activity in the solu-

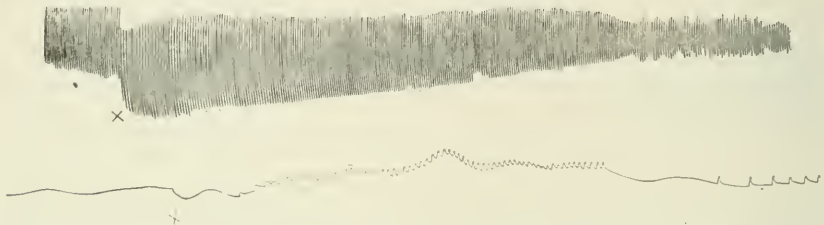


FIGURE 4 — About two-fifths the original size. *A*, tracing from the turtle's sinus twenty-four hours after removal from animal. Sinus quiescent, but exhibiting tonus rhythm. *x*, application of an isotonic solution of dextrose, showing inauguration of the fundamental rhythm by the sugar solution. *B*, tracing from the turtle's sinus immersed in an isotonic solution of dextrose. *x*, application of the dextrose solution, showing primary stimulation by the sugar solution.

tion of the same non-electrolyte.<sup>1</sup> Part of this difference is, in all probability, due to unavoidable injuries to the tissue in preparation, as it was observed in the case of the two halves of the sinus from the same specimen. But, other things equal, the sinus from a vigorous specimen continues in rhythm in any of the solutions for a longer time than the sinus from a specimen in poor condition. The rate of action of these non-electrolytes is therefore dependent on the condition of the sinus, just as Carlson found to be the case in the *Limulus* heart ganglion. This is further shown by the fact that a sinus brought to standstill in the sugar solution, then rendered active by immersion in serum, and again immersed in sugar solution, does not maintain its activity in the second sugar as long as in the first.

In this connection an interesting phenomenon, observed with one of the sinuses in urea solution, deserves mention. The sinus immediately following excision was left in the urea solution till the

<sup>1</sup> In the sugar solutions a sinus has been observed to beat as long as two hours: for urea the longest period was sixty minutes: for glycerine about sixty-eight minutes. This last sinus was from an exceptionally large and vigorous turtle.

rhythm had nearly ceased, was transferred to serum on ice for twenty-four hours, and was then again immersed in urea solution. Its period of activity for the second time was about one quarter longer before the same degree of exhaustion was obtained than for the first time. The most obvious explanation of this explanation to the general rule would be on the basis of "shock."

If we regard the irregular rhythm or delirium of the sinus as evidence of stimulation, it is obvious that these non-electrolytes have the same action on the turtle's sinus as in the *Limulus* heart ganglion, that is, a stimulating action followed by depression and paralysis. In the case of the sugars the primary stimulating action on the sinus appears both in the rate and the intensity of the beats, and comes prior to the onset of the irregular rhythm. The urea solution apparently acts in the direction of stimulation only on the rate, but this is evident before the fibrillary contractions set in, while the primary stimulating action of glycerine appears only in fibrillary contractions. It is conceivable that fibrillary contractions may be called forth by a depressor action on the co-ordinating mechanism; but they can also be produced by excessive stimulation, and this is in all likelihood their origin in these experiments, because the stimulating phase of the sugar and the urea action passes into this form of heart activity. The fibrillary contractions of the sinus in these solutions are to all appearance identical with the irregular contraction of the *Limulus* heart muscle following the immersion of the heart ganglion in similar solutions.

In the case of the *Limulus* heart these non-electrolytes primarily augment the rhythm when the whole heart as well as when only the heart ganglion is immersed in the solution. When the solution acts on the *Limulus* heart muscle alone, they depress the rhythm from the beginning. These facts show that the heart ganglion is so much more sensitive than the heart muscle that when the solution acts on both at the same time their primary action on the ganglion is first apparent. On the neurogenic theory of the vertebrate heart rhythm the action of these solutions on the turtle's sinus is brought into complete agreement with their action on the *Limulus* heart. The absence of any primary augmentation of the amplitude of the sinus beats by urea and glycerine is probably due to this depressor action on the heart muscle, while the stimulating phase results from the primary action on the sinus ganglia.

## SUMMARY.

1. Isotonic solutions of sugar, urea, and glycerine have, on the whole, the same primary action on the turtle's sinus as on the *Limulus* heart ganglion or on the entire *Limulus* heart, that is, in the direction of stimulation. This fact, in view of the further fact that these same solutions have only a depressor action on the *Limulus* heart muscle, tends to support the neurogenic theory for the vertebrate heart.

2. The ultimate cessation of the sinus rhythm in isotonic solutions of these non-electrolytes is due to some direct action of the non-electrolyte on the cells, because the duration of the rhythm in any one of the solutions depends on the condition of the sinus, and, further, because this duration does not stand in direct relation to rate of diffusion of the blood salt into the specific solution, or the rate of diffusion of the specific non-electrolyte into water or  $\frac{2}{3}$  sodium chloride.

3. It is not permissible, at least for the heart tissues, to assume that any of these non-electrolytes are without action on the tissues save through the osmotic factor.

## ON THE MECHANISM OF THE REFRACTORY PERIOD IN THE HEART.

BY A. J. CARLSON.

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

THE experiments here recorded were undertaken for the purpose of determining whether there is any causal connection between the property of so-called refractory period and the property of automatism in the heart. So far as these experiments include the invertebrate heart they are merely a continuation of the work on the systolic refractory state reported in this journal last year.<sup>1</sup> In that work it was shown that the invertebrate heart (molluscs, arthropods, tunicates) exhibits the typical refractory period at the beginning and during systole, that is, a refractory period in the sense of reduced excitability. But it is reduced excitability only, and not a condition of inexcitability. The experimental evidence in support of this thesis is conclusive. Observers who deny the presence of a refractory state in the invertebrate heart obviously understand by refractory period a state of absolute inexcitability. Such a condition does not exist in the invertebrate heart, although in some of the molluscs (octopus) and tunicates (ciona) the excitability of the heart at the beginning of the automatic beat is very slight.

Three years ago I showed that a refractory period in the sense of absolute inexcitability does not exist in the heart of one of the lowest vertebrates, the California hagfish (*Bdellostoma*).<sup>2</sup> Supermaximal contractions as well as submaximal contractions can be produced in the ventricle, auricle, and hepatic heart of this animal by strong induction shocks sent through the heart at the very beginning of systole; and not only that, but it is less difficult to obtain these supermaximal beats in the auricle than in the ventricle, although the latter

<sup>1</sup> CARLSON: This journal, 1906, xvi, p. 67.

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

exhibits much less automatism than the former. As regards the refractory state, therefore, there is no difference, or at the most only a difference in degree, between the heart of this vertebrate and the invertebrate heart. These observations have now been extended to the higher vertebrates (amphibians, reptiles) with practically the same results. The frog heart and the tortoise heart are excitable at the beginning of systole, just as is the case with the hagfish heart and the invertebrate heart. The view that the systolic refractory state in the vertebrate heart is a condition of absolute inexcitability is therefore untenable for many vertebrates, and will probably prove untenable for all.

One series of the experiments was directed towards determining whether the refractory state of the heart is a property of the heart muscle or the nervous tissue or both. The other series aimed to determine the degree of refractory state exhibited by the different parts of the vertebrate heart. If there is a causal connection between automatism and a refractory state in the sense of absolute inexcitability, we ought to find this condition in the *primus movens* of the heart, the sinus, or in mammals, the mouth of the great veins, and we would expect a less degree of refractory state or even none at all in parts of the heart not automatic, for example, the tortoise ventricle, or the apex of the frog ventricle.

#### I. THE TISSUES IN THE HEART CONCERNED IN THE PROPERTY OF THE REFRACTORY STATE.

1. *The automatic heart ganglion of Limulus exhibits the typical refractory period of the heart or a state of reduced excitability during systole.*—For the experiments demonstrating this point the dorsal nerve cord or ganglion was isolated from the heart muscle except in the first two heart segments, which were used for recording the ganglionic rhythm. The heart muscle was transected in the middle of the second segment, and the part posterior to the lesion removed. This leaves the ganglion on the posterior end of the heart free, so that it can be placed on the electrodes and stimulated without the stimulus reaching the heart muscle, while the connection of the ganglion with the musculature of the first two segments insures accurate records of the activity of the ganglion. This method of preparing the heart is described in more detail in my report on the action of temperature variations on the *Limulus* heart.<sup>1</sup>

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

An intensity of the induced shock that produces shortening of the diastole and a supermaximal contraction of the heart muscle when sent through the ganglion towards the end of the systole of the muscle produces no visible effect when sent through the ganglion at the beginning of the muscle contraction. The beginning of the contraction of the heart muscle is, of course, not the beginning of the discharge of the impulse from the ganglion. Hence, by this method it is not possible to stimulate the ganglion at the very beginning of the nervous discharge. It is not known whether the time of

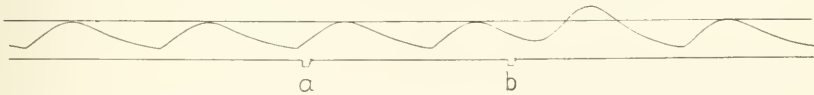


FIGURE 1.—One-half the original size. Tracing from the anterior end of the Limulus heart. The dorsal ganglion isolated from the heart muscle posteriorly and stimulated with induced shocks (make) of uniform intensity. *a*, ganglion stimulated near the beginning of systole. *b*, ganglion stimulated towards the end of diastole, showing diminished excitability of the heart ganglion for some time after the beginning of an automatic discharge.

discharge from the ganglion covers the entire time of systole of the muscle. But even if it does, it is obvious that the beginning and the end of the muscle systole lag behind the beginning and the end of the discharge from the ganglion, because the region of the ganglion that is especially automatic is in the middle third of the heart, that is, removed from the heart muscle in our preparation by a distance of three to four centimetres. And it has been shown in a previous paper that the dorsal nerve plexus in the Limulus heart conducts at the low rate of 40 cm. per second.<sup>1</sup> Whatever be the exact time relations between the ganglionic discharge and the systole of the heart muscle, it is certain that the stimulus which is effective when sent through the ganglion at the end of the latter is ineffective when applied to the ganglion at the beginning. This fact can only be interpreted in one way, namely, that the ganglion exhibits diminished excitability for an appreciable time after or during the automatic discharge.

Typical tracings illustrating this point are reproduced in Figs. 1 and 2. The make shock (down stroke of signal) is used for stimulus. The tracing in Fig. 1 is from a rapidly moving recording surface. The strength of the shock is the same at *a* and *b*. At *a*, beginning

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 99.

of systole, it is practically ineffective, while towards the end of diastole (*b*) the stimulation not only shortens the diastole, but produces a supermaximal beat.

It is equally easy to demonstrate that this refractory state of the ganglion is not a condition of absolute inexcitability. If induced shocks of sufficient intensity are used, supermaximal beats are produced by stimulating the ganglion at the beginning of the muscular systole. It may be urged that these experiments do not show that the ganglion is excitable at the very beginning of the discharge, since it is being stimulated an appreciable time after the beginning. I

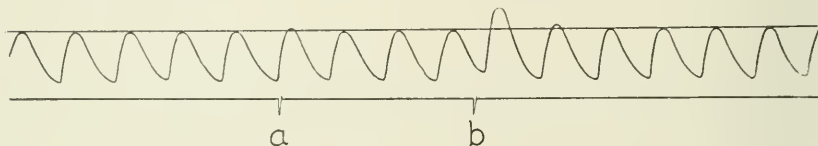


FIGURE 2.—Tracing from the anterior end of the *Limulus* heart. Heart ganglion prepared and stimulated as in Fig. 1. *a*, stimulation of ganglion and the beginning of systole. *b*, stimulation of ganglion towards the end of diastole. Showing diminished excitability of the ganglion at the beginning of systole.

have tried to demonstrate an absolute refractory state in the ganglion by stimulating it a fraction of a second before the beginning of the muscular systole so as to make the stimulation coincident with the beginning of the discharge, but if the induced shock was strong enough I never failed to get the supermaximal beat. The fact that the ganglion can be tetanized with the strong interrupted current also tends to show that refractory state of the ganglion is not absolute.

A typical tracing showing supermaximal beats following a strong induced shock sent through the ganglion at the beginning of the muscular systole is reproduced in Fig. 3.

2. *As long as the heart ganglion is in physiological connection with the heart muscle, the heart muscle and nerve plexus exhibit a condition of reduced excitability at the beginning of systole.*—This result is obtained from all regions of the heart. Nor is it necessary that the ganglion is intact in the region stimulated. The ganglion may be extirpated in the first two segments and the heart muscle removed for a distance of one or two centimetres in the third segment, leaving the anterior end connected with the ganglion by the lateral nerve plexus only, and this ganglion-free anterior end exhibits the same condition of reduced excitability at the beginning of systole as the middle region of the heart containing the ganglion.



The heart muscle and the nerve plexus in the Limulus heart respond to stimulation with induction shocks. These tissues are therefore directly excitable to the induced current. It is probable, however, that both the muscle and the nerve plexus are less readily stimulated by the induced current than is the heart ganglion, because it has been shown that on extirpation of the ganglion the response of the heart to direct stimulation is greatly diminished.<sup>1</sup> The maximal stimulus for the intact heart becomes submaximal on removal of the ganglion. This fact might suggest the following explanation of the apparent refractory state of heart muscle and nerve plexus just de-

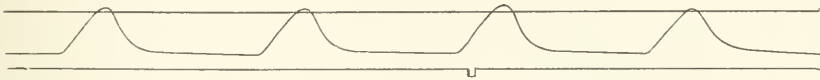


FIGURE 3.—Two-thirds the original size. Tracing from the anterior end of the Limulus heart. Ganglion isolated posteriorly, and stimulated with a strong induced shock near the beginning of systole. Supermaximal beat following strong stimulation of the ganglion at the beginning of systole.

scribed, namely, that it is really the refractory state of the ganglion and not of the muscle and nerve plexus that is brought out by these experiments. The stimulus is too weak to act on the heart muscle and the motor nerve directly, but strong enough to act on the ganglion and on the afferent reflex fibres in the plexus.<sup>2</sup> Hence the response of the muscle on stimulation during diastole is due to a reflex through the ganglion. And the failure of the response at the beginning of systole is due to refractory state of the ganglion at this period. This theory was put to the experimental test in the following way. The ganglion was extirpated in the first two segments and the heart muscle completely dissected away in the third segment, leaving the lateral nerves intact. The two ends of the heart were suspended for simultaneous graphic registration in the usual way, and the electrodes arranged for sending the current through the anterior end from side to side. Now, if the response on stimulating during systole is due to direct action of the current on the heart muscle and the motor nerves, that response should be confined to the anterior end. If it is a reflex through the ganglion, however, the posterior end of the heart should also exhibit the extra contraction. The results contradicted the proposed explanation. The extra contraction was confined to the anterior end. It is therefore not a reflex through the ganglion, but a

<sup>1</sup> CARLSON: This journal, 1905, xiii, p. 217.

<sup>2</sup> *Ibid.*, 1905, xii, p. 471.

direct effect of the current either on the heart muscle or on motor nerve plexus or on both. Hence it follows that *a stimulus strong enough to produce an extra beat by acting on the heart muscle and nerve plexus fails to produce any visible effect when sent through the same tissues, at the beginning of the normal systole.* — In other words, both ganglion, motor nerve plexus, and heart muscle exhibit the systolic refractory state or diminished excitability. In neither of the tissues is the refractory condition absolute. A strong induced shock

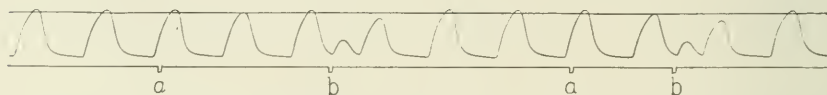


FIGURE 4. — One-third the original size. Tracing from the anterior end of the *Limulus* heart. Ganglion extirpated from the anterior end, the lateral nerves being left intact. The ganglion-free anterior end stimulated with induced shocks of uniform intensity. *a*, stimulation at the beginning of systole. *b*, stimulation towards the end of diastole. Showing diminished excitability of the heart muscle and nerve plexus at the beginning of a normal (neurogenic) automatic beat.

sent through the anterior end of the heart (prepared as just described) at the beginning of systole produces a supermaximal contraction just as when the ganglion is similarly stimulated.

3. Do the *Limulus* heart muscle and motor nerve plexus exhibit a systolic refractory state after being severed from the ganglion? Since extirpation of the ganglion abolishes the automatic contractions permanently, this question can only be attacked by means of artificial rhythms. Attempts were made to decide the point by stimulating the isolated segments by a uniform series of induction shocks at the rate of every four or five seconds and by means of a second set of electrodes test the excitability of the heart tissues at the beginning of the contraction. The results were not conclusive, mainly because of the difficulty of getting a series of contractions of absolute uniform amplitude from the *Limulus* heart after the nerve cord is removed. The interpretation of the tracings from these experiments is therefore difficult.

Since these inconclusive experiments were made I have found that the sodium chloride rhythm of the *Limulus* heart deprived of the ganglion is idio-muscular and that the superficial or dorsal nerve plexus takes no part in the rhythm.<sup>1</sup> The sodium chloride rhythm may, in rare instances, be perfectly regular in the rate and the amplitude of the contractions. It is obvious that this rhythm, when

<sup>1</sup> CARLSON: This journal, 1907, xvii, p. 478.

regular, gives the conditions for determining whether the heart muscle exhibits the refractory state during an artificial systole. It is true that during the period that the sodium chloride rhythm remains regular the heart muscle continues to respond to the stimu-

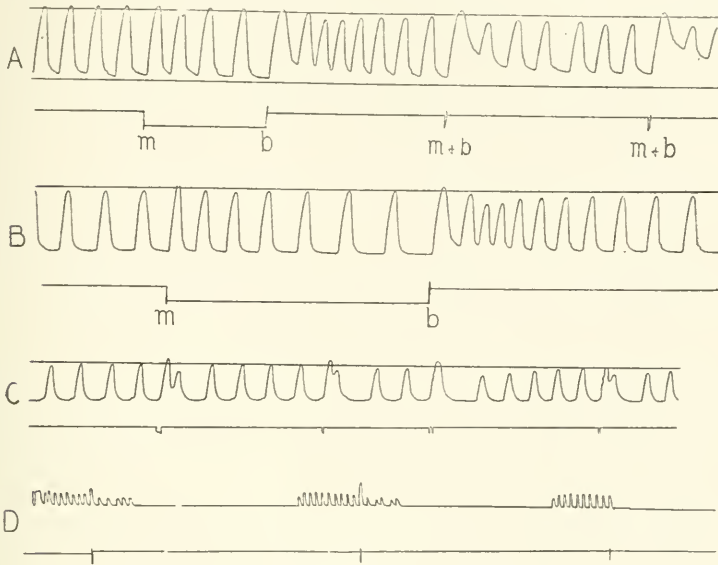


FIGURE 5 — *A-C*, tracings from the isolated frog's ventricle. *D*, tracing from the isolated toad's ventricle. *m*, make induced shock. *b*, break induced shock. Stimulation at the beginning of systole resulting in supermaximal beats at times accompanied or followed by tonus contraction. Showing excitability of these ventricles at the beginning of systole.

lation of the motor nerves, so that on direct stimulation we do not know whether we are stimulating the muscle directly or indirectly through the nerve plexus. But that does not affect this question, as the nerves or the motor plexus are not active in this rhythm and hence cannot possibly exhibit a refractory state. The question will be settled by this method as soon as material is at hand.

4. Is the refractory state in the vertebrate heart a property of the heart muscle apart from the intrinsic heart ganglia and nerve plexus?<sup>1</sup> (1) It is needless to say that this question has not so far, and perhaps never can be, attacked by direct experiments. Rohde has recently attempted to answer the question by studying the changes in the response of the frog's ventricle to direct stimulation after subjecting

<sup>1</sup> The experiments in this section were performed in conjunction with Mr. W. B. Walker.

the ventricle to the action of chloral hydrate.<sup>1</sup> According to Rohde the physiological properties peculiar to the heart tissues are abolished by chloral hydrate before contractility and irritability are abolished. The refractory state is shortened if not altogether abolished, the all-or-none law fails, and appropriate stimulation produces superposition and tetanus. Rohde interprets these results as due to the paralysis of the nervous tissue in the frog's ventricle by the drug before the muscle is paralyzed. On this assumption the refractory state and the other properties peculiar to the heart are properties of the nervous tissue or of the nervous and the muscular tissues as long as they retain their functional relations, but when the heart muscle is isolated from the nervous tissue it responds to direct stimulation like skeletal and smooth muscle. Schultz,<sup>2</sup> working in Howell's laboratory, has repeated Rohde's experiments, using the frog and the terrapin ventricle. Schultz's experiments practically confirm the results of Rohde, except on the one point touching the complete abolition of the systolic refractory state. According to Schultz, the ventricular tissue retains the characteristic refractory state in chloral narcosis as long as contractility is retained, although the drug shortens the duration of the refractory period. Thus summation of the contractions is produced by stimuli reaching the ventricle during the latter third of the systole. The tracings published by Rohde (Fig. 2 b) do not prove that the "shortened absolute refractory period" in the sense that term is employed by Schultz is abolished, because in these records some of the stimuli that give rise to the supermaximal beat reach the heart in the last third of systole.

I have shown in a previous paper that Rohde's theory is completely substantiated by the action of chloral hydrate on the tissues in the *Limulus* heart, this drug paralyzing first the ganglion, then the dorsal or superficial nerve plexus, and lastly the heart muscle.<sup>3</sup> I am not yet in position to state whether or not a systolic refractory state can be demonstrated in the *Limulus* heart muscle after paralyzing the nervous tissue by chloral hydrate. The contractions of the heart muscle after such paralysis of the ganglion are those called forth by artificial stimulation, and it is difficult to obtain a uniform series of contractions, even when great care is taken to have the stimulus of uniform intensity.

<sup>1</sup> ROHDE: *Archiv für experimentelle Pathologie und Pharmakologie*, 1905, lxiv, p. 104.

<sup>2</sup> SCHULTZ: *This journal*, 1906, xvi, p. 483.

<sup>3</sup> CARLSON: *Ibid.*, xvii, p. 1.

(2) Nerve tissue probably dies sooner than muscular tissue when respiration and nutrition are stopped. If the refractory state depends on the nervous tissue in the heart, the excised or dying heart ought to exhibit a condition of diminished or abolished refractory state in the later stages while it still retains some excitability and contractility. This hypothesis was tested on the tortoise ventricle, which under normal conditions comes nearer than the frog ventricle to having an "absolute refractory period." The excised ventricle was kept for a

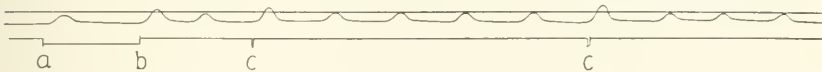


FIGURE 6. — One-half the original size. Tracing from the tortoise ventricle after being kept isolated in moist chamber at 20° C. for twenty-four hours. Rhythm started by strong break shock at *b*. *C*, ventricle stimulated with very strong break shocks at the beginning of systole, resulting in supermaximal beats.

varying number of days in the moist chamber at room temperature (18°–22° C.) or in the ice box (at a temperature of about 5° C.), and the degree of systolic excitability tested from time to time. The ventricles kept at the lower temperature, of course, retained their irritability and contractility the longest. The longest time a tortoise ventricle retained excitability and contractility after isolation was six days (at 3° C.). The ventricle, isolated from the auricles, does not continue in rhythmic activity. It is therefore necessary to resort to contractions artificially produced. A series of induction shocks of uniform intensity was usually employed. Isotonic sodium chloride was also made use of. The systolic excitability was tested on the whole ventricle as well as on ventricular strips. Our results were as follows: (a) The single induced shock of an intensity that fails to produce a supermaximal contraction when sent through the fresh ventricle at the beginning of systole causes a supermaximal contraction in a heart in the last stages of dying (Fig. 6). This is also true for the sodium chloride rhythm of the heart in the last stages of dying, and can therefore not be explained by the staircase phenomenon. The excitability of the dying heart at the beginning of systole is further shown by the fact of summation, two induction shocks following one another within a shorter interval than the latent period of the ventricle, producing a greater contraction than either stimulus separably. This form of summation cannot be accounted for by the phenomenon of staircase contractions, unless the first stimulus is too

weak to produce any contraction at all by itself, which was not the case in our experiments. In the case of ventricles kept for two or three days at the low temperature a rhythmical series of beats could sometimes be started by sending a very strong induction shock through the ventricle. Supermaximal beats were always produced by a strong break shock at the beginning of systole of this rhythm. But while the tortoise ventricle in the last stages of dying is excitable at the beginning of systole, the excitability is lower at the beginning of systole than towards the end of diastole. It is therefore evident that *even in the last stages of dying the ventricular tissue or tissues retain the property of systolic refractory state in the sense of diminished excitability.*

(b) In the last stages of dying the excitability of the tortoise ventricle to the induced current is greatly diminished if not entirely abolished before the excitability to mechanical and chemical stimulation is lost. Thus frequently the strongest induction shock available (two Edison-Lalande cells, type "S" in circuit, 1100 windings of the secondary coil) failed to produce any contraction, while mechanical stimulation or immersion in an isotonic sodium chloride solution was effective. This might suggest that the heart muscle is, even under normal conditions, but slightly affected by the induced current, but because of the greater excitability of the nervous tissues in the heart this fact does not become evident till the latter are eliminated. It is practically certain, however, that the heart muscle itself is more readily affected by all stimuli when in normal condition than when in the last stages of dying.

(3) It has been shown in a previous paper that the sodium chloride rhythm of the *Limulus* heart with the automatic ganglion extirpated is idio-muscular. The dorsal or superficial nerve plexus takes at least no part in this rhythm, although the rhythm is affected by stimulation of this nerve plexus. The systolic excitability of the *Limulus* heart muscle in sodium chloride rhythm has not yet been studied. If the corresponding rhythm of the tortoise ventricle in sodium chloride is idio-muscular, we might expect a diminution or abolition of the systolic refractory state in this rhythm, provided the refractory state is a property of the nervous tissue alone. Numerous experiments were carried out with strips from the tortoise ventricle to test this hypothesis. It is nearly as difficult to produce a supermaximal beat by a stimulus at the beginning of systole in the case of the fresh ventricular strip brought to uniform rhythm by sodium

chloride as in the case of the intact ventricle beating in response to the impulse from the auricles. It has already been pointed out that when the ventricular strips are in the last stages of dying they react differently to systolic stimulation when in sodium chloride rhythm. The fact that the fresh tortoise strips in the sodium chloride rhythm exhibit practically as great a degree of systolic refractory state as the ventricle in normal rhythm, does not prove, however, that the refractory state is a property of the muscle apart from the nervous tissue, as the sodium chloride rhythm of the tortoise ventricle may involve the nervous tissue, just as it does in the *Limulus* heart when the ganglion is intact.

We also repeated the experiments of Rohde and Schultz with the effect of chloral hydrate on the refractory state of the tortoise ventricle, obtaining practically the same result as these observers. A systolic refractory period in the sense of diminished excitability is in evidence as long as the ventricle retains excitability and contractility.

It is therefore evident that the question whether heart muscle when isolated from the intrinsic nervous tissues exhibits the property of refractory state to greater degree than skeletal and smooth muscle is still an open one, since the facts bearing on the question can be interpreted either way. In *Limulus* the heart ganglion exhibits the typical refractory period of heart tissue, and as this is a characteristic of at least many ganglion cells in the central nervous system of vertebrates, it is probable that the ganglion cells in the vertebrate heart possess a systolic refractory state similar to that of the *Limulus* heart ganglion.

## II. THE DEGREE OF REFRACTORY STATE IN THE HEART OF DIFFERENT ANIMALS, AND IN THE DIFFERENT PARTS OF THE HEART OF THE SAME ANIMAL.

- While some observers have held that the refractory period in the vertebrate heart is a condition of diminished excitability only, and not a state of absolute inexcitability, most physiologists employ the term "refractory period" in the latter sense. Thus Howell's student Schultz speaks of the "absolute refractory period" as coincident with the time of systole in the frog and tortoise ventricle. I have shown that this conception of the refractory state is not tenable for any part of the heart of the hagfish. Supermaximal beats as well as diminution of the beats are produced in this heart by strong induction shocks

at the very beginning of systole. In the hagfish heart the refractory state is therefore a condition of reduced excitability only.

1. *The ventricles of higher vertebrates do not exhibit the same degree of systolic refractory state.*— In the case of the suspended and empty ventricle of the snapping turtle and the mud turtle I have not succeeded in obtaining supermaximal beats by even the very strongest induction shocks at the beginning of systole. These strong induced shocks produce, however, a diminution of the beat without necessarily producing any change in the subsequent rhythm (Fig. 7 a). It is obvious that inhibition of a phenomenon is just as much an evidence of irritability of the tissue as the augmentation of it. Hence, when the strong induced shock sent through the tortoise ventricle at the very beginning of a normal automatic contraction diminishes the amplitude of that contraction, it is demonstrated that the tortoise ventricle responds to the strong induced shock at the beginning of systole. The tortoise ventricle is therefore excitable at the beginning of systole, and the refractory state in this ventricle is only a condition of greatly diminished excitability.

The empty and suspended ventricle of the frog, toad, and salamander (neoturus) differ from the tortoise ventricle in this regard. A break or make shock of sufficient intensity never fails to produce a supermaximal beat in the empty and suspended ventricle of the former animals when applied at the beginning of the beat (Fig. 5). The supermaximal beat may or may not be accompanied by a tonus contraction and alteration of the subsequent rhythm. But the fact that the supermaximal beat is sometimes obtained without any demonstrable tonus contraction goes to show that it is not produced by addition of the tonus to the fundamental contraction.

The fact that the frog's ventricle responds with a supermaximal beat to the very strong induction shock at the beginning of systole prevented us from using the frog's heart in the chloral hydrate experiments just described. We found, in fact, that the supermaximal beat by stimulation at the beginning of systole is almost as readily produced in the normal ventricle as in the ventricle acted on by chloral hydrate.

A certain intensity of the induced shock applied at the beginning of systole may diminish the strength of the beat of the amphibian ventricle instead of augmenting it, but the augmentation is the usual effect. Either phenomenon demonstrates the excitability of the ventricle to the induced current at the beginning of systole. This phe-



nomenon of inhibition has now been demonstrated for the heart of all vertebrates and invertebrates tested in this regard, and is therefore probably common to all hearts.<sup>1</sup> The mechanism of this inhibition is obscure. It has in all probability nothing in common with the normal inhibition of the heart. We have seen that the inhibition is obtained in the tortoise ventricle, which according to Gaskell has

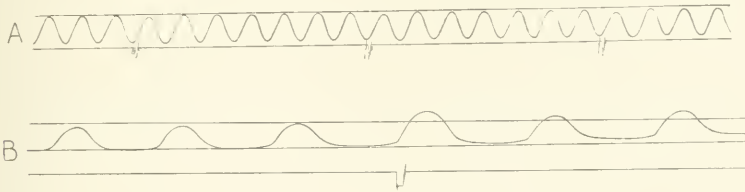


FIGURE 7.—*A*, tracing from the isolated ventricle of the tortoise (*Cistudo*). Diminution of the beat by strong induced shocks sent through the ventricle at the beginning of systole. *B*, tracing from the sinus of same specimen as *A*. Supermaximal beat by strong induced shock at the beginning of systole.

no inhibitory nervous mechanism. The same is true of the heart of many invertebrates in which inhibitory nervous mechanism appears to be absent.

2. *The degree of refractory state in the different parts of the same heart.*—The refractory state is usually considered to be a common property of all parts of the heart. If there is any causal connection between the property of an absolute refractory state and the property of automatism, it is evident that the sinus venosus in cold-blooded vertebrates and the mouth of the great veins in the mammals must exhibit an absolute refractory condition at the beginning of systole, because those are primarily the automatic parts of the heart. The auricles must also exhibit this absolute refractory state, as the auricles are invariably more automatic than the ventricles.

In the case of the hagfish heart the supermaximal contraction by stimulation at the beginning of systole is more readily obtained in the auricle than in the ventricle. It is therefore evident that the auricular tissues have a greater excitability and contractility at the beginning of systole than the ventricle, although the latter exhibits less automatism than the former.

In the amphibians (frog, toad, salamander) and reptiles (snapping turtle, mud turtle) examined by me there seems to be no appreciable difference between the degree of systolic refractory condition of the

<sup>1</sup> CARLSON: This journal, 1906. xvi, p. 100.

ventricle and the auricles. Supermaximal beats, apart from tonus contractions, are, if anything, more readily produced by stimulation during systole in the auricles than in the ventricles. In the case of the auricles of the snapping turtle, however, I have been unable to produce supermaximal beats apart from tonus contraction by stimulation at the beginning of systole. This stimulation produces diminution of the contraction just as in the ventricle of these animals.

The sinus venosus of the amphibians at my disposal (frog, toad, salamander) is small and delicate. Isolation from the auricles and the veins for accurate graphic record of the rate and amplitude of the contractions is therefore difficult without injury to the sinus. My observations on the sinus venosus of these animals were therefore few in number and inconclusive in results. The sinus of the large snapping turtle is easily isolated and strong enough for the ordinary graphic method, and the same may be said of the sinus of the smaller mud turtle. Numerous experiments were made with the sinus venosus of these animals. The sinus of the snapping turtle exhibits on the whole the same degree of systolic refractory state as the auricles and the ventricles of the same animal. That is to say, even the very strongest induced shock at the beginning of systole fails to produce a supermaximal beat unaccompanied by tonus contraction. But this stimulation may diminish the strength of that beat just as in the other parts of the heart (Fig. 9). The sinus of *Cistudo* exhibits a different response to stimulation during systole. Even moderately strong induced shocks sent through this sinus at the beginning of systole produce a supermaximal beat not accompanied by tonus contraction (Figs. 7, 8). A stronger induction shock produces in addition to the supermaximal fundamental beat a tonus contraction lasting for many minutes. But even in this case the supermaximal beat is not the result of the addition of the tonus to the normal fundamental contraction, because the added amplitude is greater than the tonus contraction, even at the end of the subsequent diastole, and the tonus contraction reaches its maximum only after the lapse of minutes. Bottazzi describes numerous smooth muscle cells among the striated heart cells of the tortoise auricles, and is inclined to believe that the tonus contractions of the auricles are due to the contraction of this smooth muscular tissue while the fundamental beat involves the striated cells only.<sup>1</sup> In case the same anatomical conditions obtain in the sinus it is obvious that if the supermaximal beats produced by stimulation

<sup>1</sup> BOTTAZZI: *Zeitschrift für allgemeine Physiologie*, 1906. vi. p. 140.

at the beginning of systole were always accompanied by tonus contractions the systolic excitability of the tissues concerned in the fundamental beat would not be proved, for the supermaximal beat might be simply the addition of the tonus (smooth muscle) to the funda-

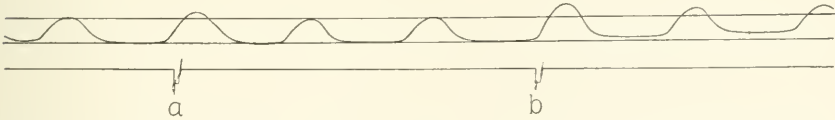


FIGURE 8. — Tracing from the sinus of *Cistudo*, showing supermaximal beats following stimulation with strong induced shocks at the beginning of systole. The stronger shock (*b*) produces also a slight tonus contraction.

mental contraction (striated muscle). In this tortoise we have, therefore, the case of a *vertebrate heart exhibiting a less degree of systolic refractory state in the automatic part (sinus) than in the non-automatic part (ventricle)*.

3. *Is there any causal connection between the property of refractory state and the property of automatism in the heart?* — The property of



FIGURE 9. — About one-half the original size. Tracing from the sinus of the snapping turtle. Showing diminution of the fundamental contraction (and tonus) by the strong induced shock at the beginning of systole.

refractory state in the heart is believed by some physiologists causally connected with the property of automatism.<sup>1</sup> If we conceive of the stimulus to the heart rhythm to be some chemical substance ever present in the active tissues, it is plain that the systolic refractory state of the automatic tissue would result in periodic response to this constant stimulus. Admitting for the sake of argument that the systolic refractory state is absolute and that the stimulus to the heart rhythm is some chemical ever present in the tissues, it is obvious that the normal maximal intensity of that stimulus fails to affect the heart during a greater part of diastole, a period in which it is admitted by all that the heart tissues are excitable to direct stimulation. This fact shows that the time of action of this stimulus does not coincide with the absolute return of excitability of the heart tissues. The

<sup>1</sup> HOWELL: The journal of the American Medical Association, 1906, lxi, Nos. 22, 23.

stimulus is able to effect a response only when the excitability of the heart tissues has reached their maximum. Hence a refractory state in the sense of diminished excitability would just as surely result in rhythmic activity as a systolic refractory state of absolute inexcitability. Thus we see that even on the above assumptions an absolute refractory state of the automatic tissues of the heart is by no means a *sine qua non* for the rhythm.

It is furthermore obvious that if the heart muscle is not the automatic tissue in the heart, we can account for the heart rhythm even if the automatic tissue exhibits no variation in excitability during the different phases of the heart beat and is being stimulated continuously by our hypothetical chemical substance. If, for example, the heart muscle possesses a systolic refractory state but no automatism, that is, the contractions are the result of impulses reaching it from the nervous tissues, we can conceive of this nervous tissue sending a continuous impulse to the muscle perhaps in a way analogous to the activity of the motor neurons resulting in the tonus of skeletal muscle, but these impulses would be effective only at the point of return of the heart muscle to a certain degree of excitability. This rhythm would not be distinguishable from a rhythm due to the automatism of the heart muscle itself. Yet the automatic tissue may not suffer any more demonstrable changes in excitability than is exhibited by a nerve fibre after stimulation. But aside from all theoretical considerations, is the above view tenable on the basis of facts? The following considerations seem to make the theory untenable:

(1) *The property of refractory state is exhibited by tissues that do not have the property of automatism under normal conditions.*—The ventricle of the turtle is not automatic, although automatism is induced in it by many artificial conditions; yet all parts of the turtle's ventricle exhibit a systolic refractory state as absolute as that of the automatic parts of the heart or that of the automatic parts of the heart of any other vertebrate. The Bernstein experiment shows that the apex of the frog's ventricle is not normally automatic, yet the degree of systolic refractory state is just as marked, if not more so, in the ventricular apex as in the auricles; and if we look for instances in support of the above thesis outside of the tissues of the heart, we find such in the motor cells of the central nervous system and in the tissues of the small intestines (mammal). The observations of Horsley and Schäfer and Richet go to show that the refractory period of the pyramidal cells of the cerebral cortex and of the motor cells in the cord may be

as great as one-tenth to one-twelfth of a second. This length of refractory period is exhibited by the nerve cells in response to the normal or physiological stimulus. Whether the refractory state of the nerve cells is absolute or only relative will probably remain an open question forever, because it is not possible to stimulate the cells directly with a very strong induction shock without at the same time stimulating the motor fibres leading from the cells. No physiologist will contend, I opine, that the pyramidal cells of the motor areas or the motor elements of the cord in connection with the skeletal muscles are in automatic activity under normal conditions of life.

The case of the mammalian intestines argues to the same effect. Magnus has shown that the cat's intestine exhibits a condition of refractory state at the beginning of a spontaneous contraction. This fact has been denied by Schultz, again reiterated by Magnus, and, at least in part, confirmed by Bottazzi.<sup>1</sup> The work of Bayliss and Starling and especially Magnus has demonstrated that none of the forms of periodic activity of the intestines are due to muscular automatism. The peristalsis, at least, is a complex reflex. The same is probably true of the so-called "pendulum movements." It would seem safe to assume that the intestinal musculature as well as the intrinsic motor nerves possess direct excitability. Nevertheless the entire organ exhibits a refractory state at the beginning of an automatic contraction.

The nerve plexus and heart muscle of *Limulus* are not automatic, yet as long as they are in physiological connection with the automatic ganglia, they exhibit systolic refractory state in the sense of reduced excitability.

(2) *Some tissues that are normally automatic do not exhibit an absolutely refractory state, but only a condition of greatly diminished excitability.* — (a) The hearts of all invertebrates are automatic. But a sufficiently strong stimulus is effective in the invertebrate heart at the very beginning of systole. This fact, however, does not prove that the automatic tissue of the invertebrate heart is devoid of an absolutely refractory state, because the supermaximal contraction produced by the stimulus at the beginning of systole may be due to direct stimulation of the non-automatic heart muscle, while the automatic heart ganglia remain unaffected. Fortunately this objection can be disproven by the *Limulus* heart. In this heart the ganglion is the automatic tissue, and we have seen that the refractory period of this ganglion is a state of diminished excitability only.

<sup>1</sup> For the literature see BOTTAZZI: *Archiv für die gesammte Physiologie*. 1906, cxiii. p. 136.

(b) The same is true of the sinus venosus of the mud turtle, as already pointed out, and will in all likelihood be found to be true for the automatic end of the heart of other vertebrates. The excitability of this sinus at the beginning of systole is not due to stimulation of the smooth or supposedly non-automatic muscle cells of the sinus walls.

(c) The tonus rhythm of the tortoise auricles does not exhibit an absolute refractory period in the systolic phase.<sup>1</sup> The mechanism of the normal heart tonus is as yet largely a matter of conjecture. The *Limulus* heart seems to show that the motor nerve-centres in the heart have the same relation to the normal tonus of the heart muscle as has the central nervous system to the normal tonus of skeletal muscle. On the side of contraction the tonus in the heart may involve the muscle cells concerned in the fundamental beat, or only the smooth muscle cells scattered throughout the heart, as suggested by Bottazzi. In either case, Porter's observation modifies the common conception of the refractory state, at least as regards the tortoise heart, a fact that appears to be overlooked by Howell and Schultz.

(3) *In the same heart the parts possessing the greatest degree of automatism may exhibit a less degree of refractory state than the part of the heart not automatic*, as shown by the heart of the mud turtle already described.

<sup>1</sup> Porter: This journal, 1905, xvi, p. 1.

# A CONTRIBUTION TO THE CHEMISTRY OF CELL DIVISION, MATURATION, AND FERTILIZATION.

BY A. P. MATHEWS.

[From the Marine Biological Laboratory, Woods Holl, Mass.]

## INTRODUCTION.

THIS paper contains the results of experiments upon the eggs of the starfish, *Asterias Forbesii*,<sup>1</sup> and of the sea-urchin, *Arbacia punctulata*, to determine something of the nature of the chemical processes involved in mitotic cell division. The work was done at Woods Holl, in 1905, in the latter part of August and early September, when the starfish eggs are at their best.

While the morphology of mitosis has now been very carefully studied, very little is known concerning the chemical processes which furnish the energy for these complex movements. Changes in the amount and staining reaction of the chromatin during cell division early attracted attention and have been studied by many investigators, of whom particular mention may be made of Lillienfeld<sup>2</sup> and Heine.<sup>3</sup> These authors drew the conclusion, now widely accepted, that during mitosis the chromatin split into an albuminous substance and into a salt of nucleinic acid. The latter substance formed the chromosomes; the fate of the former was undetermined. It is also known that chemical changes occur in the cytoplasm coincident with these changes in the chromatin, since the cytoplasm undergoes at that time marked alteration in its staining reaction, physiological properties, and physical appearance. The nuclear wall appears to be dissolved or digested, and the astral figures make their appearance. The latter, in many instances at any rate, consist of a kind of cytoplasm different from that formerly present, if we may rely upon the different affinity for stains it shows. Nothing, however, is known

<sup>1</sup> Or *Asterias vulgaris*.

<sup>2</sup> LILLIENFELD: *Archiv für (Anatomie und) Physiologie*, 1893, p. 395.

<sup>3</sup> HEINE: *Zeitschrift für physiologische Chemie*, 1896, xxi, p. 494.

concerning the nature of the chemical changes involved in its production. It is in fact still uncertain whether the astral figure and the spindle are more or less viscid than the surrounding protoplasm, although the observations of Foot and Strobell<sup>1</sup> to the effect that the spindle preserves its form after flowing out of punctured eggs of *Allolobophora fœtida* would indicate that it was coherent or jelly-like. It is not possible to say with certainty whether the act of mitosis is accompanied by an act of clotting of the cytoplasm or not, although the evidence is perhaps in favor of such a conclusion. R. Lillie has tentatively suggested that at the astral centres and chromatin hydrogen ions are set free by some auto-digestive process and by their diffusion produce the astral figure, but it cannot be said that there is as yet much evidence of this.

The first clear physiological evidence of the chemical nature of the processes involved is found in the work of De Moor<sup>2</sup> on the dividing cells of the stamen hairs of *Tradescantia*. He found that in the absence of oxygen these cells could not complete their division. If a cell was dividing when placed in an atmosphere of hydrogen, it went through the division but came to rest without the formation of cell wall. This observation indicated that free oxygen was one of the essentials of mitosis, but it did not conclusively show at what stage it was necessary. Loeb,<sup>3</sup> by similar observations on animal cells, found that free oxygen was necessary for the division of certain echinoderm eggs and eggs of the fish *Ctenolabrus*, but that it was not necessary for many divisions of the eggs of the minnow, *Fundulus heteroclitus*. In *Ctenolabrus* not only was cell division prevented, but the blastomeres already formed in some cases re-fused in the absence of oxygen.

The dependence of mitosis upon oxygen was more carefully examined by Lyon,<sup>4</sup> who found that oxygen was more necessary at certain definite periods of the process of fertilization. For the eggs of the sea-urchin, *Arbacia punctulata*, he showed that if the eggs after fertilization were placed in sea water deprived of oxygen the division came to a stop. On removing the eggs to oxygenated sea water after several hours' immersion in the oxygen-free water, the division in some cases recommenced and proceeded normally, while

<sup>1</sup> FOOT and STROBELL: *American journal of anatomy*, 1905, iv, 109.

<sup>2</sup> DE MOOR: *Archives de biologie*, 1895, xiii, 103.

<sup>3</sup> LOEB: *Archiv für die gesammte Physiologie*, 1895, lxii, p. 240.

<sup>4</sup> LYON: *American journal of physiology*, vii, 1902, p. 56.



in other cases the eggs did not recover. On further investigation it developed that if the eggs were placed in hydrogen about the time of the first cleavage they never recovered. Exactly the same phenomenon was discovered for their susceptibility to sea water containing potassium cyanide. About the time of the first division they were highly susceptible. Lyon was uncertain of the exact period at which they were susceptible, but thought it immediately after cell segmentation. Spaulding,<sup>1</sup> however, working with ether and hydrochloric acid, came to the conclusion that maximum susceptibility was just before or during segmentation, and this conclusion I have recently confirmed.<sup>2</sup> As ether, acids, and cyanides all interfere primarily with cell respiration, these results all show that about the period of first segmentation, when the aster is growing with its greatest rapidity and the nuclear wall dissolving, any agent which interferes with cell respiration is peculiarly fatal to *Arbacia* eggs. The significance of this fact will appear later. Lyon<sup>3</sup> in a subsequent paper studied the production of carbonic dioxide by these eggs during fertilization, and succeeded in showing that carbon dioxide was not produced evenly throughout the process, but that coincident with the entrance of the sperm and about the time of the first segmentation there was a marked increase in its production.<sup>4</sup>

These facts, which so clearly pointed to the conclusion that mitosis was accompanied, if not caused, by modifications in the respiratory activity of the egg, were the starting-point of this investigation in which I have tried to discover whether these respiratory changes had any causal relation with cell division. I hoped to get some evidence, also, of the cause of the differences of electrical potential assumed to exist in the egg and to cause cell division by R. Lillie, Spaulding, and Hartog.

#### THE THEORY OF RESPIRATION.

The work of many authors on the nature of respiration has led to certain general conclusions which I have embodied in my paper<sup>5</sup> on the theory of cell respiration. That theory is a modification of the

<sup>1</sup> SPAULDING: *Biological bulletin*, 1903, vi, p. 97.

<sup>2</sup> MATHEWS: *Ibid.*, 1906, xi, 137.

<sup>3</sup> LYON: *American journal of physiology*, 1904, xi, p. 52.

<sup>4</sup> LYON: *Loc. cit.*, p. 57, says that one would infer that the energy for cell division comes from fermentative rather than oxidative processes.

<sup>5</sup> MATHEWS: *Biological bulletin*, 1905, viii, p. 331.

earlier theory of Hoppe-Seyler based chiefly upon the work of Nef. The general conclusion of all the work on respiration is to the effect that respiration is probably due to at least three distinct factors: *First*, to a strong reducing substance, which must be constantly produced by cell metabolism. This substance is in all likelihood a carbon compound, and has the power, possibly by setting free two of the valencies of a carbon atom, of attacking water, oxidizing itself and setting free nascent hydrogen. What this substance is, where it is located, and whether there is one or several such substances is unknown, except that it is probably produced by, and exists primarily in, the region of the nucleus, which, as is well known, generally lies in the most strongly reducing part of the cell. *Second*, to the cell oxidases. The manner of action of these substances and their composition is still uncertain, but there is no doubt that there exist in cells substances whose presence enormously hastens the action of atmospheric oxygen on the cell contents. These oxidases are also probably formed by, or are a constituent of, the cell chromatin, since the observations of Spitzer,<sup>1</sup> Lillie,<sup>2</sup> and Croftan,<sup>3</sup> among others, indicate that they accompany the cell chromatin in its chemical separation. Croftan's observations, particularly, seem to show that one at least of these oxidases consists of nucleinic acid in combination with an albumose. And *third*, to atmospheric oxygen. If cell division is at its basis a respiratory process, we have to look for and locate in the cell the first two factors and demonstrate the importance of the third.

The mitoses chosen for study were the maturation mitoses and subsequent phenomena of the starfish egg, and the first segmentation of the starfish and sea-urchin egg.

#### THE CHEMICAL PROCESSES INVOLVED IN THE MATURATION OF THE STARFISH EGG.<sup>4</sup>

The living, ovarian eggs of the starfish when fully developed consist of a clear, viscid protoplasm containing a multitude of partially transparent granules and at one pole a very large germinal vesicle

<sup>1</sup> SPITZER: Archiv für die gesammte Physiologie, 1895, lx, p. 303; *Ibid.*, 1897, lxvii, p. 615.

<sup>2</sup> LILLIE: American journal of physiology, 1902, vii, p. 412.

<sup>3</sup> CROFTAN: Medical record, New York, 1903, lxiv, p. 9.

<sup>4</sup> For a description of the morphological processes see the paper by WILSON and MATHEWS: The journal of morphology, 1904, x, p. 319.

with a large nucleolus. The eggs do not, as a rule at any rate, mature in the ovary, but only after shedding into the sea water. As soon as the eggs are shed they become a little more opaque, and if thoroughly ripe, the germinal vesicle wall becomes irregular and disappears in the course of ten minutes. The nuclear sap then mixes with the cytoplasm; the nucleolus dissolves and disappears; the chromatin dissolves for the most part; the maturation spindles are formed, containing a small part of the chromatin; and two polar bodies are extruded. The whole process to this point takes between two and three hours. The nucleus then re-forms at first as a little group of vesicles, but later these fuse, and the nucleus grows greatly in size and moves toward the centre of the egg. For the next three or four hours it continues to increase in size until its diameter may become more than half that of the germinal vesicle. Throughout this period the cell protoplasm remains clear and transparent, but about eight or twelve hours after the maturation has begun, the cytoplasm becomes more and more opaque, and if not disturbed it is finally converted into a coarsely granular, opaque, and dead mass.

The essence of the process of maturation consists morphologically, as pointed out by Delage,<sup>1</sup> in the discharge into the cell cytoplasm of the whole of the nuclear sap, the greater part of the chromatic network and the dissolved nucleolus, and the formation of the small maturation spindles. At maturation, therefore, two portions of the egg, *i. e.*, nucleus and cytoplasm, which have formerly been held separate, are intermingled. All that happens to the unfertilized egg thereafter — its division, its early death, its growing opacity and wholly changed physiological potentialities — may be ascribed to this admixture.

The early death and opacity of the egg after maturation occurs only if the egg be not fertilized or if the cell be left undisturbed. It may be wholly prevented by the entrance of the sperm, or by the various means producing artificial parthenogenesis. The early death of the matured, unfertilized egg is in striking contrast to the fate of the eggs of which the germinal vesicle remains intact, and no admixture of nuclear and cytoplasmic matter takes place. Such eggs remain clear, transparent, and living for two or more days.

The first questions to be answered are, what causes normally the dissolution of the nuclear membrane, and thus inaugurates the process of maturation by mixing cytoplasm and nuclear contents? and what change in the egg is produced by its maturation, which leads to its early death?

<sup>1</sup> DELAGE: Archives de zoologie expérimentale et générale. 1901. ix. p. 285.

As regards the answer to the first question, my results and conclusions confirm those of Loeb<sup>1</sup> on the same object. The cause of the beginning of the process of maturation is to be found primarily in the presence of free oxygen in the sea water. If eggs are shed into water deprived of its oxygen, they will not mature. However, hydroxyl ions in any numbers are not necessary, as Loeb suggests, since eggs will mature in pure solutions of calcium chloride of  $\frac{2}{3}$  mol concentration. I attempted, however, to cause maturation in the ovary by exposing this to oxygen directly, but without success. This negative result, however, I do not regard as conclusive, owing to the fact that it was tried early in the summer at a time when the ovaries were not in very good condition. There is, however, no doubt that free oxygen is essential to the beginning of normal maturation, and that the first visible sign of maturation consists in the disappearance of the membrane of the germinal vesicle at a point where it comes nearest to the surface of the egg. It may be recalled in this connection that mechanical shock is able to inaugurate maturation in eggs which normally do not mature, and that such eggs show on section ruptured germinal vesicles.<sup>2</sup>

#### DOES AN OXIDASE ESCAPE FROM THE NUCLEUS ON THE DISSOLUTION OF THE NUCLEAR MEMBRANE ?

We come now to the second question as to the nature of the changes produced in the cytoplasm by the admixture of nuclear material, — a change which leads to the early death of the egg, if the latter is not fertilized, and which so totally changes the potentiality of the cytoplasm. That the cytoplasm after maturation does differ markedly from that existing before fertilization is shown by its behavior. It acquires the power of forming a fertilization membrane; under suitable conditions it can generate asters; it becomes capable of cell division; and, if left alone, it rapidly dies.

The early death of the egg after maturation was studied and described by Loeb,<sup>3</sup> but he undertook no experiments to determine its cause or nature. He says, however:<sup>4</sup> "I can only suggest that the

<sup>1</sup> LOEB: Biological bulletin, 1902, iii, p. 295; Archiv für die gesammte Physiologie, 1902, xciii, pp. 59-76; also The dynamics of living matter. In his last statement LOEB lays more stress on the oxidation phenomena, but suggests that certain splitting phenomena are favored by it.

<sup>2</sup> MORGAN: Anatomische Anzeiger, 1894, lx, 150.

<sup>3</sup> LOEB: Biological bulletin, 1902, iii, p. 306.

<sup>4</sup> *Loc. cit.*, p. 306.

processes underlying maturation are at least in some form of a destructive nature, one might think of autolytic processes, which the egg cannot withstand for an indefinite length of time without dying.' He conceived that the spermatozoon saved the life of the egg by accelerating a "series of chemical changes, syntheses, in the egg which do not occur sufficiently rapidly without spermatoc, chemical, or osmotic fertilization." Again he says: "The chemical processes underlying maturation are not identical with those which bring about fertilization." Delage also is uncertain of the nature of the processes involved, and makes only the very broad suggestion that possibly ferments have something to do with it. Delage did, however, clearly recognize the fundamental fact of the importance of the admixture of nuclear and cytoplasmic substance.

I first tried to discover whether oxygen had anything to do with the early death of the egg. This is shown conclusively and very simply to be the case if the eggs after maturing are brought into water freed from its oxygen by hydrogen, or if they are left in a dense mass at the bottom of a dish so that the underlying eggs are deprived of their oxygen. Eggs so treated do not become opaque for twenty hours, or even longer, but remain clear and, so far as can be judged, alive. For example, a lot of eggs were shed into sea water at 9 A. M. At 9.30 nearly all had begun to mature. At 10.10 I transferred one portion to sea water through which a stream of hydrogen gas was passing. At 2 and 4 P. M. some of the eggs were retransferred from the hydrogen to fresh sea water. The remainder were left in the hydrogen for twenty-four hours. When examined at the end of this time, all eggs which had been transferred to oxygenated water were found dead and opaque. The eggs which had remained in the hydrogen, on the contrary, although mature, were perfectly normal in appearance. The cytoplasm was clear, fertilization membranes were out, the nuclei had re-formed. On transferring these eggs to fresh sea water a few became swollen in one hour; the remainder retained their living appearance for nearly three hours and then became opaque.

The foregoing experiment shows that the early death of the egg after maturation occurs only if free oxygen is present. It may be concluded, then, that death is brought about in this case by an oxidation of the cell cytoplasm, and that this takes place much more rapidly after the germinal vesicle contents have been discharged into the cytoplasm than before, since if the nuclear wall remains intact the egg does not become opaque even in the presence of oxygen.

There can be no doubt, I think, from these experiments, that at maturation a substance which greatly hastens the action of atmospheric oxygen upon the cell cytoplasm is poured into the cytoplasm from the nucleus, or at any rate becomes active in the cytoplasm as a result of such an admixture. A substance which thus facilitates oxidation is called an oxidase. Inasmuch as no means are known of producing such a change in the cytoplasm in the absence of nuclear admixture, I believe the most probable conclusion is *that at maturation an oxidase escapes from the nucleus and spreads throughout the egg cytoplasm, and this oxidase, in the presence of oxygen, leads, if its action is not checked, to the death of the egg.*

IS THE OXIDASE THUS THROWN INTO THE CYTOPLASM ONE OF THE FACTORS IN THE PRODUCTION OF THE ASTRAL FIGURE?

That the production of the sperm aster after the entrance of the sperm is conditioned by the same factors as condition the early death of the egg after maturation, is shown by the following evidence. Sometimes sperm will enter non-mature starfish eggs, or they enter before maturation takes place. In all such cases I have found, by an examination of both living and sectioned material, that the sperm does not form its ordinary large aster, but instead all that is produced is a very minute series of radiations in the neighborhood of the sperm head and formed presumably about the middle piece or centriole of the sperm. If, however, the germinal vesicle wall has disappeared spontaneously or been ruptured by mechanical shock, the aster begins to form as soon as the sperm enters, and normally its radiations shortly extend far out into the cell protoplasm.

This observation shows very clearly that there is a fundamental chemical difference between the protoplasm of a mature and an immature starfish egg. Although the sperm carries a centriole into the egg or forms a centriole soon after entering, this is incapable of forming a good-sized aster unless the nuclear wall has disappeared and the nuclear sap has spread through the cell. Indeed it may be said that, from this point of view, the egg partially fertilizes itself by the contents of its germinal vesicle.

The impossibility of forming an aster in the immature cytoplasm, as contrasted with the cytoplasm after maturation, has been found also in artificial fertilization. Thus starfish eggs do not undergo artificial parthenogenesis by the action of acids or other agencies, unless maturation has occurred or at least begun. Similarly Yatsu<sup>1</sup>

<sup>1</sup> YATSU: Journal of experimental zoölogy, 1905, ii. p. 287.

found that in pieces of the cytoplasm cut from mature eggs of *Cerebratulus* aster formation was easily caused by magnesium chloride. It was impossible to form asters in pieces which had been cut from the eggs before maturation had occurred. Miss Foot<sup>1</sup> has made similar observations in *Allolobophora*. The cone and aster depend on the stage of maturation. No matter how far the sperm penetrates, one never sees a sperm attraction sphere before the anaphase of the first polar spindle. Similar observations have been made by many other students. It is therefore clear that the cytoplasm becomes capable of forming a good-sized aster only after the mixture with it of the nuclear contents.

I have collected many other facts which show this same relationship between aster formation and the discharge of nuclear material. Thus, in *Toxopneustes*, as described by Wilson,<sup>2</sup> and in *Arbacia* after fertilization and conjugation of the pronuclei, there ensues a stage lasting thirty to fifty minutes in the period after fertilization during which the astral radiations are diminishing and ultimately the coarser radiations die out. This is a period of retrogression of the asters, during which the nuclei are greatly increasing in size, but are sharply cut off from the cell cytoplasm. At the end of this period the nuclear membrane disappears with some abruptness opposite the asters, so that the nuclear sap here comes directly in contact with the astral substance. There ensues a sudden outburst of activity on the part of the asters. They grow enormously, and the radiations extend throughout the cell. I have observed closely parallel phenomena in the starfish egg. Starfish eggs were shed in water and fertilized at 8.45 A.M. At 10.30 the pronuclei were lying in contact side by side. At 10.45 the nuclei fused. The astral radiations were small, extending but a short distance from the centres. About 10.50 the nuclear membrane rapidly disappeared, and immediately following this disappearance great rays developed through the egg, reaching a maximum at 11 to 11.15, when the division took place. This disappearance of the rays after the nuclear membrane has re-formed and their sudden and great development after the solution of the nuclear membrane has been described for a variety of eggs.<sup>3</sup> In some cases it may go so far that the whole astral structure disappears together with the centrioles, while in other cases only a reduction in the size of the asters and

<sup>1</sup> FOOT: *Journal of morphology*, 1897, xii, p. 809.

<sup>2</sup> WILSON: *Journal of morphology*, 1895, xv, p. 452.

<sup>3</sup> See especially COE'S observations on various echinoderms and *Cerebratulus*, *Journal of morphology*, 1899, xvii, p. 455.

radiations is noted. Thus, Miss Foot<sup>1</sup> states that, after or about the time of the conjugation of the pronuclei, the sperm astral centres totally disappear; the asters of the first segmentation nucleus are new formations.

In *Thalassema*, according to Griffin,<sup>2</sup> the asters become smaller and less distinct and grow again greatly after the dissolution of the membranes of the nuclei. F. R. Lillie<sup>3</sup> states for *Unio* that the sperm asters undergo retrogressive metamorphosis, and as they disappear the yolk granules flow in and gradually obliterate all traces of the clear areas where the asters were. The centrosome disappears completely. The first segmentation asters appear as new formations. In *Bufo*,<sup>4</sup> when the pronuclei are in contact, every trace of radial systems disappears. The asters are round masses surrounded by pigment granules which formerly made the rays. When the nuclear wall disappears, the astrospheres increase enormously in volume and rays appear.

These observations indicate clearly that one of the factors of the formation of asters, both in the normally fertilized egg and in cells not fertilized, is to be found in the nucleus, presumably in the nuclear sap, since the change in the protoplasm extends throughout the egg substance. The admixture of this sap enormously stimulates the activity of the aster, whatever be the origin of the latter, whether from sperm or from the egg itself.

It is, therefore, the action of the nuclear sap on the cytoplasm which enables the sperm to perform its essential act of fertilization, namely, the development of asters, and which leads to the death of the egg if this act is not performed. I will now show that the essential processes involved in these two cases are also parallel in that each is dependent for its fulfilment upon atmospheric oxygen. This has already been shown for the death process; it is also necessary for the astral formation.

THE ASTRAL FIGURE DEPENDS FOR ITS EXISTENCE IN *ARBACIA* AND *ASTERIAS* UPON THE PRESENCE OF FREE OXYGEN ON THE SURFACE OF THE EGG.

Wilson<sup>5</sup> showed that ether and presumably other anesthetics caused the astral rays in *Toxopneustes* eggs to disappear. The

<sup>1</sup> FOOT: *Journal of morphology*, 1897, xii, p. 809.

<sup>2</sup> GRIFFIN: *Journal of morphology*, 1898, xv, p. 594.

<sup>3</sup> LILLIE, F. R.: *Ibid.*, 1899, xvii, p. 232.

<sup>4</sup> KING: *Ibid.*, p. 333.

<sup>5</sup> WILSON: *Archiv für Entwicklungsmechanik*, 1902, xiii, p. 365.



rays reappeared when the anesthetic evaporated. This observation is suggestive, since ether checks cell respiration. It suggested to me that the absence of oxygen should produce the same result. I examined accordingly the action of hydrogen gas on a lot of living starfish eggs and sea-urchin eggs at the time of the first cleavage. I used a Leitz  $\frac{1}{2}$  oil immersion ocular 4, the eggs being under slight compression by the cover glass.

The eggs of *Arbacia* and *Asterias* were fertilized and transferred after different intervals to sea water which contained no oxygen. The sea water had been boiled for two minutes, and hydrogen gas passed through for two to three hours, precautions being taken to avoid evaporation. After remaining in the oxygen-free water for ten to thirty minutes, the eggs were lifted out with a little of the water, placed quickly on a slide, and covered at once with a cover glass and examined. The result was always the same. If the eggs were introduced into the hydrogen before the formation of a large aster, they did not develop the aster; if they had been introduced during the height of astral formation, the rays had faded out and disappeared, and only clear areas in the egg marked where the nucleus, spindle, and aster substance was. I then lifted the cover glass, mixed fresh water with the eggs, left them exposed to the air for a minute to permit the entrance of oxygen, and re-examined. In from three to five minutes the radiations began to appear, and in ten to fifteen minutes or longer, depending somewhat on the length of time they had been exposed to the hydrogen, the egg became filled with great radiations.

These experiments show clearly that the coarse, peripheral, astral radiations of the first segmentation amphiaser of these eggs depend for their existence upon the presence of oxygen. They disappear if the oxygen is cut off; they reappear if the oxygen is readmitted. We see, therefore, that even though a substance be discharged from the nucleus into the cytoplasm, this is unable to form a large aster in these eggs unless oxygen is present. *This observation indicates that the substance escaping from the nucleus and active in the formation of an aster is an oxidase, since it is active only in the presence of oxygen.*

As additional evidence of this conclusion, the peculiar action of quinine toward these eggs may be cited. Quinine, as is well known, has a very remarkable power of checking certain oxidations. Thus

very small amounts of it prevent the oxidation of guaiac by ozonized turpentine and blood. The Hertwig brothers<sup>1</sup> long ago observed the peculiarly poisonous action of quinine on dividing ova. In repeating and extending their observations, I was astonished at the very rapid fading out of the astral figure produced by this poison. The radiations disappeared as if by magic, and the cell disintegrated very rapidly. No other poison which I examined was quite so fatal to dividing cells as quinine.

The following experiment illustrates the action of various drugs on the astral radiations and the life of dividing starfish eggs.

A very fine lot of starfish eggs which matured very uniformly were fertilized at 8.30. They were placed in the various solutions at 11.14, about ten minutes before division. Large asters had appeared in some cells; in others the nuclear membranes had not disappeared, and the asters appeared as small, clear areas with small radiations at the nuclear poles.

SOLUTION.	RESULT.
1. Quinine sulphate .1%	Radiations disappear at once. Eggs become opaque and swell in 20 minutes.
“ “ .01%	Astral radiations disappear at once. No segmentation. Cells darker. 24 hours all dead.
“ “ .005%	Radiations disappear. Astral centres as clear areas. No segmentation. 24 hours all dead.
“ “ .001%	Three-fifths of eggs slowly segment. Far later than control. A few not killed in 24 hours.
2. Atropine sulphate .1%	About $\frac{1}{2}$ segment. 24 hours dead.
“ “ .05%	All segment and form morulæ. Next morning dead.
“ “ .02%	Segmentation somewhat retarded. 24 hours nearly all dead.
“ “ .01%	All segment. Majority form abnormal swimming blastulæ.
3. Caffeine hydrochlorate .1%	Eggs remain clear many hours. Several centres in unsegmented eggs. $\frac{1}{3}$ segment in 2 hours. Next morning many living.
“ “ .05%	Segmentation delayed. Radiation somewhat reduced. Many divide at once into 4 cells. 24 hours living.
“ “ .02%	Segmentation retarded, but all develop. Abnormal gastrulæ in 24 hours.
“ “ .01%	Segmentation at first not retarded. Gastrulæ not so good as control.
4. Physostigmine (alkaloid)	Many eggs partially divide, but blastomeres refuse. At
Fresh solution: .05%	1.53 no centres visible in any eggs, but periphery of egg buds off small pieces of protoplasm.
“ “ .025%	A few cells divide completely. Radiations strong. Remainder show incomplete division. In 24 hours all dead.

<sup>1</sup> HERTWIG, O. and R.: Jenaische Zeitschrift, 1887, xx, p. 120.

Fresh solution.	.001%	Many divide. Centres visible. Stop after one division. Blastomeres concave toward each other. Dead in 24 hours.
"	" .0005%	First division in all, but no further. Peculiar change in cytoplasm as noted.
5. Aconitine sulphate	.05%	Nearly all divide and develop.
"	" .025%	Nearly all divide and develop to gastrulæ. Die after 48 hours.
"	" .001%	All develop. 48 hours alive and swimming gastrulæ.
"	" .005%	Development nearly normal.
6. Cocaine hydrochloride	.1%	Division retarded. Centres divide. Rays reduced. Dead in 24 hours.
"	" .05%	First segmentations retarded, but not prevented. Rays reduced. 24 hours dead.
"	" .02%	Like control at first. Dead in 24 hours.
"	" .01%	Some retardation. Dead in 24 hours.
7. Strychnine sulphate	.05%	Centres divide. Eggs not. Radiations reduced.
"	" .025%	A few segment. Majority not.
"	" .01%	Nearly all divide, but retard. Alive after 24 hours.
"	" .005%	Like control, but dies in 48 hours.
8. Adrenaline chloride	.007%	First two divisions take place, but protoplasm altered like physostigmine. All dead in 24 hours.
"	" .0035%	Some 4 cell. Do not develop farther. Same protoplasmic change.
9. Veratrine chloride	.03%	All divide and develop, but retarded. Dead in 24 hours.

The preceding experiment shows the astonishing fact that quinine sulphate toward dividing eggs is more poisonous than atropine, pilocarpine, caffeine, physostigmine, aconitine, cocaine, veratrin, or strychnine. Its power of checking cell division is even greater than sodium cyanide. It is indeed surprising that quinine, a drug which may be taken with impunity in relatively enormous doses by mammals, should be so extraordinarily poisonous toward dividing cells of the sea-urchin and starfish. The effect of this drug on the astral figure is almost instantaneous. A dilution of one part of the sulphate to 17,000 of sea water is sufficient to wipe out the large astral radiations and to prevent segmentation. On the other hand, veratrin and aconitin, two of the most fatal and powerful poisons known, are toward the process of segmentation relatively inert.

#### THE EFFECT OF COLD ON THE ASTRAL RADIATIONS.

It is well known that cold checks the respiratory processes. It ought, hence, if the aster is a dynamic and not a static structure, to cause a disappearance of the rays.<sup>1</sup>

<sup>1</sup> BOVERI has tried similar experiments from a different point of view (Sitzungsberichte der physikalischen-medizinischen Gesellschaft, Würzburg, 1897).

A lot of ripe starfish eggs were fertilized just after the germinal vesicle membrane had disappeared. Just before division, when most of the eggs had very large asters in them and radiations extending to the periphery of the egg, I put them in sea water cooled to  $+2^{\circ}$  C. After twenty-five minutes' exposure to the cold I examined them. Nearly all eggs were undivided. The great astral radiations had disappeared, and only clear areas without rays could be seen, generally two in each egg. I then carefully warmed the slide on my hand. The rays began to appear almost at once, and very soon enormous astral radiations appeared about each centre, and division took place.

Some eggs were left for three hours at  $+2^{\circ}$  to  $+5^{\circ}$  C. In these eggs three or four clear areas without rays could be seen. On warming rays appeared about each centre.

#### THE NATURE OF THE CENTRIOLE.

We have thus far been able to trace two elements of respiration in cell division: the oxidase, formed by the nucleus and thrown into the cytoplasm, and free oxygen. There remains the other element, *i. e.*, the reducing substance, the substance producing nascent hydrogen, to be accounted for. It occurred to me that that substance might be the centriole. If that were the case, the astral centre should show a strong reducing action.

I can find very little evidence of the chemical nature of the centriole. From its staining reaction and digestibility in pepsin-hydrochloric acid, it is not apparently a nuclein, but, as Zacharias<sup>1</sup> maintains, an albuminous substance. It may, however, be like the oxidase, an albuminous derivative of the chromatin. It is not even certain that all the centrioles in the cell have the same chemical composition. If, as is possible, the centriole has a reducing action and causes the astral figure in virtue of that fact in the way shortly to be considered, any reducing substance, whatever its chemical composition, would have a similar action. As there are certainly several reducing substances in every cell, it will be seen that it will not do to assume that all centrioles have the same composition.

In the literature I find but a few scattered observations on the possible reducing action of the centriole, or centropiasm. It is of course well known that the interior of the egg in which the asters normally develop has during life a strong reducing action. Ehrlich's

<sup>1</sup> ZACHARIAS: Berichte der deutschen botanischen Gesellschaft, 1898, xvi pp. 185-198.

work clearly shows this fact, which was indeed known long before. Evidence for it in the starfish egg will shortly be presented.

The centrioles first appear ordinarily close to the nuclear wall or even in the nucleus. It is exactly about the nucleus that the strongest reducing action is to be found. Thus Beyer<sup>1</sup> observed that if tellurium oxide was injected it was deposited as metallic tellurium about the nucleus. Methylene blue and other stains are reduced particularly in this region. The centrioles and asters therefore normally arise in the region of most intense reduction, even though they may not be the cause of that reduction.<sup>2</sup>

Foot and Strobell<sup>3</sup> report that in the eggs of *Allobobophora foetida*, fixed in chrome-acetic and then exposed to osmic acid vapor, the centrosomes appear as brownish yellow granules, not so black as certain other granules in the cytoplasm. These observations indicate that the centrosomes fix and reduce osmic acid. Field<sup>4</sup> reports also that if the sperm of echinoderms be exposed to osmic vapor the tip and middle piece of the sperm reduce the osmic acid far more than the nucleus.

To determine this point more directly, eggs with large asters were placed in a 0.01 per cent solution of methylene blue in sea water. The methylene blue enters the egg with great ease. In two to three minutes the granules in the outer third of the protoplasm become stained a light blue. If the eggs are left in the solution beneath a cover glass, the interior of the egg does not color even after the outer third is an intense blue. This fact indicates that the interior of the cell is strongly reducing, and as rapidly as the stain penetrates it is reduced. The clear protoplasm at the centre or at one side of the egg which represents the asters, spindle, and the chromatin will not stain in methylene blue for a very long period. Inasmuch as there is no membrane about the spindle or in the egg to prevent the penetration of the stain, and as the cell stains rapidly and completely if boiled first, I am of the opinion that the centres and the spindle will not stain, owing probably to their strong reducing action. However it might also be urged that the stain will not attack the centres, because there are no granules in them with which it ordinarily combines. This evidence is not, therefore, conclusive.

<sup>1</sup> BEYER: *Archiv für Physiologie*, 1895, p. 231.

<sup>2</sup> It is possibly on account of this intense chemical action that the chromatin will not stain during "life."

<sup>3</sup> FOOT and STROBELL: *Zoölogical bulletin*, 1899, ii, p. 131.

<sup>4</sup> FIELD: *Journal of morphology*, 1895, xi, p. 240.

Further evidence of some value was obtained in the following way. The protoplasm of these eggs consists of a homogeneous matrix in which myriads of minute granules of varying sizes are embedded. If we knew whether these granules were electronegative or electropositive, we might infer from their behavior whether the astral centres were oxidizing or reducing, since all oxidizing regions would be electropositive.

The behavior of these granules is extremely interesting. Originally they are distributed evenly all through the cytoplasm. Just as soon, however, as the centriole appears, the clear homogeneous protoplasm begins to gather around it, and the granules move away or disappear in the neighborhood of the aster. The result is that finally the asters and spindles are left in the midst of a clear homogeneous protoplasm which is entirely free from these granules.

This clear area can only have arisen by the granules moving outward and the clear substance inward toward the centre or by the dissolution of the granules in the clear substance. I was unable to satisfy myself which of these processes took place, but I am inclined to think that both occur, although I could not actually observe with entire certainty the solution or movement of any single granule.

It is well known that if the centre is reducing, it will act as a negative electrode; if it is oxidizing, as a positive electrode. If the granules are electropositive and the centre is a reduction centre, the granules should be attracted toward the centre, and should fuse into larger granules near the centre, following the well-known behavior of colloidal particles and suspensions toward electrodes. If, on the other hand, the centres are reducing, the granules, if electronegative, will be repelled from the centre, they will move outward, and should grow larger toward the periphery of the end, and those near the centre should dissolve if they cannot move away. The first point, therefore, was to determine accurately the character of the charges on the granules.

To determine this point I used the various stains, and I quickly found, as had been discovered for other cells already by Fischer and Overton, that the granules united only with basic dyes. Indeed this is the easiest method I know of for telling an acid from a basic dye. One puts the starfish eggs in a .03 per cent solution of the dye, allows them to remain five minutes, and then examines in fresh sea water. No acid dye stains. I used the following dyes:

BASIC.	ACID.
Methylene blue.	Methyl blue.
Thionin.	Acid fuchsin.
Dahlia.	Acid violet.
Methyl green.	Acid green.
Methyl violet.	Carminate of sodium.
Safranin.	Indigo-carmin.
Toluidin blue.	Erythrosin.
Neutral red.	Eosin.

That the basic stains form a combination with the granules and are not simply dissolved in an oil drop, as suggested by Overton, is shown by the fact that in some cases, particularly with neutral red, the granules form insoluble red granules.

These observations show that the granules in the living cell without exception, so far as I could see, are electronegative. These same granules are electropositive in fixed specimens, owing to the action of the acid in the fixing fluids. Their behavior toward the astral centre indicates, therefore, that this is the region of intense reduction, since here they are either dissolved or are repelled from it, or, as is more probable, are in part dissolved and in part repelled.

Conversely, when the asters die out, the granules move again toward the centre or reappear there, as is seen, for example, in the eggs of *Unio*, and as may easily be seen in many other eggs where the sperm or maturation asters die out before the segmentation spindle is formed.

The behavior of these granules, in connection with the other observations already mentioned, indicates, in my opinion, that the substance of the centrioles, or, at any rate, the substance of the astropasm and presumably of the centriole is a reducing agent, and is accordingly, like all reducing agents, probably setting free nascent hydrogen.

#### CONCLUSIONS.

The foregoing observations strongly support the view that the chemical processes involved in mitosis are the processes of respiration, and that the phenomena shown are the result of localized changes in respiratory activity. All the facts indicate that the formation of an aster in the starfish and sea-urchin eggs is not a purely physical process, but that the physical change is underlain by a more fundamental chemical change which supplies the energy for the process. It

appears that the protoplasm of these eggs cannot produce a typically large aster, unless at least three factors coexist, (1) a substance derived from the nucleus and only active in the presence of oxygen, probably therefore an oxidase; (2) free oxygen; and (3) the substance of the centriole. No two of these factors alone can in these eggs produce the formation of an aster. Concerning the nature of the substance of the centriole, no conclusive evidence exists, but what facts there are indicate that it is probably an albumin and that it has a reducing action.

The facts suggest that the egg during its period of growth forms in the nucleus, by its metabolism, two important substances, an oxidase and the substance known as the centriole. As long as the egg remains in the ovary of the starfish, the germinal vesicle wall persists, and these substances cannot find access to the cell protoplasm with sufficient rapidity to produce cell division. When the egg is shed, however, oxygen gets entrance to the egg, and owing to the eccentric position of the nucleus, it gets access to the wall of the germinal vesicle. A chemical change, possibly an oxidation in the latter, is at once set up, leading to its dissolution. Upon the dissolution of the membrane a mingling of the nuclear contents with the cell cytoplasm takes place. Among the substances thus set free are the substances of the centriole<sup>1</sup> and an oxidase. The latter spreads through the cell protoplasm, and in the presence of oxygen produces in it an oxidation, which if not checked leads to the death of the cell. Meanwhile the centriole acting on the cytoplasm in the presence of the oxidase and free oxygen gives rise to the astral figure, possibly owing, as Lillie suggests, to differences in electrical potential. At the close of maturation the centriole substance is itself either completely oxidized or otherwise rendered inert. It disappears. The nucleus re-forms and at once begins the same cycle over again. It grows actively; it forms more oxidase and particularly more reducing substance.

There is, however, now no agent to cause the necessary destruction of the nuclear wall. While the nucleus continues to form its metabolic products, the cytoplasm is being oxidized by the oxidase discharged into it and is finally killed. If, however, the nuclear wall be ruptured by mechanical shock at the right instant, when the re-formation has reached the proper stage, and the protoplasmic

<sup>1</sup> Earlier observations show that the centriole substance precedes aster formation and that it escapes from the nucleus. MATHEWS: *Journal of morphology*, 1894, p. 335.



changes have not gone too far, the centriole or reducing substance gets free in the egg. It sets up cell division of a whole or a part of the egg substance, and thereafter alternations of activity carry the process on to the formation of an embryo. Confirming this conclusion are my observations on artificial parthenogenesis by mechanical agitation, shortly to be considered.

The action of the sperm is clear on the basis of these conclusions. The sperm brings into the egg cytoplasm, which already contains the oxidase, two important substances. In the first place it brings a reducing substance — the centriole — which by its activity counteracts the action of the oxidase of the egg cytoplasm; and in the second place it brings a very active nucleus, which grows rapidly when immersed in the egg cytoplasm, and forms more reducing substance and possibly oxidase. By the entrance of the sperm there is thus set up that extraordinary series of opposite actions of oxidation and reduction which accounts for the sudden outburst of respiratory activity coincident with the entrance of the sperm, and which probably underlies many of the most important syntheses and chemical transformations in protoplasm (see Drechsel, Baumann, Hoppe-Seyler).

The main difference between parthenogenetic and non-parthenogenetic ova upon this theory would lie in the different activity of their respective nuclei after maturation. This is well illustrated if we contrast *Arbacia* with *Asterias* eggs. The former is a typical non-parthenogenetic ovum; the latter is almost parthenogenetic. The nucleus of an *Arbacia* egg after maturation remains small for days, and shows almost no growth or other indication of activity; the nucleus of an *Asterias* egg, on the other hand, grows with great rapidity after the extrusion of the second polar body and moves toward the centre of the egg. In fact, if the egg is partially deprived of oxygen so as to prolong its life, the diameter of the re-formed nucleus may ultimately be half that of the germinal vesicle. In fact, there is little doubt that the nucleus is almost able by itself to inaugurate cell division in this egg and to counteract the destructive action of the oxidase thrown into the cytoplasm from the germinal vesicle.

My observations on the production of artificial parthenogenesis in these eggs by mechanical shock clearly support this conclusion. Loeb has suggested that this parthenogenesis is not really due to the shock, but to changes in its gaseous environment. I have,

since the publication of that paper, made additional observations which throw some more light on this process. I have found that this parthenogenesis is brought about with greatest ease about six to nine hours after the eggs have begun to mature. If one keeps the eggs in a fairly thick layer so that they are not so freely exposed to oxygen and then waits until the egg nucleus is re-formed and grown to a large size, a slight disturbance is all that is necessary to set up cell division in some of the eggs. If the eggs are transferred very carefully to a watch glass and then the watch glass struck sharply on the desk two or three times, the changes in the eggs may easily be seen, under the microscope, beginning immediately after the shock. The changes involve the throwing out of the fertilization membranes and the solution of the nuclear membrane, and often, at any rate, the development of a large monaster about the nucleus. A considerable portion of the cytoplasm, particularly in the periphery of the egg, often disintegrates, while that about the nucleus, which is less oxidized, remains clear, rounds itself off, divides, and forms swimming embryos, generally dwarf and abnormal in shape. The essential point in this observation is to show that the change brought about by mechanical shock involves primarily the nucleus and leads to the mingling of its contents with the cytoplasm, the indirect result being cell division. It is impossible to cause artificial parthenogenesis in *Arbacia* by mechanical shock, for the reason that such shock does not disrupt the nucleus, and the nucleus itself is apparently quite inert. There is no question of the efficacy of mechanical shock in producing artificial parthenogenesis in the starfish egg, and I think there can be no doubt that it is not a question of  $\text{CO}_2$  formation, as Loeb suggests, but the shock itself which causes this result.

The conclusions of this paper are also of interest in the matter of artificial parthenogenesis by salt solutions and the formation of asters by such solutions in pieces of the mature egg, as described by Wilson. In my opinion the action of the various agencies which set up artificial parthenogenesis cannot be explained if these fundamental chemical changes are neglected. The opinion expressed by Morgan that we are dealing with one fundamental chemical process, and that this may be set up in a variety of ways by various means or stimuli, appears to me well founded. Anything is a stimulus which effects this fundamental process, the respiratory process, in a certain way, although the direct physical effect of the agent on the protoplasm cannot, of course, be neglected.

I would suggest the following explanation of the physiological and morphological phenomena observed in cell division and artificial parthenogenesis. The reducing substances destined at times to give rise to the centrioles are being formed constantly by the metabolism of the nucleus. These substances are normally shed into the cytoplasm so slowly and in such small quantities that they suffice only to keep up normal respiration and to keep the cell body in its reduced state. They are consumed at the periphery of the cell, or otherwise rendered inert as rapidly as they are produced, so that no local accumulation of them in the cytoplasm in an active form is possible. The amount of these substances in the nucleus is greater than that in the cytoplasm, and at times a larger mass than usual, owing to the dissolution of the nuclear wall, gets into the cytoplasm. Under favorable conditions of oxygenation this mass sets up the localized, marked disturbance in respiration and forms the aster, it itself being the centriole. The centriole, in other words, is simply an abnormal amount of the active reducing substance localized in one place. It may also be assumed, from analogy with other chemical substances, that this centriole substance exists in two forms, an active dissociated and an inactive undissociated form, and that it readily passes from the inactive to the active condition. It is, for example, possibly inactive when hydrated, just, for example, as  $\text{NH}_4\text{OH}$  is inactive and the dehydrated radical  $\text{NH}_3$  is active. It is converted into the active form by taking out water by strong solutions or in other ways, and the active form is the strong reducing substance as assumed for cell respiration. This is, of course, only a suggestion to explain the production of asters in enucleated fragments. These two states of the centriole or reducing substance may be in equilibrium with the water present in the cell and with each other. That is, the larger the active mass of the water, the more of the particles will be in the hydrated or inactive form; the smaller the active mass of the water, the more of the particles which will become active, that is, strongly reducing. By the addition of salts such as  $\text{MgCl}_2$  the active mass of the water is reduced, owing to the combination of the water with the ions and molecules of the salt, and consequently more of the particles become active, with the result that they form scattered asters, or one large monaster if they are diffused. The effects of the dehydration in increasing the per cent of active particles would be the same as the production and discharge by the nucleus of a large quantity of the stuff composing the particles. We cannot cause asters in immature

eggs, owing to the fact that not sufficient oxidase and centriole substance is in the cytoplasm.

According to this view, then, all agencies for artificial parthenogenesis are effective primarily because they succeed in liberating in the cell cytoplasm active reducing centriole substance. They may liberate it there either by causing a rapid discharge of it from the nucleus or by rendering the inert substance already present active.<sup>1</sup> The sperm is able to fertilize because it brings in active centriole substance itself, and a nucleus capable of forming more.

This hypothesis appears to me to give a tentative explanation of both natural and artificial fertilization and to harmonize the experimental results with the morphological changes observed.

I may be permitted to point out, in closing, that these results are supported by the observations of F. R. Lillie<sup>2</sup> on the discharge of substances from the cell nucleus into the cytoplasm, and that they furnish a basis for the explanation R. Lillie has given for the astral rays. The rays appear to stretch from regions of intense reduction to regions of oxidation, — in other words, between an oxygen and a hydrogen electrode, — and hence possibly to be of electrostatic origin.

#### SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

1. The inauguration of the maturation of the egg of *Asterias* when shed into sea water consists in the dissolution of the nuclear membrane at the point where the germinal vesicle comes nearest to the surface of the egg. The dissolution of the wall is due to the oxygen in the sea water, since it does not occur if oxygen is absent.

2. By the dissolution of the nuclear wall all but a minute portion of the contents of the vesicle are mixed with the cytoplasm. Coincident with this admixture the cytoplasm undergoes chemical and physical changes. The matured egg becomes opaque and dies in ten hours; the egg where the germinal vesicle remains intact lives for days. The early death of the matured egg is greatly delayed if oxygen is prevented access to the egg. It is therefore clear that among the substances set free through maturation is one which greatly facilitates the action of atmospheric oxygen on the egg. Presumably,

<sup>1</sup> See WILSON'S account of Asters in fragments of eggs. *Archiv für Entwicklungsmechanik*. 1901. xii, p. 4.

<sup>2</sup> LILLIE, F. R. : *Journal of experimental zoölogy*, 1906, iii, p. 153.

therefore, an oxidase escapes from the cell nucleus into the cytoplasm on rupture of the nucleus.

3. The cytoplasm of the mature egg can form asters when the sperm enters or when subjected to dehydration. The cytoplasm of the non-maturated egg will not form asters under these conditions. The cytoplasm of the maturated starfish and sea-urchin egg will only form large asters after entrance of the sperm if free oxygen is present. The astral radiations fade out and disappear if oxygen is withdrawn, if quinine is given, if cold or ether is applied. They reappear if oxygen is readmitted. I infer from these observations that the sperm requires to develop an aster oxygenated cytoplasm of a suitable kind containing an oxidase. The astral figure is hence the product in these eggs of three factors: (1) centriole substance; (2) an oxidase; (3) free oxygen.

4. The centriole substance is probably a strong reducing substance and the substance which lies at the bottom of the cell respiration. This is indicated by the fact that the centrosome lies in the part of the cell where reduction is strongest; by the behavior of the electro-negative granules in the cell which are repelled from or dissolved by it; and by other observations of its action on osmic acid.

5. The chemical basis of cell division is probably the process of respiration, the astral figure being a localized region at the centre of which is intense reduction.

6. It is suggested that the various methods employed to produce artificial parthenogenesis do not do so by their direct physical action on the cell, but indirectly by producing in one way or another active centriole substance in the cell cytoplasm, or by causing its discharge from the nucleus.

7. A basis is thus given for the electrostatic differences in potential assumed to exist by Hartog, Lillie, and Spaulding to explain the astral figure.

8. These conclusions support those of Delage in most particulars, but in addition give some evidence of the nature of the substances acting on the cytoplasm and formed by the nucleus.

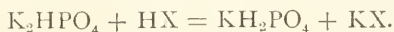


## CONCERNING THE EXCRETION OF PHOSPHORIC ACID DURING EXPERIMENTAL ACIDOSIS IN RABBITS.

By R. FITZ, C. L. ALSBERG, AND L. J. HENDERSON.

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IN a previous paper<sup>1</sup> one of us has pointed out certain reasons for seeking in the phosphates a special activity in neutralizing acids in the body. Briefly these reasons are as follows: In protoplasm phosphates are present in very great amount, undoubtedly as mixtures of mono- and di-potassium phosphates and similar salts; such mixtures constitute a nearly neutral solution which has the remarkable property of being able to take up large quantities of acid or alkali without becoming acid or alkaline. This behavior is easily explained by the facts that acid sufficient to convert all the di-potassium phosphate of such a mixture into mono-potassium phosphate must be added before the slight acidity of the pure mono-potassium phosphate is obtained, and that enough alkali to convert all the acid-potassium phosphate into di-potassium phosphate must be added before the faint alkaline reaction of the latter substance is obtained, while, in accordance with the requirements of the concentration law, all mixtures of the two substances are much more nearly neutral than either alone. Accordingly, if acid is introduced into protoplasm in a form more strongly acid than acid potassium phosphate, it must immediately react with di-potassium phosphate and similar substances forming a salt and acid potassium phosphate according to the reaction.



Other substances, of course, such as carbonates and proteids, are not without concern in the readjustment of equilibrium, but on the whole there is good reason to attach to them a minor importance, at least in the first stages of acid intoxication when little acid has been neutralized. Nevertheless a proper understanding of the division of

<sup>1</sup> L. J. HENDERSON: This journal, 1906, xv, p. 257.

function among such substances is an important matter, and at present it is being investigated in this laboratory.

In the process of getting rid of acid from the body the next step would be to remove from the cells the excess of acid potassium phosphate or more precisely of  $\text{H}_2\text{PO}_4^-$  ions formed by the above process, thereby restoring the equilibrium between mono- and di-potassium phosphate at its former level. If such a process should occur, either by a selective activity of the cell, or, in accord with the experiments of Maly,<sup>1</sup> through the great diffusibility of acid phosphates, that is to say, of  $\text{H}_2\text{PO}_4^-$  ions, this phosphate, entering the blood stream, should rapidly pass out of the body through the kidneys. It can hardly be doubted that the cell will endeavor to restore the normal ratio between mono- and di-potassium phosphates in view of the desirability of restoring the full normal power to neutralize acids. Moreover, though a considerable increase in the amount of acid phosphate at the expense of the other salt must involve a very slight change in hydrogen ionization at most,<sup>2</sup> even this slight change can hardly be without influence upon the delicate catalytic reactions of protoplasm. Moreover, too, slight changes of this sort probably influence the colloidal organization of protoplasm to a certain extent. Finally, it is clear that if acid is to be removed from protoplasm without preparatory chemical change it must leave in the form of mono-potassium phosphate chiefly, for there can be no doubt that in this form chiefly it exists in protoplasm.

Concerning the excretion of phosphates in acidosis, there is little satisfactory information, and nothing which conclusively indicates a direct influence of acid upon phosphate excretion. Interesting in this connection are the observations of Teissier,<sup>3</sup> who noted in certain cases of diabetes inverse variations in the excretion of glucose on the one hand and phosphoric acid on the other. It is far from improbable that in these cases the period of diminished glucose excretion and increased excretion of phosphoric acid was also a period of increased acid formation.

From the above considerations it seemed possible that acid feeding would produce an increased phosphoric acid excretion in the urine, which might be followed by a falling off in the excretion of phosphoric acid when the body could no longer spare that important constituent.

<sup>1</sup> MALY: *Zeitschrift für*

<sup>2</sup> See HENDERSON: *Loc. cit.* *Physiologische Chemie*, 1877, i, p. 174.

<sup>3</sup> TEISSIER: *Du diabète* p. 260.

Paris, 1876.



The following experiments have been carried out to decide this question.

Four rabbits were fed on a nearly constant diet of cabbage, such variations as were incidentally introduced being quite too small materially to influence the results. They received gradually increasing amounts of 0.9 per cent hydrochloric acid through a stomach tube daily, and in the urine the phosphoric acid was estimated day by day. The choice of 0.9 per cent hydrochloric acid was made in accordance with the experiments of Walter.<sup>1</sup> Rabbits were used because it was desired so far as possible to obviate the neutralization of acid by ammonia so readily accomplished by the carnivora. Phosphoric acid was estimated by diluting the urine to 500 c.c. and titrating the amount of phosphoric acid in 50 c.c. of the solution, using uranyl nitrate and potassium ferrocyanide as an indicator, a high degree of accuracy in the determinations being unnecessary for the matter in hand.

The rabbit's weight, food, acid ingestion, and phosphoric acid excretion day by day are recorded in Table I.

It is clear that in all these cases there occurred, for a period of varying length, not long after the beginning of the acid feeding, a material increase in the phosphoric acid excretion by the kidney. The average increase for all four rabbits during the first fifteen recorded days after the beginning of the acid treatment is 55 per cent. On closer scrutiny it is easy to see that there is a considerable regularity both in increase and in a later decrease in phosphate excretion in the cases of all four rabbits. At first the excretion is relatively little increased, in the gray rabbit, No. 2, indeed, slightly diminished; later, as the amount of hydrochloric acid ingested rises, there is a large increase in the excretion of phosphoric acid. There follows a period of diminished excretion, the diminution in some cases being so great that materially less phosphoric acid is excreted than normally. Finally, there is to be noted in two of the three rabbits, No. 1 and No. 3, just before death a marked increase in the phosphoric acid excretion, not easily to be explained by the present considerations. This phenomenon we hope to study in connection with the nitrogen metabolism in a later investigation. One rabbit,<sup>2</sup> No. 4, bore a relatively large

<sup>1</sup> WALTER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1877, vii, p. 148.

<sup>2</sup> It is thought that this rabbit was a young one. Experiments to test the influence of acid upon the excretion of phosphates in young animals will soon be undertaken in this laboratory.

TABLE I.  
LARGE WHITE RABBIT.

Date.	Weight.	Food. Cabbage.	Acid ingestion. 0.9% HCl.	P <sub>2</sub> O <sub>5</sub> in urine.
1906	grams	grams	c.c.	grams
August 1	1585	150	0	0.077
" 2	....	350	0	0.064
" 3	....	350	25	0.064
" 4	1550	250	25	0.085
" 5	....	300	25	0.068
" 6	....	300	25	0.078
" 7	....	300* <sup>1</sup>	35	0.124
" 8	....	300	35	0.144
" 9	....	300	35	0.160
" 10	....	300	35	0.077
" 11	1530	300	35	0.164
" 12	....	300	35	0.072
" 13	....	300	50	0.141
" 14	....	300	50	0.070
" 15	....	300	70	0.114
" 16	....	300	70	0.064
" 17	....	300	70	lost
" 18	1445	300	70	0.285
" 19	....	300	70	0.064
" 20	....	300	70	0.136
" 21	1335	300	70	0.226
" 22	Dead			

<sup>1</sup> In the tables the three stars indicate that on the days indicated the rabbits received from ten to twenty grams of wheat. The phosphate content of this food is much too small to produce an appreciable effect upon the results of the investigation.

TABLE II.  
GRAY RABBIT.

Date.	Weight.	Food. Cabbage.	Acid ingestion. 0.9% HCl.	P <sub>2</sub> O <sub>5</sub> in urine.
1906	grams	grams	c.c.	grams
August 2	....	300	0	0.107
" 3	2410	250	0	0.077
" 4	....	300	0	0.043
" 5	....	300	0	0.145
" 6	....	300	25	0.068
" 7	....	300	25	0.081
" 8	....	300	35	lost
" 9	....	300	35	0.092
" 10	2265	300	35	lost
" 11	....	300	35	0.072
" 12	....	300	35	0.081
" 13	....	300	50	0.081
" 14	....	300	50	0.092
" 15	....	300	70	0.043
" 16	....	300	70	0.067
" 17	....	300	70	0.067
" 18	2195	300	70	0.105
" 19	....	300	70	0.269
" 20	....	300	70	0.034
" 21	....	300	70	0.045
" 22	....	300	70	0.031
" 23	....	300	70	0.053
" 24	2110	300	70	0.051
" 25	....	300	70	0.068
" 26	....	300	70	0.038
" 27	....	300	70	0.052
" 28	1960	300	70	0.053
" 29	....	300	70	0.045
" 30	Dead			

TABLE III.  
BLACK RABBIT.

Date.	Weight.	Food. Cabbage.	Acid ingestion. 0.9% HCl.	P <sub>2</sub> O <sub>5</sub> in urine.
1906 August 1	grams 1245	grams 150	c.c. 0	grams 0.064
" 2	....	250	0	0.043
" 3	....	250	25	0.038
" 4	....	250	25	0.031
" 5	....	200	25	0.053
" 6	....	100*	0	0.136
" 7	....	200*	25	0.124
" 8	1255	200	0	0.098
" 9	....	200	25	0.196
" 10	....	200	25	0.139
" 11	....	200	25	0.154
" 12	....	200	25	0.201
" 13	....	200	35	0.181
" 14	....	200	35	0.160
" 15	1155	250	50	0.179
" 16	....	300	50	0.205
" 17	....	300	50	0.205
" 18	....	300	50	0.164
" 19	....	200	50	0.081
" 20	940	200	50	0.064
" 21	870	200	50	0.416
" 22	Dead			

TABLE IV.  
SMALL WHITE RABBIT.

Date.	Weight.	Food. Cabbage.	Acid ingestion. 0.9% HCl.	P <sub>2</sub> O <sub>5</sub> in urine.
	grams	grams	c.c.	grams
1906 August 2	1430	250	0	0.108
" 3	....	250	0	0.108
" 4	....	200	0	0.149
" 5	....	200	0	lost
" 6	....	200	0	0.068
" 7	....	200	25	0.226
" 8	....	200	25	0.115
" 9	1405	200	25	0.064
" 10	....	200	25	0.186
" 11	....	200	25	0.171
" 12	....	200	25	0.201
" 13	....	200	35	0.273
" 14	....	300	35	0.188
" 15	1275	300	50	0.115
" 16	....	300	50	lost
" 17	....	300	50	0.262
" 18	....	300	50	0.215
" 19	....	200	50	} 0.128
" 20	....	200	50	
" 21	....	200	50	} 0.031
" 22	1070	200	50	
" 23	....	200	50	} 0.164
" 24	....	200	50	
" 25	....	200	50	} 0.041
" 26	....	200	50	
" 27	....	200	50	} 0.049
" 28	....	200	50	

TABLE IV (continued).

Date.	Weight.	Food. Cabbage.	Acid ingestion. 0.9% HCl.	P <sub>2</sub> O <sub>5</sub> in urine.
	grams	grams	c.c.	grams
1906 August 29	1065	200	50	0.228
" 30	....	200	50	0.136
" 31	....	200	50	0.053
September 1	....	200	0	0.038
" 2	....	200	0	0.060
" 3	....	200	0	0.077
" 4	....	200	0	lost
" 5	1105	200	0	} 0.089
" 6	....	200	0	
" 7	....	200	0	} 0.058
" 8	....	200	0	
" 9	....	200	0	} 0.038
" 10	....	200	0	
" 11	....	200	0	} 0.055
" 12	1105	200	0	
" 13	....	200	0	} 0.111
" 14	....	200	0	
" 15	....	200	0	} 0.309
" 16	....	200	0	
" 17	....	200	0	} 0.049
" 18	....	200	0	
" 19	1125	200	0	} 0.154
" 20	....	200	0	
" 21	1160	200	0	

amount of acid for a relatively long time, and manifested particularly great variation in the phosphoric acid output. In this case the acid feeding was finally discontinued, whereupon the excretion of phosphoric acid sank even lower than before, and then gradually rose

toward the normal, as would be expected if the animal had lost more phosphate than it could spare.

These facts are assembled in the following table in which are recorded the average acid income and phosphoric acid output per day during the various periods above indicated. As was to be expected,

SUMMARY OF RESULTS.

Rabbit.	Period.	Average Acid Ingestion per day. 0.9% HCl.	Average P <sub>2</sub> O <sub>5</sub> excretion in urine per day.
		c.c.	grams
Table I Large White Rabbit	August 1-2	0	0.070
	" 3-6	25	0.074
	" 7-11	35	0.134
	" 12-17	55	0.092
	" 18-21	70	0.178
Table II Gray Rabbit	" 2-5	0	0.093
	" 6-15	50	0.078
	" 16-19	70	0.127
	" 20-29	70	0.047
Table III Black Rabbit	" 1-2	0	0.053
	" 3-8	17	0.080
	" 9-17	35	0.180
	" 18-20	50	0.103
	" 21	50	0.416
Table IV Small White Rabbit	" 2-6	0	0.108
	" 7-11	25	0.152
	" 12-18	40	0.209
	" 19-31	50	0.063
	Sept. 1-12	0	0.038
" 13-21	0	0.069	

these periods are of different lengths in the different animals, and in a certain degree the choice of limits for the different periods is an arbitrary one; this has, however, no material effect upon the results.

These results materially strengthen the theory that the phosphates are intimately concerned not only with the neutralization of acid within the body in experimental acidosis of rabbits but also with its removal from the body.

Experiments are being undertaken in this laboratory to test the effect of acid feeding on the phosphate excretion of the carnivora and also to test the effect of the feeding of alkalis on phosphate excretion. It is planned also to study the phosphate excretion during diabetic acidosis in man.

SUMMARY.

It is shown that the feeding of acid to rabbits produces first a marked increase, then a marked decrease, in the excretion of phosphoric acid through the urine.

It is pointed out that these observations are in accord with the theory that in the body phosphates are primarily concerned with the neutralizing of acid and with its removal from the body, and that they strongly support that theory.

A sudden premortal rise in the excretion of phosphoric acid in two cases of experimental acidosis is reported.

One case of experimental acidosis is reported in which after a great rise and great fall in the excretion of phosphoric acid, upon discontinuing the feeding of acid, the excretion of phosphoric acid sank even lower than before and then gradually rose toward the normal value. This observation further supports the theory.



## A NEW DECOMPOSITION PRODUCT OF GLIADIN.<sup>1</sup>

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

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

BY the action of trypsin (pancreatin) on various protein substances Emil Fischer and Emil Abderhalden<sup>2</sup> obtained a substance of polypeptide nature which contained all of the proline, glycocoll, and phenylalanine that could be detected in the hydrolysis solution.

Levene and Wallace<sup>3</sup> have recently investigated the prolonged action of trypsin on gelatin and succeeded in isolating an exceedingly interesting substance which on hydrolysis yields proline and glycocoll and in which those two substances appear to be linked with anhydride binding. Levene and Beatty<sup>4</sup> have further investigated the action of 25 per cent sulphuric acid on gelatin and reached the conclusion that after boiling a solution of 400 gm. of gelatin in 3 litres of 25 per cent sulphuric acid for twelve hours the hydrolysis is not effected to the amino-acids, but there still remain substances of a peptide character (gelatoses).

In hydrolyzing our preparation of gliadin for the tyrosine<sup>5</sup> determination by protractedly boiling the solution of the protein in 25 per cent sulphuric acid, we have succeeded in isolating a crystalline substance of definite character which on vigorous hydrolysis yields proline and phenylalanine. We think it highly probable that the substance is a dipeptide, as the hydrolysis indicates, and we propose to investigate it more fully as soon as we have sufficient material at our disposal. The amount of this substance that is obtained from

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

<sup>2</sup> FISCHER and ABDERHALDEN: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 81.

<sup>3</sup> LEVENE and WALLACE: *Ibid.*, 1906, xlvii, p. 143.

<sup>4</sup> LEVENE and BEATTY: *Ibid.*, 1906, xlix, p. 247.

<sup>5</sup> OSBORNE and CLAPP: This journal, 1906, xvii, p. 245.

gliadin is by no means insignificant, as from 1 kg. we were able to isolate about 4 gm. of nearly pure substance.

One thousand grams of air-dried gliadin were treated with a mixture of 2500 c.c. water and 500 c.c. of concentrated sulphuric acid and heated at 100° for about six hours, until solution was effected. The solution was then boiled in an oil bath for thirteen hours. After the quantitative removal of the sulphuric acid with baryta the filtrate was concentrated and allowed to crystallize slowly. The first crystallization consisted almost entirely of the new substance, while the filtrate separated more but mixed with varying amounts of tyrosine and leucine. From the latter substances the new decomposition product was freed by precipitation with phosphotungstic acid.

The crude fractions were dissolved in a small volume of 5 per cent sulphuric acid and phosphotungstic acid added as long as a precipitate formed.<sup>1</sup> The process was repeated until the filtrate no longer gave the Millons reaction. The free acid was then regenerated by decomposing with baryta in the usual manner and after recrystallizing from water was obtained pure. It is very difficultly soluble in cold water, much more soluble in water at 100°, and crystallizes from this solvent in long flat prisms, sometimes perfectly rectangular, more often with modified ends. When filtered dry by suction, the crystals exhibit a beautiful mother-of-pearl lustre much resembling valine. Dried in the air, the substance contains a molecule of water, which it, for the most part, loses in vacuum over sulphuric acid, completely at 120°.

- I. 0.2909 gm. substance, air dry, lost 0.0195 gm. H<sub>2</sub>O at 127°.  
 II. 0.3791 gm. substance, air dry, lost 0.0249 gm. H<sub>2</sub>O at 127°.  
 III. 0.2789 gm. substance, air dry, lost 0.0182 gm. H<sub>2</sub>O at 122°.

Calculated for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> · H<sub>2</sub>O ; H<sub>2</sub>O = 6.43 per cent.

Found	I. . . . .	H <sub>2</sub> O = 6.70 per cent.
"	II. . . . .	" = 6.57 " "
"	III. . . . .	" = 6.53 " "

- I. 0.2853 gm. substance, dried at 125°, gave 0.6697 gm. CO<sub>2</sub> and 0.1745 gm. H<sub>2</sub>O.  
 II. 0.2714 gm. substance, dried at 125°, gave 0.6362 gm. CO<sub>2</sub> and 0.1723 gm. H<sub>2</sub>O.  
 III. 0.2606 gm. substance, dried at 125°, gave 0.6114 gm. CO<sub>2</sub> and 0.1653 gm. H<sub>2</sub>O.

<sup>1</sup> Cf. E. FISCHER and E. ABDERHALDEN: Zeitschrift für physiologische Chemie, 1904, xlii, p. 540.

IV. 0.2432 gm. substance, dried at 125°, gave 23.1 c.c. moist N<sub>2</sub> at 754.5 mm. and 20.5°.

V. 0.1553 gm. substance required 12 c.c.  $\frac{H}{10}$ HCl (Gunning-Arnold).

Calculated for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>; C = 64.12; H = 6.87; N 10.69 per cent.

Found	I. . . . .	C = 64.02; H = 6.79	per cent.
"	II. . . . .	C = 63.93; H = 7.05	" "
"	III. . . . .	C = 63.99; H = 7.05	" "
"	IV. . . . .		N 10.75 per cent.
"	V. . . . .		N 10.82 " "

The substance, on rapid heating, decomposes at about 249° (uncorr.) with evolution of gas to a red oil. The melting varies, however, considerably with the rate of heating. Whether it thereby is converted into a piperazine we have not as yet ascertained.

On boiling the aqueous solution with copper hydroxide a blue color at once appears, and on concentration the copper salt separates in well-developed crystals belonging to the orthorhombic system. On exposure to the air the deep blue crystals gradually lose their lustre and on prolonged standing disintegrate to a green powder. As a consequence the water determinations were somewhat below the calculated for three and one-half molecules. The copper salt can also be recrystallized from absolute alcohol.

- I. 0.2778 gm. substance, air dried, lost 0.0436 gm. H<sub>2</sub>O at 115°.
- II. 0.3390 gm. substance, air dried, lost 0.0535 gm. H<sub>2</sub>O at 115°.
- III. 0.1526 gm. substance, air dried, lost 0.0238 gm. H<sub>2</sub>O at 115°.
- IV. 0.2731 gm. substance, air dried, lost 0.0434 gm. H<sub>2</sub>O at 115°.

Calculated for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>Cu · 3½H<sub>2</sub>O; H<sub>2</sub>O = 16.30 per cent.

Found	I. . . . .	H <sub>2</sub> O = 15.69	" "
"	II. . . . .	H <sub>2</sub> O = 15.78	" "
"	III. . . . .	H <sub>2</sub> O = 15.60	" "
"	IV. . . . .	H <sub>2</sub> O = 15.89	" "

0.2617 gm. substance, dried at 115°, gave 0.0640 gm. CuO.

0.2341 gm. substance, dried at 115°, gave 0.4453 gm. CO<sub>2</sub> and 0.1086 gm. H<sub>2</sub>O.

Calculated for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>Cu; C = 51.92; H = 4.94; Cu = 19.65 per cent.

Found	. . . . .	C = 51.88; H = 5.15; Cu = 19.53	" "
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The following crystallographic measurements we owe to the kindness of Prof. W. E. Ford of the Mineralogical Laboratory of Yale University:

The crystals of the copper salt belong to the orthorhombic system and show the simple combinations of prism,  $m$  (110), macro-dome,  $d$  (101) and macro-pinacoid,  $a$  (100), as shown in the figures. The prism faces are vertically striated, and the pinacoid faces are always small, often being entirely wanting.

The faces were not of the character to yield very good reflections, but the results obtained by taking the average of a number of measurements cannot be far from correct. The following angles were measured, those marked with an asterisk being used as the fundamental angles from which the axial ratio was calculated:

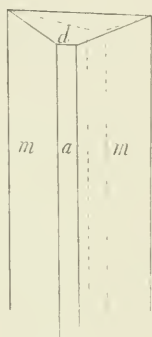


FIGURE 1.

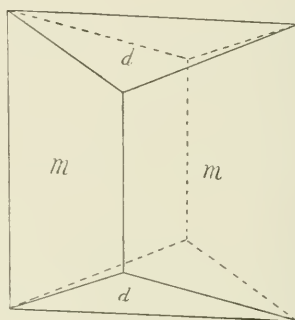


FIGURE 2.

$$m \wedge m''' = 62^\circ 31' *$$

$$d \wedge d = 64^\circ 44' *$$

$$m \wedge d = 62^\circ 52' \text{ (calculated} = 62^\circ 46')$$

$$a : b : c = .6071 : 1.00 : .3848.$$

Under the microscope with crossed nicols the crystals showed parallel extinction, confirming the crystallographic evidence of their orthorhombic character.

Crystals made at two different times showed the different types of development illustrated by the figures. Fig. 1 represents a slender prismatic habit, the average dimensions of the crystals being 5 mm.  $\times$  .5 to .75 mm.; the crystals of the other type as shown in Fig. 2 were usually doubly terminated and much shorter and thicker, their average dimensions being 2 to 3 mm. long by 2 mm. in thickness.

The free substance is readily soluble in dilute alkalis and acids. Its aqueous solution possesses no pronounced taste. It gives the xanthoprotein reaction and the pyrrol-test with the spruce splinter on sublimation. The substance is *lævo*-rotatory in 20 per cent hydrochloric acid.

0.9293 gm. dried at  $115^{\circ}$  dissolved in 17.94 c.c. of 20 per cent hydrochloric acid rotated  $4.24^{\circ}$  to the left at  $20^{\circ}$ , in a 2 dcm. tube, from which is calculated  $(\alpha)_{D}^{20} = -40.93^{\circ}$ . 1.2288 gm. of another preparation of anhydrous substance dissolved in 17.94 c.c. 20 per cent hydrochloric acid rotated  $5.69^{\circ}$  to the left at  $20^{\circ}$ , in a 2 dcm. tube, from which is calculated  $(\alpha)_{D}^{20} = -41.55^{\circ}$ . After standing for two hours at  $20^{\circ}$  there was no appreciable change in rotation.

**Hydrolysis.**—0.9300 gm. substance were heated with 35 c.c. of 20 per cent hydrochloric acid in the sealed tube for three hours at  $118^{\circ}$  and for three hours at  $125^{\circ}$ . The solution, which was only very slightly colored, was concentrated on the water bath to small volume. On cooling there separated 0.42 gm. of phenylalanine hydrochloride. This was recrystallized from strong hydrochloric acid, and 0.34 gm. obtained, which was converted to the free acid by evaporation with ammonia and the phenylalanine recrystallized from water. It separated in the characteristic crystals of phenylalanine, which heated side by side with a preparation of the pure substance from phaseolin decomposed simultaneously with the latter at about  $280^{\circ}$  (uncorr.).

It gave on boiling with potassium bichromate and dilute sulphuric acid the characteristic odor of phenylacetaldehyde. For identification a small portion was converted into the characteristic copper salt.<sup>1</sup>

0.0644 gm. substance, dried at  $110^{\circ}$ , gave 0.0130 gm. CuO.

Calculated for  $C_{18}H_{20}O_4N_2Cu$ , Cu = 16.23 per cent.

Found . . . . . Cu = 16.15 “ “

The phenylcyanate derivative melted at  $179^{\circ}$ – $180^{\circ}$  (corr.) and gave the following analysis:

0.2135 gm. substance, dried at  $100^{\circ}$ , gave 0.5298 gm.  $CO_2$  and 0.1103 gm.  $H_2O$ .

Calculated for  $C_{16}H_{16}N_2O_3$ ; C = 67.61, H 5.63 per cent.

Found . . . . . C = 67.68, H 5.74 “ “

The filtrate from the phenylalanine hydrochloride was freed from hydrochloric acid and the dried crystalline residue extracted with boiling alcohol.

For identification the solution of the proline in alcohol was evapo-

<sup>1</sup> Cf. SCHULZE and WINTERSTEIN: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 210.

rated to dryness, and the proline racemized by dissolving in water and heating with baryta in the autoclave at  $140^{\circ}$  for six hours. On boiling the racemic substance with alcohol there remained undissolved 0.11 gm. of pure phenylalanine, while the weight of alcohol soluble product was 0.44 gm., while the calculated is 0.41 gm. of proline. The total weight of pure phenylalanine obtained was 0.39 gm., or 67 per cent of the calculated quantity for the dipeptide.

The racemized proline was identified as the copper salt. It crystallized in plates, and when dried at  $110^{\circ}$  assumed the characteristic lilac color. It gave on sublimation the pyrrol-test.

0.0893 gm. substance, air dried, lost 0.0099 gm.  $H_2O$  at  $110^{\circ}$ .

0.0780 gm. substance, dried at  $110^{\circ}$ , gave 0.0211 gm.  $CuO$ .

Calculated for  $C_{10}H_{16}N_2O_4Cu \cdot 2 H_2O$ ;  $H_2O = 11.00$  per cent.

Found . . . . .  $H_2O = 11.09$  " "

Calculated for  $C_{10}H_{16}N_2O_4Cu$ .  $Cu = 21.79$  per cent.

Found . . . . .  $Cu = 21.64$  " "

We are at present engaged in further investigation of the properties of this decomposition product of gliadin. In conclusion we wish to express indebtedness to Professor Ford for the measurements of the crystals of the copper salt.

# THE INFLUENCE OF DIGITALIS, STROPHANTHUS, AND ADRENALIN UPON THE VELOCITY OF THE BLOOD CURRENT.

BY CHARLES WALLIS EDMUNDS.

[From the Pharmacological Laboratory of the University of Michigan.]

## INTRODUCTION AND METHOD.

**I**N considering the factors which influence the velocity of the blood current, it may be said that there are two which completely overshadow all others in importance, namely, the efficiency of the heart and the peripheral resistance. From the pharmacological point of view, no drugs have such a characteristic action on the two factors mentioned as the members of the digitalis series, and it seemed desirable, therefore, to study them a little further in their relation to the velocity of the blood stream and to try, if possible, to explain their effects.

The first to investigate this question was Kramnik,<sup>1</sup> who noted that in small doses digitalis increased the rate of flow, while in larger doses the current was slowed.

Hemmeter,<sup>2</sup> using various preparations of digitalis, found only great retardation in the blood flow, the mean velocity falling from a normal of 165.16 mm. per second to 98.6 mm. He explained this as being due to a lessened activity of the heart with an increased peripheral resistance. Gottlieb and Magnus<sup>3</sup> in their works on the vascular action of this group, say that all the digitalis bodies hold this in common, that they produce a strong acceleration of the blood current, first through the blood pressure increase and second through the narrowing of the stream beds. Both Kramnik and Hemmeter employed

<sup>1</sup> KRAMNIK: Moskauer pharmacologische Arbeiten, p. 143; cited in Virchow-Hirsch's Jahresbericht. 1876, ii, p. 433.

<sup>2</sup> HEMMETER: Medical Record, 1891, xl, p. 292.

<sup>3</sup> GOTTLIEB und MAGNUS: Archiv für experimentelle Pathologie und Pharmacologie, 1901, xlvii, p. 163.

Ludwig's stromuhr, and while this instrument has proved about as satisfactory as any devised for the purpose, many objections have been urged against it, and Stewart<sup>1</sup> has introduced a very ingenious electrical method of measuring the velocity of the blood flow which offers many advantages over the stromuhr.

This method rests upon the fact that the electrical conductivity of the blood depends mainly upon the salts dissolved in it. By the injection, at one point of the circulatory system, of a small amount of a strong salt solution which will be carried in the blood stream to another point where the electrical resistance of the blood is being measured by a galvanometer, the stronger salt solution, at its moment of arrival, will alter the resistance, giving a deflection of the galvanometer. The time between the moment of injection and that of deflection is measured by a stop watch. It is true this does not give the actual circulation time,<sup>2</sup> but rather the shortest time it takes the blood to pass between any two given points in the circulatory system, and would be more nearly the velocity of the axial stream which, according to Poiseuille and v. Kries, is double the mean velocity.

The method is carried out as follows, fuller details being found in the excellent description in Stewart's article referred to. After the animal is anæsthetized a cannula for injecting the salt solution is tied into any vessel that it may be desired to start measuring the time from. Another vessel where it is wished to place the electrodes for the galvanometer connection, is then dissected free from its surrounding tissue for a distance of two or three centimetres according to its location.

The best form of non-polarizable electrode is probably the kaolin and zinc sulphate combination in glass tubing which may be drawn out into a hook with an opening in it against which the vessel may rest in contact with the exposed kaolin. The electrodes, held by clamps, are placed about 1 centimetre apart; this distance being varied according to the location of the vessel used and the length isolated. The vessel is then insulated from the surrounding tissues by means of thin rubber dam, unless, as can be done in some cases, the neighboring tissues are drawn back from all contact with the electrodes. The zinc terminals which rest in the sulphate solution of

<sup>1</sup> STEWART: *Journal of physiology*, 1894. xv. p. 1.

<sup>2</sup> It might be explained that in this paper the phrases "velocity of the blood" and "circulation time" are used in a loose sense, as convenient terms, rather than in the strict sense, as defined in works on physiology.



the electrodes are connected in the circuit with a Wheatstone bridge and a resistance box of the post-office type and a D'Arsonval galvanometer. The resistance in the arms of the bridge in most of my experiments was in the ratio of 100:1000, although in a few it was 1000:1000. The galvanometer was arranged to throw a beam of light on a scale which was located about two feet from the instrument, so that the deflection could be seen by an assistant, who at the moment of injection closed the key of an electric signal which wrote on the kymograph directly under the blood pressure tracing. In my first experiments I did not record the blood pressure, but very soon found that it was necessary to do so in order to get the heart rate and the height of the blood pressure, that they might help explain the changes in the circulation time found, as well as indicating the stage of action of the drug employed.

The animals used were dogs and rabbits. The latter I found very satisfactory to work with when I was investigating the effects of adrenalin and digitalis. With digitalein and strophanthin I used dogs in working on the pulmonary and peripheral circulations, but rabbits in investigating the portal area, for reasons which will be mentioned later. The rabbits were anæsthetized with paraldehyde, given by the stomach, 1.7 c.c. per Kg. of body weight, while the dogs were given morphine and chloretone, about 0.20 G. of the former being administered subcutaneously three or four hours before the operation and 2 to 4 G. of chloretone dissolved in a few cubic centimetres of alcohol given by the stomach at the time of the operation. These anæsthetics have proved very satisfactory during the several years that they have been used in this laboratory, giving a uniform anæsthesia and having little effect on the circulation.

To maintain the body temperature of the animals and to lessen shock, I used a galvanized iron tank operating board, which was filled with warm water kept at a fairly even temperature during the experiment. The animals were then partially covered by another similar water tank, or, in case this could not be used for lack of room, they were covered by towels. By these means the rectal temperature was kept at about 38° or 39° C., as was proved by frequent observation.

Perhaps the most important matter in the manipulations was the salt solution used. In the first place it had to be injected at as nearly a constant temperature as possible. By means of a water bath it was always kept at 39° C., thus allowing for a slight cooling during the time it was drawn up into the warmed syringe preparatory to its

injection. The constant temperature was very important as a difference of  $1^{\circ}$  would cause a distinct alteration in the circulation time. The strength of the solution was varied with the different animals, using it only strong enough to cause a distinct deflection of the galvanometer. With medium-sized rabbits (2500 G.) 2 c.c. of a  $2\frac{1}{2}$  per cent solution was very good provided it had to pass through only one set of capillaries before reaching the electrodes. With larger rabbits (3500 G.) 4 per cent or 5 per cent solutions had to be used, as will be noted in the protocols to be given later. If solutions stronger than these were employed, the effects were at once shown by the animal giving a slight convulsive movement accompanied by an alteration in the respiration. These disturbances had to be avoided, and the blood pressure tracings rarely showed any irregularity except that due to mechanical influences. In the dogs stronger solutions had to be utilized. In the smaller animals 4 c.c. of a 10 per cent solution was strong enough, while in larger dogs (15 to 20 K.) it was necessary to use 4 c.c. of a 20 per cent solution. The greater capacity of the heart and the larger bulk of blood allowed so much greater opportunity for dilution of the salt solution that even in these strengths it caused no circulatory disturbance, except in one or two animals, where an occasional irregularity of the heart appeared.

I found it necessary to measure the velocity of the blood stream at three points. In the greater circulation it had to be measured twice, namely, in the splanchnic area and in the peripheral vessels proper. The necessity for these two measurements is due to the fact that the resistance in these two channels may be altered in opposite directions under vasomotor influence, or constriction may occur in one area while the other may not be affected, so that the velocity of the current may be affected differently in the different parts of the vascular system. The total effect of the drug would be shown in the pulmonary circulation, which would require but one measurement, as here all the blood takes the one route.

The pulmonary circulation time was measured in the same way in all the experiments—from one jugular vein where the salt solution was injected to a carotid artery where the electrodes were placed. This, as has been pointed out by Stewart, will give more than the true pulmonary time, because in rabbits there is a distance of about 1.5 to 2 cm. from the cannula to the right auricle and about 4 cm. from the left ventricle to the electrodes, and figuring the velocity of the current in the arteries and veins and adding the fraction of a

second it would spend in the heart, he considers the actual readings to be about 0.35 second over the exact pulmonary time. However, in my work this small excess would be of no importance, as it would be the same under the drug as it was in the controls.

To measure the portal (splanchnic) circulation time, the injection was made in rabbits into the left carotid, while the electrodes were placed on the superior mesenteric vein. This vessel was reached by a longitudinal incision made about 2 cm. to the right of the median line. The intestines being packed off to the left, the vessel could be isolated very easily, as for about 2 cm. of its course it has no side branches. Several attempts were made in dogs to get the portal time in the same way, but the great difficulty was that the mesenteric vessel was so close to the liver that it was impossible, on account of the respiratory movements, to prevent slight movement of the vessel on the electrodes, thus causing alterations in the resistance, producing constant swinging of the galvanometer. Efforts were also made to use smaller branches of the mesenteric veins, but the same difficulty was experienced, although to a less degree, depending upon the length of the mesentery. The main trouble experienced here was injury to the vein walls produced when separating the vein from the artery. This traumatism was followed at once by extensive hemorrhage into the vascular or perivascular tissues and immediate clotting in the vessel. All the portal measurements were therefore made on rabbits.

The peripheral circulation time in rabbits was taken from the left carotid artery, where the salt was injected, to the inferior vena cava, which was isolated for a distance of about 2 cm., as near to its origin as convenient. The most satisfactory point found to measure the peripheral time in dogs was from the external carotid to the femoral vein just below Poupart's ligament. Criticisms might be made to the injections being given into the carotid on the ground that the injected salt solution is propelled by the force of the syringe and not carried by the blood stream itself. This objection cannot be entirely overcome, but was minimized by using no undue force at each injection and trying to make them as uniform in this respect as possible. The only way to avoid this difficulty completely with the portal and peripheral systems would have been to inject into a vein. This could not be done for two reasons: it would have required stronger salt solution for the two sets of capillaries, and then it would not have given the pure peripheral or portal circulation time, as the pulmonary

vessels would be included, and any marked change here might obscure alterations in the other two areas. That this latter objection is not purely hypothetical was proved in some of the experiments in which a modification of this method was used.

The drugs employed were adrenalin (Takamine) dissolved in boiling salt solution; the U. S. P. fluid extract of digitalis; and the glucosides, digitalein (Merck), and strophanthin dissolved in water, the solutions being made up fresh each time. A number of experiments were made with each drug, but only one protocol of each need be given, as they were all essentially alike, except as noted.

In the protocols given, all readings are omitted which there was any reason to call in question. Difficulties arose many times during an experiment, the most common cause being slight movement of the vessel on the electrode with a consequent swinging of the galvanometer that made accurate readings impossible. All such readings were recorded at the time as untrustworthy and are therefore omitted.

#### ADRENALIN (ACTION ON RABBITS).<sup>1</sup>

**Protocol I.**—January 30, 1906. Pulmonary circulation time. Rabbit 2900 G. 4.9 c.c. paraldehyde. Salt solution (2 c.c., 2½ per cent. temperature 39° C.) injected into left external jugular vein. Electrodes on right carotid 3½ cm. from arch of aorta. Blood pressure taken from left carotid. Adrenalin injected into jugular vein. Temperature of animal during experiment 39° C. Arms of resistance box 100:1000.

Number.	Time.	Circulation time.	Heart rate.	Blood pressure.
1	3.05	2½ sec.	285	66 Hg mm. Control.
2	3.10	3 "	315?	74 " "
3	3.12	2½ "	285	70 " "
4	3.15	2½ "	285	70 " "
5	3.20	4 "	255	98 " Adrenalin
6	3.23	4½ "	195	135 " "
7	3.27	4¾ "	210	132 " "

**Protocol II.**—January 30, 1906. Peripheral circulation time. Rabbit same as Protocol I. Salt solution (2 c.c., 10 per cent. temperature 39° C.) injected into left carotid. Electrodes on inferior vena cava. Adrenalin injected into branch of external jugular vein.

<sup>1</sup> For convenience the discussion of the effect of adrenalin on dogs will be given later, p. 147.

Number.	Time.	Circulation time.	Heart rate.	Blood pressure.	
17	4.45	5 $\frac{2}{3}$ sec.	255	120 Hg mm.	Adrenalin
18	4.50	3 $\frac{1}{3}$ "	255	90 "	"
19	4.52	2 $\frac{2}{3}$ "	255	34 "	Control
20	4.55	2 $\frac{1}{3}$ "	270	28 "	"
21	4.58	5 "	255	86 "	Adrenalin
22	5.00	5 $\frac{1}{3}$ " <sup>1</sup>	270	110 "	"
23	5.03	2 $\frac{1}{3}$ "	210	30 "	Control
25	5.10	6 $\frac{2}{3}$ "	240	124 "	Adrenalin

**Protocol III.**— February 5, 1906. Portal circulation time. Rabbit 2570 G. 4.4 c.c. paraldehyde. Injection of salt solution (2 c.c., 5 per cent) into left carotid, 3 $\frac{1}{2}$  cm. from arch of aorta. Electrodes on superior mesenteric vein. Blood pressure from right carotid. Adrenalin injected into jugular vein.

Number.	Time.	Circulation time.	Heart rate.	Blood pressure.	
1	2.30	3 $\frac{1}{5}$ sec.	300	24 Hg mm.	Control
2	2.40	3 $\frac{2}{5}$ "	285	24 "	"
4	2.45	3 $\frac{4}{5}$ "	285	28 "	"
8	3.12	3 $\frac{3}{5}$ "	240	28 "	"
10	3.20	6 $\frac{1}{5}$ "	195	116 "	Adrenalin
11	3.25	6 $\frac{1}{5}$ "	195	118 "	"
13	3.35	3 $\frac{1}{5}$ "	210	30 "	Control
16	3.45	4 $\frac{1}{5}$ "	225	60 "	Adrenalin
17	3.52	4 $\frac{1}{5}$ "	225	82 "	"
18	3.56	2 $\frac{1}{5}$ "	240	22 "	Control

There can be no doubt but that the effect of adrenalin upon the velocity of the blood current in rabbits is to slow it very greatly. This result was confirmed in many other experiments not given here.

The cause of the slowing which takes place in the entire blood stream, as shown by the pulmonary observations, must primarily lie in the greater circulation. It is there that the vessels are contracted and the blood pressure increased, mainly on account of the increased peripheral resistance. This increased pressure in the aorta, with which for a short time the ventricle is able to cope, finally prevents the heart from emptying itself completely, and it dilates, as has been shown in plethysmographic tracings by Elliott,<sup>2</sup> who found great distention of the chambers, the volume of the heart increasing proportionately with the rise of blood pressure. The blood backs up into the pulmonary vessels, and, as Elliott found, there is a rise in the venous pressure.

<sup>1</sup> Slow reading.

<sup>2</sup> ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 407.

There can be little doubt that this back pressure is the cause of the retardation in the pulmonary area. Bradford and Dean<sup>1</sup> found that "back pressure" is produced if the systemic blood pressure is raised in other ways, as, for instance, by clamping the aorta. Here the systemic blood pressure is enormously raised, but no change takes place in the pulmonary pressure unless the compression lasts longer than ten seconds, in which case it will be slightly increased. This result they no doubt rightfully ascribe to "back pressure." A factor which would tend to overcome this obstruction to the pulmonary outflow would be the direct action of the adrenalin on the right side of the heart increasing its efficiency. The back pressure through the left side of the heart added to the action on the right heart would doubtless raise the pulmonary pressure considerably were the pulmonary vessels not able to dilate and take care of the excess of blood. Also it must not be overlooked that the right heart may be rendered less efficient by vagus action, dilate and give a rise in venous pressure, as described by Elliott.

The pulmonary vessels themselves probably are not acted upon by the adrenalin or only to a very slight degree. Brodie and Dixon<sup>2</sup> say that it dilates them, while Gottlieb<sup>3</sup> argues on theoretical grounds that an action by adrenalin on the lung vessels is very improbable, and quotes Gerhardt in support of his contention to the effect that while the vagi are intact adrenalin exerts on the vessels only a very slight influence, but when the vagi are cut the vessels remain completely inactive. The slowing in the pulmonary time under adrenalin can scarcely therefore be primary but secondary to the systemic changes, and here the retardation might be produced either by lessened efficiency, or slowing of the heart, or increased peripheral resistance. The latter, being such an important result of adrenalin action, was first investigated. The readings of the three experiments were arranged in the order of blood pressure heights with the results given in table on page 137.

The readings arranged in this manner show with very few exceptions that with increasing pressure there is progressive slowing of the blood current, the two apparently standing in very close relations. This does not exclude the rate of the heart from being of importance,

<sup>1</sup> BRADFORD and DEAN: *Journal of physiology*, 1894, xvi, p. 34.

<sup>2</sup> BRODIE and DIXON: *Ibid.*, 1904, xxx, p. 476.

<sup>3</sup> GOTTLIEB: *Archiv für experimentelle Pathologie und Pharmacologie*, 1900, xliii, p. 300.

as indeed it must be. However, in the many attempts that were made to arrange the readings in accordance with this factor, in most cases no close relation between them could be made out, probably because the pressure changes were so marked as to conceal the other influence. This was not always the case, as may be seen in revised Protocol I given below, in which with increasing rate of the heart

From Protocol I.			From Protocol II.			From Protocol III.		
Height.	Rate.	Time.	Height.	Rate.	Time.	Height.	Rate.	Time.
135	195	sec. 4 $\frac{1}{2}$	124	240	6 $\frac{2}{3}$	118	195	6 $\frac{1}{3}$
132	210	4 $\frac{2}{3}$	120	255	5 $\frac{2}{3}$	116	195	6 $\frac{1}{3}$
98	255	4	110	270	5 $\frac{1}{3}$ <sup>2</sup>	82	225	4 $\frac{1}{3}$
70 <sup>1</sup>	285	2 $\frac{2}{3}$	90	255	3 $\frac{1}{3}$	60	225	4 $\frac{1}{3}$
			86	255	5	30	210	3 $\frac{1}{3}$
			30 <sup>1</sup>	245	2 $\frac{2}{3}$	22	240	2 $\frac{2}{3}$
<sup>1</sup> Average of controls.						<sup>2</sup> Recorded slowly.		

there was a lowering of the circulation time. Protocols II and III show no such close relation.

In considering further the effect of the heart rate there were found scattered through the experiments many readings in which the pressure was practically exactly alike in two succeeding readings, the only difference being in the rate of the heart. In such cases, if the two rates did not differ from one another more than 5 per cent or 8 per cent, the readings were usually found the same, while if there was a difference of, say, 10 per cent or over, the velocity of the current would be found to be changed. This may be readily understood when we consider that in many of the animals the heart was beating about 250 times to the minute. Contracting at this rate, it may be questioned whether it had time to fill properly during its short diastole, and if not, a change of fifteen or twenty beats to the minute would have little effect on its efficiency. If, on the other hand, the slowing exceeded more than 10 per cent, then it began to make its influence felt on the rate of the blood stream, although in some of these cases even with the slower heart there was an increased velocity of the stream.

This question of the influence of pressure and heart rate upon velocity was studied in a more direct way by slowing the heart with pilocarpine and maintaining at the same time a uniform blood pressure. The latter was accomplished by applying tightly around the animal's abdomen a rubber bag 10 cm. wide, and then inflating the bag with air until the pressure on the animal was sufficient to raise the blood pressure enough to overcome the fall due to the pilocarpine. This experiment gave the following readings:

**Protocol IV.**—January 11, 1906. Rabbit 3100 G. 5.2 c.c. paraldehyde. Injection 2 c.c., 2½ per cent NaCl into left jugular vein. Electrodes on left carotid. Uniform blood pressure maintained by means of rubber bag to abdomen.

Heart rate.	Circulation time.
315	2½ sec.
4 mg. pilocarpine in salt solution injected.	
270	2⅔ sec.
225	3¼ "
150	3⅔ "
1 mg. atropine injected.	
300	2⅔ sec.

These results show in general that with slowing of the heart there is an increase in the circulation time. It will be noticed that the readings for the various heights are not arranged in an exactly regular order, but this point will be taken up a little later.

The influence on the circulation time of changes in blood pressure with a uniform heart rate was investigated in a similar way. The rubber bag was applied to the rabbit's abdomen as before, and was inflated to varying degrees in order to bring the blood pressure to any required point which would correspond more or less closely with a pressure which might be obtained with adrenalin. The results of these observations with a heart rate of 270 are arranged in the order of the pressure heights.

Blood pressure.	Pulmonary circulation time.
116 Hg mm.	2⅔ sec. (2 readings).
112 "	2⅔ "
106 "	2½ "
104 "	2½ "
102 "	2⅔ "
96 "	2⅔ "
86 "	2⅔ "
80 "	2 "



Here, as in the previous experiments, two of the readings may be said to be irregular, but with those exceptions there is a remarkably close relationship between the peripheral resistance and the velocity of the blood current; with increasing resistance there is progressive slowing of the stream.

As there can be in this experiment no question of a direct action on the lungs, the slowing in the pulmonary circulation must be secondary, as discussed above, being either due to "back pressure" through the heart, or it might possibly be due to interference to the passage of the blood from the arteries to the veins and thus to the right side of the heart.

In regard to the "irregular readings" which have been mentioned from time to time and which suddenly come in, apparently without any relation to heart rate or blood pressure, it may be said that it is not surprising that they should be found, but is rather surprising that they are not more frequent. For instance, Dogiel found the mean velocity of the blood in a dog's carotid to vary greatly from second to second, one series of his readings being as follows: 489, 733, 386, 349, and 489 mm. per second. In a rabbit he found it to vary from 94 mm. per second to 226 mm. per second. Also Schäfer says "the velocity in any particular artery bears no absolute relation to pulse frequency or general arterial tension." Such may indeed be, and no doubt is the case, but in the very large number of observations made in the course of this research it has happened very frequently that many readings that were alike in the course of one experiment and which might be taken an hour or more apart, were found, on studying the blood pressure tracing later, to be associated with practically or exactly the same heart rate and pressure. Again a number of consecutive readings taken at intervals of two or three minutes would usually be exactly alike, certainly giving the idea that the velocity of the blood was very much more uniform than Dogiel's readings would indicate. Nevertheless, as pointed out, there are occasionally these sudden isolated readings, the cause of which it is impossible to explain. They might be due to altered efficiency of the heart, but this would be expected to show itself in the blood pressure, or perhaps to some temporary disturbance in the ability of the heart to receive its normal amount of blood. Then also a contraction of the arterioles supplied by the main vessel under consideration at the time might be entirely compensated by a local dilatation of some vessels elsewhere, the effects of the two balancing one another and

so making no change in the blood pressure, but an alteration in the velocity of the current; retardation in the contracted area with acceleration through the dilated area. The vast majority of the observations, however, are very uniform; any changes being usually easily explained by variations in the blood pressure or pulse rate.

The action of adrenalin, then, upon the velocity of the blood stream in rabbits is to slow it; this retardation being dependent partly upon increased peripheral resistance and partly upon slowing of the heart.

#### STROPHANTHIN.

The action of strophanthin was studied in the same way as that of adrenalin, dogs being used instead of rabbits, except in the study of the portal circulation. The pulmonary time, as stated earlier, was taken from the external jugular vein to the carotid artery; the portal time from the carotid to the mesenteric vein, and the peripheral from the carotid to the femoral vein.

**Protocol V.**—March 21, 1906. Pulmonary circulation time. Large dog. 200 mg. morphine. 2 G. chloretone in 8 c.c. alcohol. Salt solution (4 c.c., 20 per cent, temperature 39° C.) injected into left jugular vein. Electrodes on right carotid artery. Blood pressure from left carotid. Strophanthin injected into saphenous vein.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
2	3.23	$4\frac{4}{5}$ sec.	140	86 Hg mm.
3	3.25	$4\frac{3}{5}$ "	140	82 "
4	3.28	$4\frac{3}{5}$ "	140	100 "
5	3.35	$5\frac{3}{5}$ "	110	80 "
6	3.36	5 "	140	84 "
7	3.45	$5\frac{2}{5}$ "	130	88 "
8	3.46	$5\frac{2}{5}$ "	140	94 "
9	3.47	$4\frac{1}{5}$ "	144	100 "
10	3.48	$4\frac{1}{5}$ "	156	100 "
	3.49	1 mg. strophanthin in salt solution injected.		
11	3.49 <sup>2</sup>	$3\frac{4}{5}$ sec.	156	106 Hg mm.
12	3.50 <sup>2</sup>	$3\frac{4}{5}$ "	156	114 "
13	3.52	$4\frac{1}{5}$ "	130	114 "
14	3.53	$4\frac{1}{5}$ "	120	110 "
15	3.56	$4\frac{2}{5}$ "	130	108 "
16	3.59	$4\frac{2}{5}$ "	130	110 "
17	4.02	$4\frac{2}{5}$ "	120	110 "
	4.04	0.5 mg. strophanthin injected.		
18	4.05	5 sec.	100	128 Hg mm.
19	4.06 <sup>2</sup>	5 "	95	140 "
	4.15	1 mg. atropine sulphate injected.		
20	4.17	$4\frac{4}{5}$ "	150	130 Hg mm.

From the results of this experiment there cannot be the slightest doubt that strophanthin first accelerates the blood current and then retards it. The acceleration is only temporary, and occurs in spite of the increasing peripheral resistance due to contraction of the blood vessels in splanchnic area, this contraction and therefore increased resistance being indicated by the rising blood pressure (Observations 11, 12, etc.).

In some experiments (Protocol VII, page 143) an increase in the heart rate might partially account for the acceleration, but in this experiment the rate in observation No. 10 before the strophanthin injection was the same as in Nos. 11 and 12, so that there must be another factor present to produce this change. This is the increased efficiency of the heart brought about by the direct action of the strophanthin upon the cardiac muscle.

The secondary retardation is dependent upon two factors also, the increased resistance and the slowing of the heart. It is clear that a narrowing of the arterioles must have a tendency to obstruct the outflow of blood from the vessel supplying them, and in this way to retard the stream, as will be pointed out below; but this increased resistance does not seem to be such an important factor in the dog as in the rabbit. This is shown by the fact above mentioned that in the early stage of the drug's action there is acceleration, together with a contraction of the arteries.

The second factor mentioned seems of much more importance, as the retardation in the stream seems to stand in more or less direct relation with the cardiac slowing, as will be shown a little later and as may be seen by a comparison of observations 15 to 19. The more marked effect on the velocity of changes in the rate of the dog's heart in comparison with the rabbit is doubtless due to the greater capacity of its ventricle, and therefore the greater bulk of blood expelled at each systole. The effect of the rate is shown in a striking manner by a comparison of Nos. 18 and 19 with No. 20. Here, by the use of atropine, the vagus effect was removed and by the increased heart rate the pulmonary circulation was shortened by three-fifths of a second.

For purposes of further comparison, all the readings may be arranged according to heart rate irrespective of pressure height, merely giving the average where there was more than one reading at a certain height.

Number of readings.	Average heart rate.	Average blood pressure.	Circulation time.	
			Control.	Strophanthin.
2	156	110 Hg mm.		$3\frac{1}{5}$ sec.
1	156	100 "	$4\frac{1}{5}$ sec.	
1	144	100 "	$4\frac{1}{5}$ "	
5	140	90 "	$4\frac{1}{5}$ "	
3	130	110 "		$4\frac{2}{5}$ "
1	130	88 "	$5\frac{2}{5}$ "	
2	120	110 "		$4\frac{3}{5}$ "
1	110	80 "	$5\frac{3}{5}$ "	
1	100	128 "		5 "
1	95	140 "		5 "

A comparison of this sort shows with only one exception (and in that case there was only one reading) that the heart rate and circulation time bear an inverse ratio to one another. The strophanthin readings also stand in this case in direct ratio to the pressure, although the control readings do not. But more striking is the fact that a comparison of each strophanthin reading with the nearest (as far as height and heart rate are concerned) control shows in every case that the former is much quicker, thus showing the effect of the increased efficiency of the heart on the velocity of the current. This is illustrated in the readings with the heart rate at 156 and also at 130, in both of which cases, in spite of a higher blood pressure, the strophanthin time was much the quicker. The other readings show the same thing. This table then shows in a very clear manner the effect on the velocity of the three factors by which it is chiefly affected, namely, efficiency and rate of the heart and peripheral resistance.

**Protocol VI.** — December 7, 1906. Portal circulation time. Rabbit 2,400 G. 4 c.c. paraldehyde. Salt solution (2 c.c.,  $3\frac{1}{2}$  per cent,  $39^{\circ}$  C.) injected into left carotid. Electrodes on the superior mesenteric vein. Blood pressure from right carotid.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
6	3.42	$2\frac{1}{5}$ sec.	240	80
7	3.44	$2\frac{1}{5}$ "	235	85
	3.46			
8	$3.46^2$	$3\frac{2}{5}$ sec.	240	85
9	3.47	$3\frac{1}{5}$ "	230	85
10	3.48	$3\frac{1}{5}$ "	230	94
11	3.50	$3\frac{3}{5}$ "	225	94
12	3.52	$3\frac{3}{5}$ "	210	85
13	3.56	$3\frac{1}{5}$ "	220	75
	3.57			
14	3.58	$3\frac{2}{5}$ sec.	210	90
15	4.00	3 "	225?	80
16	4.02	$3\frac{2}{5}$ "	205	75

**Protocol VII.** — November 16, 1906. Peripheral circulation time. Medium-sized dog. 0.2 G. morphine; 3 G. chloretone. Salt solution (4 c.c., 10 per cent) injected into left carotid. Electrodes on left femoral vein. Blood pressure from right carotid.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
9	3.16	4 $\frac{4}{5}$ sec.	100	130
10	3.18	5 "	105	120 heart irregular
11	3.21	4 $\frac{3}{5}$ "	100	120
	3.22	1 mg. strophanthin into jugular.		
12	3.24	4 $\frac{2}{5}$ "	105	130
13	3.26	4 $\frac{4}{5}$ "	110	145
14	3.27	3 $\frac{4}{5}$ "	125	150
16	3.32	4 $\frac{3}{5}$ "	105	60
17	3.37	3 $\frac{3}{5}$ "	120	30
	3.38	$\frac{1}{2}$ mg. strophanthin.		
18	3.39	4 "	125	40
19	3.40	3 $\frac{4}{5}$ "	115	60
21	3.43	3 $\frac{2}{5}$ "	120	80
	3.56 <sup>2</sup>	$\frac{1}{2}$ mg. strophanthin.		
24	3.57	6 $\frac{1}{2}$ "	140	55
25	4.00	5 $\frac{2}{5}$ "	130	80

These two protocols (VI and VII) show that the final effect of strophanthin is to retard the blood stream, but that in the case of the peripheral circulation this is preceded by an acceleration. The portal area shows no such acceleration, the retardation coming on very quickly and definitely. It might be urged, perhaps, that this is due to the difference in animals, but against this objection is the fact that practically the same state of affairs is found under digitalis, as will be pointed out later, and with that drug rabbits were used entirely.

The results stand in complete agreement with those of Gottlieb and Magnus,<sup>1</sup> who found under strophanthin the vessels of the internal organs were contracted and those of the periphery were dilated. The blood is therefore diverted from the interior of the body to the periphery, where, owing to lack of obstruction, it would flow faster on account of the increased pressure; while in the splanchnic area it would be delayed by the increased resistance against which it has to contend. If this is correct in those cases where there is only slight contraction in the vessels supplied by the splanchnics, as shown by a small rise in blood pressure, there should be only very slight retardation in the current in this area, and there might be an acceleration due to increased efficiency of the heart. This is well illustrated under the action of digitalis and digitalein.

<sup>1</sup> GOTTLIEB und MAGNUS: *Archiv für experimentelle Pathologie und Pharmacologie*, 1901, xlvii, p. 144.

The acceleration in the pulmonary area with strophanthin is due, first, to the right heart being acted upon by the drug; second, the vessels of the lungs are not contracted; and lastly, owing to the acceleration of the current through the peripheral vessels, the blood can reach the right heart easily, although this last factor may be balanced by the contraction of the vessels of the splanchnic area.

## DIGITALIS AND DIGITALEIN.

**Protocol VIII.**—December 8, 1905. Pulmonary circulation time. Rabbit 2050 G. 3.4 c.c. paraldehyde. Injection of salt solution (2 c.c., 2½ per cent) into jugular vein. Electrodes on carotid artery. Blood pressure from carotid.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
4	2.40	2⅔ sec.	240	74
5	2.43	2⅔ "	240	74
	2.45		2 m. fl. ext. digitalis into jugular.	
6	2.49	2⅔ sec.	225	74
7	2.50	2⅔ "	255	78
	2.51		2 m. fl. ext. digitalis.	
8	2.54	2⅔ sec.	255	74
9	2.57	2⅔ "	255	76

The effect of digitalis upon the pulmonary circulation time is apparently not very marked, but consists of primary acceleration, followed later by slowing, which in this experiment was merely a return to normal.

This result was also confirmed in a dog with digitalein, the details of which experiment may be given shortly as follows:

**Protocol IX.**—March 29, 1906. Dog. Morphine and chloretone. Average readings.

	Circulation time.	Heart rate.	Blood pressure.
Normal	5⅔ sec.	155	80
Digitalein	4⅓ to 5⅓ "	150-160	90 to 96

**Protocol X.**—February 9, 1906. Peripheral circulation time. Rabbit 2800 G. 4.7 c.c. paraldehyde. Salt solution (2 c.c., 3½ per cent) injected into left carotid. Electrodes on inferior vena cava. Blood pressure from carotid.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
4	3.48	2⅔ sec.	300	46
			0.1 c.c. fl. ext. digitalis into jugular.	
5	3.50	2⅓ sec.	300	54
6	3.52	2⅔ "	270	54
7	3.54	2⅓ "	285	52

Digitalein had about the same effect upon the peripheral circulation in the dog. The results were not very marked, averaging only about  $\frac{1}{5}$  of a second, but during this time the blood pressure was greatly increased (30 to 40 mm. Hg). Such an increase in pressure it would have been supposed would have caused much greater acceleration through the periphery, but this is no doubt prevented by the contraction of the peripheral vessels which Gottlieb and Magnus have shown is produced by digitoxin, which differs in this respect from strophanthin. However the results obtained on the peripheral current in both dogs and rabbits with both digitalis itself and with digitalein stand in complete agreement, namely, an acceleration which was most marked in the case of digitalis on the rabbit. In no experiment was the acceleration as marked as with strophanthin.

**Protocol XI.** — February 16, 1906. Portal circulation time. Rabbit, 3650 G. 6.2 c.c. paraldehyde. Salt solution (2 c.c., 5 per cent) injected into left carotid. Electrodes on superior mesenteric vein.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
2	2.55	$3\frac{1}{5}$ sec.	270	54
5	3.00	$2\frac{4}{5}$ "	270	40
7	3.20	$2\frac{3}{5}$ "	255	40
9	3.25	$3\frac{1}{5}$ "	225	44
12	3.37	3 "	255	40
13	3.40	$2\frac{4}{5}$ "	240	42
		0.1 c.c. fl. ext. digitalis into jugular.		
14	3.45	$2\frac{4}{5}$ sec.	255	62
15	3.47	$3\frac{2}{5}$ "	270	71
17	3.52	$2\frac{3}{5}$ "	240	76
18	3.54	$2\frac{3}{5}$ "	270	66

In another experiment as to the effect of digitalis on the portal time practically no acceleration was found, the most marked change being lengthening in the time of from one-fifth to three-fifths of a second, but in this case the blood pressure was raised considerably, about 15 mm. Hg. Digitalein in the experiment given below also caused an acceleration in the current which was very distinct, but it will be noted that the blood pressure was scarcely changed, showing the absence of any marked contraction of the splanchnic vessels.

**Protocol XII.** — November 22, 1906. Portal circulation time. Rabbit 1900 G. 3.4 paraldehyde. Salt solution (2 c.c., 5 per cent) into left carotid artery. Electrodes on superior mesenteric vein.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
6	3.26	$3\frac{2}{5}$ sec.	310	44
7	3.29	$3\frac{3}{5}$ "	290	48
8	3.34	$3\frac{3}{5}$ "	280	46
	3.36		0.5 mg. digitalein into jugular.	
9	3.39	$2\frac{1}{5}$ sec.	285	50
10	3.43	$2\frac{1}{5}$ "	270	44
11	3.45	$2\frac{1}{5}$ "	270	42
	3.49		0.5 mg. digitalein.	
12	3.51	$2\frac{2}{5}$ sec.	260	34
13	3.53	$2\frac{2}{5}$ "	260	30
14	3.56	$2\frac{2}{5}$ "	260	26
	3.59		1 mg. digitalein.	
15	4.00	$2\frac{3}{5}$ sec.	240	29
16	4.02	3 "	240	30
17	4.04	$3\frac{1}{5}$ "	240	30
	4.07		0.5 mg. digitalein.	
18	4.11	$2\frac{1}{5}$ sec.	230	24
19	4.16	$2\frac{2}{5}$ "	220	24
20	4.18	$2\frac{3}{5}$ "	220	24
	4.20		1 mg. digitalein.	
21	4.22	$2\frac{3}{5}$ sec.	240	45
22	4.23	$2\frac{3}{5}$ "	210	35
23	4.25	$2\frac{3}{5}$ "	210	35
	4.27		1 mg. digitalein.	
24	4.30	$3\frac{2}{5}$ sec.	210	30

This protocol is given fully to illustrate another point than that noted above, namely, the necessity of the blood pressure record. By it can be seen that the only readings which can be considered of importance as showing the effect of the drug are Nos. 9, 10 and 11. Later the animal passed into a state of more or less shock, and it scarcely reacted to the digitalein by any increase in pressure. After observation number 12 the circulation time is probably almost entirely influenced by the steadily falling pressure modified by the gradual slowing of the heart.

Digitalis then produces an acceleration of the blood stream which, as seen in the pulmonary area, is apparently not quite so marked as is the acceleration under strophanthin. The variation may partially be due to differences in the action in the lung itself, strophanthin probably having no effect whatever upon the pulmonary vessels while digitalis probably constricts them, raising the pulmonary pressure (Bradford and Dean<sup>1</sup> for digitalin). Probably the greatest source of difference is in their action on the peripheral vessels, with strophan-

<sup>1</sup> BRADFORD and DEAN: *Journal of physiology*, 1894, xvi, p. 87.



thin the increased blood pressure due to the constriction in the splanchnic area hurries the blood toward the right side of the heart through the peripheral arteries which are not contracted.

On the other hand, with digitalis the blood is prevented somewhat from entering the venous system, and thus also the right heart, by the peripheral arteries which are contracted by digitalis. The acceleration through the portal area will depend upon how much these vessels are contracted; — if only slightly, acceleration may be found; if the vessels are strongly acted on, retardation may be the only change met with.

#### ADRENALIN (EFFECT ON DOGS)<sup>1</sup>

The fact that acceleration of the blood current in the dog is produced by both strophanthin and digitalein suggested that perhaps adrenalin might have the same action on this animal, and an experiment was carried out to investigate this point.

In this experiment the salt solution was injected into the jugular vein, and the electrodes were placed upon a branch of the superior mesenteric artery, which, with the coil of intestine, was supported outside the body and protected from cooling by warm towels. The readings obtained by this method which was used in connection with some other experiments may for our purposes here be considered as an excess over the pulmonary time, but this excess is of no importance here, so it need not be discussed.

**Protocol XIII.** — May 4, 1906. Pulmonary circulation time. Small dog. 0.2 G. morphine. 1.5 G. chloretone. Salt solution (4 c.c., 20 per cent) into left jugular vein. Electrodes on branch of superior mesenteric artery. Adrenalin injected into saphenous vein.

Observation.	Time.	Circulation time.	Heart rate.	Blood pressure.
5	3.15	5 $\frac{2}{5}$ sec.	105	70 Hg mm. Control.
6	3.25	5 $\frac{1}{5}$ "	105	70 " "
7	3.27	5 $\frac{2}{5}$ "	105	75 " "
8	3.30	5 " .	130	110 " Adrenalin.
9	3.31	5 $\frac{1}{5}$ "	110	80 " "
10	3.33	5 $\frac{1}{5}$ "	135	110 " "
11	3.35	5 $\frac{1}{5}$ "	120	90 " "
12	3.37	5 $\frac{2}{5}$ "	115	90 " "
14	3.44	6 $\frac{1}{5}$ "	85	50 " Control.

As would be expected from the points discussed above under adrenalin and digitalis, we would have here to do with back pressure

<sup>1</sup> See footnote, p. 134.

and difficulty for the blood to reach the right side of the heart due to the contraction of both splanchnic and peripheral vessels, and yet the adrenalin causes a slight acceleration, putting the drug in line with others of the series. That this acceleration is not found in the rabbit is probably to be explained by the fact, pointed out before, that the peripheral resistance is increased so quickly that any increased efficiency of the heart is at once hidden by the great rise in blood pressure.

#### CONCLUSIONS.

The members of the digitalis series, then, produce an acceleration of the blood stream followed by a retardation which appears under larger doses of the drug. In the greater circulation the peripheral stream is accelerated, while in the portal area there may be a similar effect if these vessels are not strongly contracted. If there is a very great contraction here, as shown by a marked rise in blood pressure, the portal stream is probably retarded, although a certain amount of resistance may be overcome by the increased efficiency of the heart. In some animals the only demonstrable effect of adrenalin on the current is great slowing, probably due to the greatly increased blood pressure.

The acceleration of the blood stream which is produced by many of the series during the early stage of their action may possibly explain some of the benefits derived from their administration. The fact that the blood is flowing more rapidly through the tissues would indicate an improved blood supply, giving not only more nourishment, but also providing for more complete removal of waste products. This does not overlook the benefits derived from the characteristic action of the series upon the cardiac muscle itself.

# ON THE MECHANISM OF THE STIMULATING ACTION OF TENSION ON THE HEART.

By A. J. CARLSON.

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

## I.

IN the excised heart increased tension within limits acts as a stimulus to the heart rhythm, as shown by the fact that the rhythm is augmented in case the heart is active, and in case the heart is quiescent tension may start rhythmic activity. This is true both for the vertebrate and the invertebrate heart. The well-known experiments of Bernstein and of Porter seem to show that it is also true for isolated parts of the vertebrate ventricle, but this has recently been denied by Lingle for isolated strips from the tortoise ventricle. From the recent experiments of Magnus, Sollmann, and Guthrie and Pike,<sup>1</sup> it is evident that in the mammalian heart the important factor is the tension in the coronary vessels rather than the tension in the heart cavities. In the invertebrates hydrostatic pressure in the heart cavities is efficient; and, in fact, in the heart of some invertebrates the mere mechanical tension on the heart walls is almost as efficient stimulus as hydrostatic pressure in the heart cavities.<sup>2</sup>

In the intact heart this direct relation between tension and rhythm is obscured through the nervous control of the heart by the central nervous system.

The mechanism by which tension augments the rate and the intensity of the heart beat is largely a matter of conjecture. It is well known that within limits tension augments the amplitude of the contraction of skeletal muscle. Tension alone does not cause contraction, however. As it seems to me probable that tension has the same effect on heart muscle as on skeletal muscle, the increased amplitude of the ventricular beat following an increase of the hydro-

<sup>1</sup> For the literature see GUTHRIE and PIKE: This journal, 1907, xvii, p. 14.

<sup>2</sup> CARLSON: This journal, 1906, xvi, p. 62.

static pressure in the cavity of the ventricle is probably due to the direct influence of tension on the ventricular musculature. The mechanism by which tension augments the rate of the heart rhythm is less obvious. Sollmann suggests that the stimulating action of tension in the coronary vessels of the excised heart can be more readily explained on the neurogenic than on the myogenic theory of the heart rhythm. Because of the arrangement of the ventricular musculature it is obvious that pressure in the coronary vessels does not produce the degree and uniform distribution of the tension on the muscle cell that is affected by pressure in the cavity of the ventricle; nevertheless the latter is a less efficient stimulus to the rhythm than the former.

It is clear, from the experiments on the isolated mammalian heart referred to above, that tension in the coronary vessels augments the rate of the rhythm of the isolated ventricle. This in all probability never takes place in the intact heart, as such an action directly on the ventricles would disarrange the co-ordination of the heart. Whatever direct action tension in the coronary vessels may have in the regulating of the heart rate in the intact animal, must accordingly be exerted through region of the mouth of the great veins or at least through the auricles. There is anatomical basis for such a mechanism, at least in so far as branches of the coronary arteries pass to the mouths of the great veins as well as to the sinus venosus in the lower vertebrates.

If the myogenic theory of the heart beat is the true one, it seems highly probable that both the increased amplitude and the augmented rate is due to a direct action of the tension on the heart muscle. The only other alternative is that it is, in whole or part, due to a stimulating action of the tension on the augmentor nerves or nerve endings. I know of no evidence which even by analogy would render this alternative probable. There is no evidence, for example, that tension in the arterioles is able to directly stimulate the vasoconstrictor nerves or nerve endings. The possibility of the latter alternative cannot readily be eliminated, however, since we know of no drug or device by which the augmentor nerves may be thrown out of function without greatly injuring the heart tissues.

Assuming the correctness of the neurogenic theory, the mechanism of action of tension on the heart rhythm is probably as follows: The augmentation of the strength of the beat is, at least in part, due to a direct action of tension on the heart muscle; it may also be in part

due to the action of tension on the motor nervous tissue. The augmentation of the rate can only be due to the tension action on the motor nervous tissue. This action is on the automatic ganglion cells directly, or on afferent nerve and nerve endings in the heart walls; or both of these mechanisms may be involved. This working hypothesis has now been put to the experimental test in the only heart which, so far as we know, permits of such experiments, namely, the heart of *Limulus*. The results are in brief the following.

## II.

1. *The preparation of the heart.*—To demonstrate the different points in the hypothesis just stated the *Limulus* heart was prepared in four different ways: (1) The automatic ganglion was extirpated throughout the whole length of the heart. The heart minus the ganglion decides the question whether tension is able to start or augment the rhythm apart from the automatic ganglion by acting on the motor nerve plexus and the heart muscle. (2) The lateral nerves were isolated from the heart muscle in the second segment, the nerve cord extirpated in the first two segments, and the lumen of the heart obliterated in the second segment by compressing the heart walls with a stout thread in the manner shown in Fig. 1. The lateral nerves are, of course, not included within the thread. The anterior pair of lateral arteries may now be tied, and a cannula fixed in the anterior end of the heart by means of which the hydrostatic pressure within this isolated anterior end may be varied at will. This preparation decides the question whether tension increases the strength of the beat by a direct action on the heart muscle and the motor nerve plexus, and whether tension acts on the ganglion in an indirect way by stimulation of afferent nerves or nerve endings in the heart walls. (3) The nerve cord and the lateral nerves were isolated and the walls of the heart compressed in the second segment in the manner shown in Fig. 1. The anterior end is arranged for graphic registration. The three posterior pairs of lateral arteries are tied, and a cannula fixed into the posterior end by means of which the hydrostatic pressure in the posterior end of the heart can be varied at will. This pressure does not affect the anterior or recording end, except indirectly through the local nervous system of the posterior end. This preparation decides the question whether tension augments the intensity as well as the rate of the nervous impulses by acting directly on the

ganglion. (4) The ganglion was isolated from the heart muscle except in the two anterior segments. These two segments were arranged for graphic registration in the usual way. The action on the ganglion of hydrostatic pressure in the heart cavity was imitated by mechanical pressure and tension on the isolated ganglion directly. This prepara-

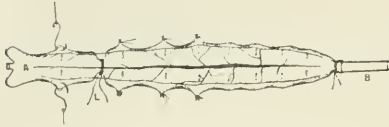


FIGURE 1. — Diagram of the preparation of the *Limulus* heart for determining the effect of tension on the heart ganglion apart from that on the heart muscle. Dorsal view. *A*, anterior or recording end of heart; *B*, cannulated in posterior end of heart for varying tension in the heart cavity. *L*, ligature compressing the muscle of the heart walls in the second segment, so as to confine the tension variations to the posterior end of the heart.

tion helps to decide the question whether the tension in the heart cavity that alters the rate acts directly or indirectly on the ganglion.

2. *After the automatic ganglion has been extirpated pressure in the heart cavity fails to inaugurate or maintain a rhythm.*—This fundamental fact has already been reported.<sup>1</sup> The experiment has been repeated many times and at varying periods after removal of the ganglion, but no exception has ever been noted. It is therefore evident that the ganglion is essential for the stimulating action of pressure in the heart cavity on the rhythm.

3. The action of tension on the heart, muscle and nerve plexus. *Moderate tension on the heart walls increases the amplitude of the contractions of the ganglion-free anterior end of the heart.* This is shown best, not by hydrostatic pressure in the heart, but by mechanical tension on the opposite sides of the heart. When the recording end of the heart is filled with plasma, under pressure, the bulging of the heart walls displaces the recording lever in such a way that a direct comparison of the amplitude of the beats cannot always be made. It is not difficult to demonstrate, however, that within limits the greater the load or tension of the recording lever the greater the amplitude of the beat. But it is also true that the greater load causes greater degree of relaxation of the recording end during diastole, so that the absolute height of excursion of the lever in case of the heavier load may not exceed or may even fall short of the height maintained with the lighter load, even though the relative amplitude of the beats is much greater in the former case. In some preparations the greater load produced an actual rise of the initial height of contraction despite the synchronous fall of the base line.

<sup>1</sup> CARLSON: This journal, 1905, xii, p. 487.

Mechanical tension or hydrostatic pressure confined to the anterior ganglion-free end of the heart which is connected with the automatic ganglion by the lateral nerves only, has no effect on the rate of the rhythm of either end of the heart. The stimulating effect manifests itself in

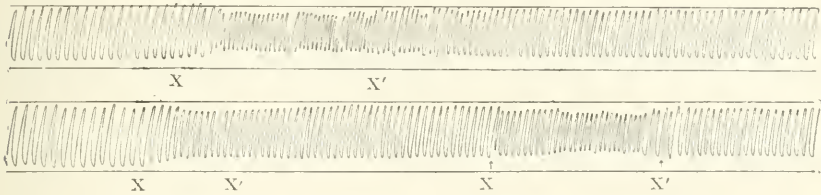


FIGURE 2.— One-half the original size. Tracings from the anterior end of the Limulus heart prepared as in Fig. 1. X, moderate increase of the hydrostatic pressure in the posterior end of the heart. X', the pressure reduced to zero, showing that the main effect of tension on the ganglion is an augmentation of the rate of the nervous discharges accompanied by slight tonus.

the strength of the beats alone, and is confined to the anterior end. The only way that tension thus applied could affect the rate would be in establishing an independent rhythm in the anterior end by acting directly on the heart muscle or the nerve plexus; or by acting indirectly on the ganglion in the posterior end by the stimulation of

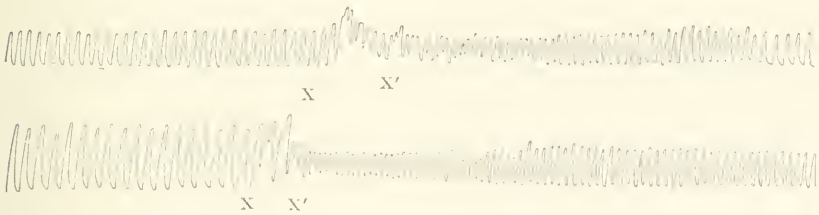


FIGURE 3.— Four-sevenths the original size. Tracings from the anterior end of the Limulus heart prepared as in Fig. 1. X, sudden and great increase of blood pressure in the heart cavity (posterior end). X', rapid diminution of the pressure to zero. Showing excessive tension and prolonged after effects after cessation of the tension.

afferent nerve fibres in the heart walls. As the fact that tension fails to cause contraction in the heart after extirpation of the ganglion precludes the former possibility, this limitation of the effect of the tension of the amplitude of the contraction in the part of the heart immediately acted on goes to show that *the tension on the heart walls does not act on the ganglion by the stimulation of afferent nerve fibres or nerve-endings in the heart wall.* I have shown in a previous paper that such afferent fibres run in the lateral nerves. They are appar-

ently not stimulated by the degree of tension that may be applied to the heart without rupturing the walls.

The augmentor effect of tension on the strength of the heart beat may be due either to a direct action in the muscle cells, or to increased excitability of the motor nerve fibres. Both factors are probably involved.

4. *When the pressure in the heart cavity is confined to the posterior end of the heart in the manner shown in Fig. 1, the most striking effect on the isolated anterior end is the augmentation of the rate of the rhythm.*—A slight increase in the intensity of the contraction is obtained with moderate tension, but when the pressure is considerable the augmentation in the rate is so great that the intensity factor becomes submerged. In fact, the amplitude of the beats is usually greatly diminished. With still greater pressure in the heart cavity the augmentation of the rhythm of the anterior end may approach incomplete tetanus and delirium or fibrillary contractions. This great tension obviously breaks down the co-ordination of the ganglion with the result of a typical delirium cordis.

A degree of tension that appreciably affects the rate of the rhythm produces also an increased tonus of the isolated anterior end, and, within limits, the greater the tension the greater the tonus contraction. But as this tonus is invariably associated with an increased rate of the beats, it may simply be failure of complete diastolic relaxation because of the rapid rhythm.

*The stimulating action of tension on the rate persists for a considerable time after the tension is reduced to zero.* The greater the tension the greater the persistence of the augmented rhythm after removal of the tension. In cases where the tension was increased to the point of producing delirium cordis and this degree of tension was kept up for five or ten minutes, the normal rhythm very seldom returned. Within ten or fifteen minutes after cessation of the tension the rhythm has usually returned to regularity, but it persists to be abnormally rapid and feeble till the final cessation. The stimulating action of the tension is on the ganglion. The persistence of the effect after cessation of the stimulus goes to show that the increased activity is not due to the mere mechanical pressure on the ganglion or the ganglion cells, as this pressure ceases with the withdrawal of the plasma from the heart cavity.

The posterior end of the heart is unable to contract to any extent against the pressure that by action on the ganglion sends the anterior



end into delirium cordis. But the feeble contractions suffice to bring out the fact that the delirium involves the posterior end of the heart also. This is necessarily the case since it is only an expression of the inco-ordinated activity of the ganglion. *The pressure in the posterior end of the heart may be raised to a point where no contractions appear, except in the anterior end, showing that the ganglion is still active, although greatly inco-ordinated.* Such excessive pressure soon stops all activity of the ganglion.

It is clear from these experiments that a certain degree of tension on the heart ganglion augments both the rate and intensity of the nervous discharges, the augmentation of the rate being the most prominent.

#### SUMMARY.

1. Tension, within limits, acting on the heart muscle and motor nerve plexus alone, augments the amplitude of the contractions, but does not affect the rate.

2. Tension does not inaugurate or maintain a rhythm in the heart after extirpation of the automatic ganglion.

3. Tension does not appear to act on the heart ganglion by stimulating afferent nerves or nerve endings in the heart walls.

4. Moderate tension acting on the heart ganglion alone increases slightly the intensity of the nervous discharges and greatly augments their rate. The tonus of the heart muscle appears also to be slightly increased. These effects may outlast the duration of the tension.

5. Excessive tension on the ganglion greatly accelerates the rate of the rhythm, increases tonus, and by destroying co-ordination in the ganglion sets up delirium cordis in the heart. If excessive tension on the ganglion is maintained for a few minutes, the ganglion can usually not be restored to its normal activity.

6. The ganglion may continue in inco-ordinated activity for a time under a degree of tension against which the heart muscle cannot contract.

## ON ABSORPTION FROM THE PERITONEAL CAVITY.

BY H. GIDEON WELLS AND LAFAYETTE B. MENDEL.

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

IN a study of the influence of adrenalin upon absorption from the peritoneal cavity, Alfred Exner<sup>1</sup> has drawn conclusions concerning the mechanism of absorption from the peritoneal cavity which are not only surprising in themselves, but also at variance with results obtained by other observers. Exner found that the appearance of toxic symptoms after the intraperitoneal injection of strychnine, physostigmine, and potassium cyanide was much delayed if a small quantity of adrenalin was injected into the peritoneal cavity a short time (nine to forty-five minutes) before the poisons were introduced. The absorption of sodium indigosulphate, as determined by the time of its appearance in the urine, was also somewhat delayed by adrenalin; but the absorption of potassium iodide did not seem to be similarly influenced. As an explanation for this failure to affect the absorption of potassium iodide, the idea was advanced that possibly this salt is absorbed from the peritoneal cavity by a different route than that taken by the substances whose absorption is delayed by adrenalin; and an attempt was made to study the influence of adrenalin upon the absorption of substances through the lymphatic channels. On the assumption that insoluble particles are absorbed from the peritoneal cavity by way of the lymphatics, four experiments were performed to ascertain the effect of adrenalin upon the absorption of an emulsion of paraffinum liquidum from the peritoneal cavity. These experiments were conducted in the following manner: The emulsion was injected into the peritoneal cavity of rabbits, and after a time the animals were killed by bleeding from the carotid. All the blood obtained (quantity apparently not determined) was shaken repeatedly with ether; the ethereal extract was evaporated, and the residue saponified with alcoholic potash. The product was

<sup>1</sup> EXNER: Zeitschrift für Heilkunde (Abtheilung Chirurgie), 1903, xxiv. p. 302.

shaken with ether, the latter driven off, and the extract weighed. This residue was considered to be a mixture of cholesterin and paraffin, chiefly the latter, since the cholesterin reaction was given slowly and less intensely than with a smaller quantity of pure cholesterin. The material examined was oily, turbid, cleared up at 70°, and when burned on platinum smelled of paraffin and left almost no residue.

Since in these four experiments Exner obtained a smaller amount of this residue from the blood of the two rabbits into which adrenalin had also been injected intraperitoneally than from the blood of their respective controls, the conclusion was drawn that adrenalin interferes with absorption from the peritoneal cavity through an interference with lymphatic absorption, it being assumed that the emulsionized paraffin is absorbed through the lymphatics and not into the blood vessels directly. Consequently, it is argued, substances which are absorbed by the lymphatics have their absorption delayed by previous injection of adrenalin; whereas, if the absorption of a substance from the peritoneum is not delayed by adrenalin, this fact indicates that it is not absorbed into the lymphatics, but directly into the blood stream.

As further support for the hypothesis that adrenalin interferes with absorption through the lymphatics is cited an experiment in which the mesentery and diaphragm of a guinea pig were stained with silver nitrate half an hour after intraperitoneal injection of adrenalin, and it seemed that the number of stomata was perhaps somewhat less than in normal animals, although the results were not at all decisive. It may be remarked here that histologists are by no means agreed as to the existence of these stomata.<sup>1</sup> More marked were the results obtained in two experiments on the absorption of bacteria from the peritoneal cavity. Adrenalin solution was injected into the peritoneal cavity of two rabbits, and *Protæus vulgaris* cultures were injected after an interval, in one experiment, of one hour; in the other, of eight minutes. Cultures were made from the blood at frequent intervals thereafter, and plated, and the number of colonies obtained was found to be much smaller than was the case with control animals; the results were more striking in the experiment in which the injection of adrenalin preceded the bacteria by an hour. The conclusion inferred is that adrenalin prevents the entrance of bacteria into the peritoneal lymphatics, and their subsequent appearance in the blood.

It must be admitted that Exner's idea that a highly soluble crystal-

<sup>1</sup> Cf. BUXTON and TORREV: *Journal of medical research*, 1906, xv, p. 53.

loid, such as potassium cyanide, is absorbed through the lymphatics, while another soluble crystalloid, potassium iodide, takes a different route and is absorbed through the blood vessels, is rather remarkable; and one is impelled to investigate the evidence upon which this opinion is based. A careful reading of the original paper leaves us convinced of the inadequacy of the proof offered.<sup>1</sup>

The chief support advanced by Exner for the belief that lymphatic absorption is specifically impaired by adrenalin (upon which is based the view of the difference in the routes of absorption of potassium iodide and the other substances tested) is furnished by two experiments on the absorption of an emulsion of liquid paraffin from the peritoneal cavity. The results of these two experiments, performed on four rabbits, were as follows:

*Experiment 1.*—Animal No. 44. No adrenalin given. Paraffin emulsion injected into the normal peritoneal cavity, and blood withdrawn from the carotid forty-five minutes later. Obtained 0.0138 gm. of the supposed mixture of paraffin and cholesterin, as previously described.

Animal No. 45. An injection of adrenalin preceded the emulsion injection by ten minutes. Blood withdrawn after forty-five minutes contained 0.0052 gm. of the "mixture."

*Experiment 2.*—Animal No. 46. No adrenalin injected. Blood withdrawn fifteen minutes after injection of emulsion contained 0.0904 gm. of the mixture.

Animal No. 47. Adrenalin injection preceded the emulsion by six minutes. Blood withdrawn after fifteen minutes contained 0.0216 gm. of the mixture.

To us these results do not seem of sufficient significance to afford a basis for an hypothesis as to the routes of absorption of substances from the peritoneal cavity. Even if we were to grant that the mixture obtained from the ethereal extract of the blood consisted largely of paraffin (which we are by no means convinced was the case) the results obtained do not prove that adrenalin interferes with absorption of paraffin emulsion through the lymphatics. First, it may be pointed out that the blood from one of the animals which received no

<sup>1</sup> MELTZER and AUER (Transactions of the Association of American Physicians, 1904) have also questioned the validity of some of EXNER'S conclusions, — not, however, the factors which we have subjected to an experimental critique. Cf. also FREYTAG: Archiv für experimentelle Pathologie und Pharmakologie, 1906, lv, p. 306.

adrenalin (No. 46) yielded, fifteen minutes after the injection of the emulsion, 0.090 gm. of "paraffin mixture," whereas in the other control rabbit (No. 44) the blood withdrawn forty-five minutes after injection of the emulsion yielded only 0.013 gm., — less than one-sixth as much. Are we to believe that the absorption of such a substance as emulsified liquid paraffin from the peritoneum is so rapid that it is at its height in fifteen minutes, and that after forty-five minutes most of the paraffin has been taken out of the blood and stored up in the organs? Our own experience does not indicate any such rapidity of absorption. The results obtained in the two sets of experiments cited above vary so much among themselves that any conclusions drawn from them seem entirely gratuitous. For example, the blood of the two rabbits which had not received adrenalin yielded 0.013 gm. (No. 44) and 0.090 (No. 46) of the "mixture," while the two animals injected with adrenalin yielded 0.005 (No. 45) and 0.021 gm. (No. 47). If, instead of comparing control animal Number 44 with adrenalin animal Number 45, we compare it with the other adrenalin animal, Number 47, we could then state that whereas the blood of a normal animal injected intraperitoneally with liquid paraffin contained but 0.0138 gm. of paraffin mixture after forty-five minutes, a similar animal which had received a previous intraperitoneal injection of adrenalin yielded 0.0216 gm. after but fifteen minutes. From this we might conclude that adrenalin injection *hastens* the rate of absorption of emulsified particles very greatly, with just as much propriety as Exner concludes the opposite. To us, therefore, these experiments seem to have yielded too inco-ordinate results to be of any significance whatever.

Secondly, the chemical evidence of the absorption of liquid paraffin and its appearance in the blood is not so conclusive as might be desired, and it is particularly faulty as to quantitative details. In two experiments performed by us, and cited below, we were unable to obtain any indication that a mineral oil emulsion was absorbed from the peritoneal cavity of dogs during three and one-half and five hours, respectively, in sufficient quantities to be detected in either the blood or lymph.

Thirdly, the statement that the paraffin emulsion is absorbed from the peritoneal cavity by way of the lymphatics rather than through the blood vessels, is purely an assumption, however probable it may be. In the two experiments performed by us we were unable to demonstrate the presence of absorbed oil in the entire lymph flowing

from the thoracic duct of large dogs during three and one-half and five hours, nor could we find this material in the blood of the same animals. On the other hand, when the animals were killed, the emulsion was found still present in the peritoneal cavity, not apparently reduced in amount. Soluble indigo carmine injected with the emulsion, however, had largely disappeared from the peritoneal cavity, and was found early and in large quantities in the blood and urine, and later also in the lymph.<sup>1</sup>

Furthermore, the experiment which Exner considers as indicating that adrenalin prevents the absorption of bacteria, supposedly by preventing their entrance into the lymphatics, is open to an entirely different and much more likely explanation. It is a well-known fact, long accepted in studies of the problems of immunity, that the injection of a mildly irritating substance into the peritoneal cavity of an animal greatly increases its resistance to bacteria injected a short time later. This fact has been repeatedly demonstrated with many sorts of bacteria and many types of irritants; and the explanation which seems to have been indisputably established is that the increased resistance of the animal is due to the local leucocytosis caused by the injected substance, which greatly favors prompt phagocytosis of the bacteria introduced. If instead of adrenalin the experimenter had injected bouillon, or urine, or tuberculin, or any one of a large number of other substances, before injecting his cultures of *Protus*, the same diminution in the number of bacteria in the blood would probably have been observed. Indeed, it is possible that part, at least, of the decreased effect of the poisons injected intraperitoneally after a preliminary injection of adrenalin, depends upon this local leucocytosis, since it has been shown that leucocytes have the power to take up soluble poisons as well as bacteria, and to decrease and delay their effects.

We therefore question the evidence advanced by Exner to support the hypothesis that such physically similar substances as potassium iodide and potassium cyanide are absorbed from the peritoneum by entirely different routes, and the consequent hypothesis that adrenalin injected into the peritoneal cavity interferes with absorption by the lymphatics, while not affecting absorption by the vessels. On the other hand, we have the evidence brought forward by Starling and

<sup>1</sup> The relatively greater participation of blood vessels in the absorption of indigo carmine from the peritoneal cavity has been demonstrated by MENDEL: *American journal of physiology*, 1899, ii, p. 342.

Tubby<sup>1</sup> and by Mendel,<sup>2</sup> that various substances injected into the peritoneal cavity are absorbed into the blood vessels, whereas Exner concludes that strychnine, physostigmine, potassium cyanide, sodium indigosulphate, and presumably many other substances, are absorbed rather by the lymphatics than into the blood vessels directly.

The protocols of our own experiments follow :

**Protocol I.** — A dog, weighing 18.5 kilos, which had not been fed since the previous day, was given morphine, and when drowsy anæsthetized with A. C. E. mixture. A cannula was placed in the thoracic duct, and as soon as the lymph flow had become steady (11.50 A. M.) 100 c.c. of an emulsion containing 25 per cent of mineral oil, held in fine suspension with gum acacia, and 25 c.c. of a solution of indigo carmine, were introduced into the peritoneal cavity from a blunt pipette. The lymph flow was carefully watched. It remained steady at the rate of about 2.0 to 2.8 c.c. per ten-minute periods. At 12.35 P. M. a sample of blood was withdrawn from the femoral artery, poured into strong alcohol, filtered, and the filtrate evaporated until sufficiently concentrated to show the greenish color of the absorbed indigo carmine. No greenish tint was noted in the lymph flowing from the thoracic duct until 12.55, and this color never became more pronounced than a trace. At no time did the lymph show any increase in turbidity to indicate the presence of absorbed particles of the paraffin emulsion.

At 3.20 P. M., three and one-half hours after the injection of the emulsion, 350 c.c. of blood were withdrawn for analysis, and the dog was killed. Autopsy showed the emulsion still present in the peritoneal cavity, but diluted to fully 300 c.c., whereas the indigo carmine color had almost entirely disappeared. The serosa was reddened and showed evidences of inflammation. The urine in the dog's bladder was colored deep green from excreted indigo carmine.

The 350 c.c. of blood and 23 c.c. of the lymph collected after injection of the emulsion were both examined for the presence of the mineral oil, according to the following method: The material was mixed with three volumes of clean sand, and dried down over a water bath. The dried mixture was ground well in a mortar, and extracted thoroughly with ether (in the Soxhlet apparatus in the case of the lymph; in a large flask with much shaking and frequent changes of ether in the case of the blood). The ethereal extract was in each case evaporated to dryness, the residue taken up with fresh ether, and saponified with sodium alcoholate, the mixture being allowed to stand over night to insure completion of the

<sup>1</sup> STARLING and TUBBY: *Journal of physiology*, 1894, xvi, p. 140.

<sup>2</sup> MENDEL: *American journal of physiology*, 1899, ii, p. 342.

saponification.<sup>1</sup> It was then added to three volumes of salt and dried, first at 35°, then 80°, then 100°. When thoroughly dried, this salt mixture, containing soaps, cholesterin, and any mineral oil that might be present, was extracted eight hours with ether in the Soxhlet apparatus. The use of salt, a procedure advocated by Ritter,<sup>2</sup> prevents the possible absorption of some of the soaps by traces of water in the ether. From the ethereal extract was obtained, on slow evaporation, a small amount of yellowish white, crystalline material, which gave Salkowski's reaction for cholesterin very distinctly. From the lymph only a minute amount of this material was obtained, just sufficient to afford the cholesterin test. From the blood the residue obtained on evaporating off the ether seemed to contain some sodium alcoholate. This was removed by shaking out with water and ether together. After evaporation of this ethereal extract, abundant crystals were obtained with typical cholesterin form, which gave the Salkowski reaction distinctly, and melted at 135°. After recrystallization from hot alcohol typical cholesterin plates with a melting point of 142° (uncorr.) were obtained. No oily material of any kind was isolated, either from the blood or lymph.

**Protocol II.** — A dog, weighing 20 kilos, was prepared as in the preceding experiment. At 11.22 A. M., 50 c.c. of a 25 per cent emulsion of mineral oil and 25 c.c. of indigo carmine solution were injected into the peritoneal cavity. In this dog the rate of lymph flow was much faster than in the first animal, being equal to 8.8 c.c. per ten minutes before the injection of the emulsion. At 11.50 the lymph began to show the indigo carmine color. The first blood sample examined was not withdrawn until after this time (12.18 P. M.) and showed a distinct indigo carmine coloration. In this experiment the lymph became much more deeply colored than in the previous one, possibly because of a hemorrhagic inflammation of the peritoneum found at autopsy, which also caused the lymph to become bloody after 2.15 P. M. The rate of flow continued at from 8 to 9 c.c. per ten minute period. At 4.22 P. M., five hours after the emulsion was introduced, the animal was bled to death. Autopsy showed approximately 200 c.c. of fluid in the peritoneal cavity, which contained the oil emulsion, apparently unabsorbed, although the indigo carmine had nearly all disappeared. There was a great deal of ecchymotic infiltration of the peritoneum, which probably caused the blood-stained appearance of the lymph.

330 c.c. of the blood drawn at the time of killing the animal, and all the lymph (236 c.c.) collected after the injection of the emulsion, were

<sup>1</sup> See KOSSEL and OBERMÜLLER: *Zeitschrift für physiologische Chemie*, 1890, xiv, p. 599; and KOSSEL and KRÜGER: *Ibid.*, 1891, xv, p. 321.

<sup>2</sup> RITTER: *Zeitschrift für physiologische Chemie*, 1902, xxxiv, p. 430.



dried down upon sand, and treated as previously described. In neither specimen was any evidence of the presence of absorbed mineral oil obtained. About 100 c.c. of blood was extracted with ether and treated after the method followed by Exner, in which saponification is accomplished by alcoholic potash, to see if an oily product similar to that described by him could be obtained. The residue obtained on slowly evaporating the ethereal extract was crystalline, reacted for cholesterin, but melted at  $100^{\circ}$ , while the material obtained by the Kossel and Ritter methods melted at from  $115^{\circ}$  to  $120^{\circ}$ , as first obtained, without further purification. Recrystallization from hot alcohol gave nearly pure cholesterin from all three products.

From these two experiments we find no evidence that a finely divided suspension of mineral oil is absorbed from the peritoneal cavity of the dog, in the course of three to five hours, in sufficient quantities to be detected in the systemic blood or in the lymph flowing from the thoracic duct.

# THE EFFECT OF CARBOHYDRATES ON RESISTANCE TO LACK OF OXYGEN.

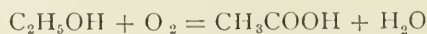
By WALES H. PACKARD.

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## I. INTRODUCTION.

IN a theory of the nature of protoplasmic respiration Mathews<sup>1</sup> has recently brought forward the hypothesis that respiration is the dissociation of water with the liberation of hydrogen. In the protoplasm there is some substance (or substances) of unknown nature which splits off water from itself and sets free active particles. These active particles attack the water of the protoplasm, decomposing it into oxygen and hydrogen. The oxygen combines with the substances of the protoplasm, thus oxidizing them; the hydrogen is either united with atmospheric oxygen to form water (aerobic respiration), or is set free in gaseous form, or it combines with other substances in the protoplasm (anaerobic respiration). According to this hypothesis the atmospheric oxygen acts the part only of a depolarizer to take care of the nascent hydrogen formed from the water, and any other substance which unites readily with nascent hydrogen can replace atmospheric oxygen and permit respiration to go on in the absence of air. Maze<sup>2</sup> found that acetic acid could be formed by certain bacteria from alcohol in the absence of air if lævulose were present; the lævulose at the same time being converted into mannite.

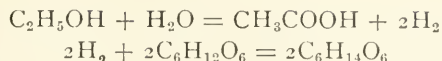
In the presence of air alcohol is oxidized to acetic acid as follows:



In the absence of air and in the presence of lævulose the oxygen is derived from the water, and the hydrogen thus set free joins itself to the lævulose and forms mannite.

<sup>1</sup> MATHEWS: Biological bulletin, 1905, viii, p. 331.

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



In this case the lævulose acts as a depolarizer by uniting with the nascent hydrogen and permits oxidation to go on in the absence of air.

If this principle is capable of a wide application, it ought to be possible by the injection of certain substances into the blood of animals which will thus act as depolarizers, to enable the tissues to carry on respiration in the absence of atmospheric oxygen, or, in other words, to greatly increase their power of resistance to a lack of oxygen.

In a previous paper<sup>1</sup> the author presented a series of experiments which showed that the power of resistance of the common Minnow (*Fundulus heteroclitus*) to lack of oxygen may be increased by increasing the alkalinity of the blood by the injection of sodium bicarbonate.

In the same paper was also reported the attempt to increase their power of resistance by the injection of lævulose, but without apparent success. It was, however, stated that the experiments were necessarily brought to a close before the author was satisfied in regard to this, and that further experiments would be taken up another summer. It was further suggested that perhaps enough time had not been allowed for the absorption of the lævulose from the body cavity into the blood stream. The experiments were repeated with the changes indicated during the past summer and were further extended to include other carbohydrates than lævulose.

## II. EFFECT OF THE INJECTION OF CARBOHYDRATES ON RESISTANCE TO LACK OF OXYGEN.

The experiments were performed in a manner similar to those described in the previous paper. The animals were first assorted according to size and sex, and in any one experiment only animals of the same sex and approximately the same size were used. This was afterwards proved to be an entirely unnecessary precaution, as experiments showed that there was no constant difference in resistance to lack of oxygen in males and females or in large and small animals. The fish were injected in the body cavity with from five to eight drops of a 0.5 molecular solution of the carbohydrate whose effect was to be tested. The above solution is approximately isotonic with the blood

<sup>1</sup> PACKARD: This journal, 1905, xv, p. 30.

of marine teleosts,<sup>1</sup> and was used in that strength to avoid osmotic effects with the blood.

For an experiment ten of the injected fish were placed in an aquarium jar with running water along with ten others properly marked to serve as controls. They were then left in the running water for about twenty-four hours to allow ample time for the absorption of the carbohydrate which previous experiments had shown to be quite slow. The effect was not shown if less than eighteen hours' time was allowed for absorption, nor did the effect seem to last beyond thirty-six hours after injection. The fish (10 injected and 10 controls) were then placed in a litre flask which was immediately filled with sea water and tightly stoppered so that all air was excluded. Under these conditions the animals quickly exhausted the available supply of air and were thus under conditions of lack of oxygen. In the previous paper<sup>2</sup> it was shown that this method was as effective as when elaborate methods were used to remove the oxygen from the water, and also that we are here dealing with phenomena of lack of oxygen and not with the effects of the accumulation of carbon dioxide. The length of time the animals were left in the flask is given in the tables in connection with each experiment. It was usually until all movements of the animals had ceased. The animals were then removed to fresh running sea water and left several hours for reviving. Enough time was allowed for this so that all animals not actually killed in the experiment were given a sufficient chance to recover.

Table I shows the results of the experiments with the injection of lævulose.

It will be seen from the summary that out of 194 live individuals 135, or 70 per cent, were those which had been injected; while only 59, or 30 per cent, were controls. Of the 166 dead, only 45, or 27 per cent, had been injected, while 121, or 73 per cent, were the controls.

The very great individual variation in resistance to lack of oxygen in different individuals renders it necessary to use many experiments and large numbers of individuals. This individual variation also explains why some (27 per cent) of the injected animals were killed by the lack of oxygen and a similar number (30 per cent) of the controls were left alive.

In the table all the experiments which were made are given, and an examination of each will show that while the increase in resistance

<sup>1</sup> GARREY: Biological bulletin, 1905, viii, p. 257.

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

conferred by the lævulose was in some cases not very marked, yet in every experiment the effect was shown, for in each case a greater number of the injected animals than of the controls were left alive, and a greater number of the controls than of the injected were dead.

TABLE I.  
FUNDULUS INJECTED WITH 0.5 MOL. LÆVULOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 22 50	hrs. min. 2	9	1	7	3
22 40	3 30	7	3	2	8
23 40	3 30	2	8	0	10
23 20	3 35	4	6	2	8
22 30	3	10	0	4	6
21 20	3	9	1	0	10
21 35	3	9	1	4	6
22 20	3	7	3	4	6
17 10	3	6	4	1	9
16 25	3	10	0	7	3
15 55	3	10	0	6	4
18 35	3	6	4	0	10
35 50	3	10	0	4	6
35 40	3	7	3	4	6
26 10	3 15	4	6	1	9
23 30	3	9	1	5	5
20 35	3 15	8	2	4	6
19 55	3 15	8	2	4	6
Total . . . . .		135	45	59	121

SUMMARY.

Alive, 194 . . . . . Injected 135 (70 per cent). Controls 59 (30 per cent).  
 Dead, 166 . . . . . Injected 45 (27 per cent). Controls 121 (73 per cent).

Table II shows the effect of the injection of glucose. The results are almost identical with those with lævulose. 71 per cent of the injected and only 29 per cent of the controls were alive, while only 27 per cent of the injected were dead as contrasted with 73 per cent controls.

TABLE II.  
FUNDULUS INJECTED WITH 0.5 MOL. GLUCOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 25 30	hrs. min. 3 15	5	5	3	7
24 15	3 15	8	2	2	8
24 10	3 15	8	2	5	5
24 05	3 15	6	4	2	8
23 15	3	8	2	5	5
23 40	3 15	8	2	3	7
23 20	3 30	9	1	3	7
23 30	3 30	7	3	2	8
23 45	3	6	4	3	7
20 05	3 15	8	2	3	7
20 15	3 15	6	4	3	7
22 00	2 30	9	1	3	7
Total . . . . .		88	32	37	83

SUMMARY.

Alive, 125 . . . . . Injected 88 (71 per cent). Controls 37 (29 per cent).  
Dead, 115 . . . . . Injected 32 (27 per cent). Controls 83 (73 per cent).

Maze<sup>1</sup> was unable to obtain any results with glucose such as he obtained with lævulose. If glucose were used instead of lævulose, he found no production of mannite, and hence but a trifling oxidation of alcohol into acetic acid. Perhaps plant respiration may be slightly different in this respect from animal respiration.

Table III gives the results of the injection with maltose. The

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

effect of maltose is even greater than either of the monosaccharides used. Of those left alive 78 per cent were injected and 22 per cent were controls, while of the dead only 23 per cent were injected and 77 per cent were controls.

TABLE III.  
FUNDULUS INJECTED WITH 0.5 MOL. MALTOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 20 45	hrs. min. 3 15	10	0	4	6
21 00	3 15	6	4	2	8
21 25	3 00	9	1	4	6
21 15	3 00	9	1	3	7
22 45	3 30	7	3	1	9
23 00	3 30	4	6	2	8
24 00	3 30	7	3	1	9
24 15	3 30	8	2	1	9
22 30	2 30	8	2	2	8
Total . . . . .		68	22	20	70

SUMMARY.

Alive, 88 . . . . . Injected 68 (78 per cent). Controls 20 (22 per cent).  
 Dead, 92 . . . . . Injected 22 (23 per cent). Controls 70 (77 per cent).

Tables IV and V give the results of the injection with two other common disaccharides — cane sugar and lactose. It will be seen from the summary of each that approximately an equal number of controls and injected were alive. The difference in percentage between injected individuals and controls both left alive and dead is well within the limits of individual variation. Another fact is to be noted. The average length of time the animals injected with lactose were left in the flask is about the same as that in the experiments with lævulose, glucose, or maltose, and yet a much larger proportion of the animals died. The same is even more strikingly shown in the experiments with cane sugar, where the average length of time in the flask is much less than that in the other experiments, and still a much

larger proportion of the animals died. This fact adds evidence to the conclusion to be drawn from Tables IV and V, that the injection of cane sugar and lactose does not confer any increased power of resistance to lack of oxygen. The animals left alive were those

TABLE IV.

FUNDULUS INJECTED WITH 0.5 MOL. CANE SUGAR.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 27 10	hrs. min. 2 45	5	5	4	6
27 30	2 45	0	10	1	9
27 15	2 45	5	5	5	5
27 30	2 45	4	6	4	6
24 45	2 30	5	5	2	8
24 30	2 30	3	7	4	6
24 15	2 30	3	7	1	9
24 15	2 30	3	7	1	9
22 30	2 00	9	1	5	5
22 30	2 00	6	4	7	3
22 30	2 00	5	5	7	2
Total . . . . .		48	62	42	68

## SUMMARY.

Alive, 90 . . . . . Injected 48 (53 per cent). Controls 42 (47 per cent).  
 Dead, 130 . . . . . Injected 62 (47 per cent). Controls 68 (53 per cent).

whose individual variation in resistance was great enough to enable them to live that length of time in lack of oxygen.

It is commonly accepted that carbohydrates cannot be absorbed and assimilated until they have been converted into monosaccharides.

If polysaccharides or disaccharides are introduced into the circulation they are immediately eliminated in the urine unless the blood contains the necessary enzymes to convert them into the simpler sugars.



Dastre, Voit, Blumenthal, and others have studied the assimilation of carbohydrates when introduced into the body with the avoidance of the alimentary tract.

TABLE V.  
FUNDULUS INJECTED WITH 0.5 MOL. LACTOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 20 00	hrs. min. 3 15	4	6	1	9
20 00	3 15	3	7	5	5
22 45	3 15	2	8	3	7
23 00	3 15	0	10	2	8
24 15	2 45	1	9	2	8
24 30	2 45	3	7	3	7
21 00	3 15	2	8	0	10
21 00	3 15	1	9	1	9
22 10	3 15	3	7	4	6
22 15	3 15	4	6	4	6
23 15	3 15	1	9	1	9
22 55	2 15	2	8	2	8
23 10	2 15	2	8	3	7
Total . . . . .		28	102	31	99

SUMMARY.

Alive, 59 . . . . . Injected, 28 (48 per cent). Controls, 31 (52 per cent).  
Dead, 201 . . . . . Injected, 102 (51 per cent). Controls, 99 (49 per cent).

Dastre and Bourquelat<sup>1</sup> found that maltose when introduced subcutaneously and intravenously was absorbed nearly as well as dextrose and much better than cane sugar, nearly all of the cane sugar being recovered in the urine.

Dastre<sup>2</sup> later reported that lactose was scarcely absorbed at all.

<sup>1</sup> DASTRE and BOURQUELAT: *Comptes rendus*, 1884, *xcviii*, p. 1604.

<sup>2</sup> DASTRE: *Archives de physiologie*, 1889, p. 718; 1890, p. 103.

Voit,<sup>1</sup> in some studies on the excretion of various carbohydrates by the kidneys in man after subcutaneous injection, gives the same results. Practically no dextrose, lævulose, or maltose was recovered in the urine, while cane sugar and lactose were thrown out in almost the same quantities in which they were injected.

Our experiments plainly coincide with the above results. Dextrose, lævulose, and maltose were absorbed and produced an increased resistance to lack of oxygen; cane sugar and lactose were not absorbed, and hence produced no effect. The blood of *Fundulus* evidently contains a maltase, but no invertase or lactase.

### III. EFFECT OF FEEDING WITH PROTEID FOOD ON RESISTANCE TO LACK OF OXYGEN.

A possible objection to the conclusions drawn from our experiments with carbohydrates may be this; the effect of the injection of carbohydrates in increasing resistance to lack of oxygen is not due to the rôle they may play in respiration, but is merely the effect which any food would have in increasing general bodily strength and resistance to adverse conditions, especially when compared with animals used as controls which had been kept in an aquarium for some days, and hence in a condition of partial starvation.

In order to test this objection the following experiments were carried out. The *Fundulus* were kept in an aquarium without food for several days. For experimentation a series of animals were then placed in aquarium jars and fed with common mussels (*Mytilus*) for a period of about twenty-four hours. As they were in a partly starved condition, they were all seen to eat greedily, and they were kept supplied with food during the whole period. This diet of course consists very largely of proteids, with only a very small amount of carbohydrate material. Another series of animals were kept under similar conditions, but without food, for controls.

The fed animals and the controls were then placed in flasks, as in the other experiments, to test their resistance to lack of oxygen.

Table VI gives the results. From the summary it will be seen that approximately an equal percentage of the fed animals and the controls were left alive. These experiments prove that feeding with proteid food does not increase the power of resistance to lack of oxygen, and

<sup>1</sup> VOIT: Münchener medicinische Wochenschrift, 1896. p. 717; Deutsches Archiv für klinische Medicin, 1897, lviii, p. 521.

hence answer the objection that carbohydrates act by merely increasing bodily strength.

The general conclusion to be drawn from all the experiments is that those carbohydrates which can be absorbed by the animal increase its

TABLE VI.  
FUNDULUS FED WITH MUSSELS.

Time between feeding and placing in flask.	Time in flask.	Fed.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 21 00	hrs. min. 2 30	5	5	7	3
		5	5	5	5
19 50	2 45	4	6	5	5
		6	4	7	3
		7	3	7	3
19 15	2 45	4	6	5	5
19 15	2 45	3	7	4	6
20 00	2 45	4	6	4	6
		6	4	3	7
19 15	2 30	8	2	5	5
21 00	3 15	5	5	3	7
24 00	2 45	5	5	7	3
24 00	2 45	6	4	7	3
22 00	2 45	5	5	4	6
22 00	2 45	3	7	5	5
22 00	3 00	4	6	4	6
22 30	3 00	4	6	7	3
22 30	3 00	3	7	8	2
Total . . . . .		87	93	97	83

SUMMARY.

Alive, 184 . . . . . Fed 87 (48 per cent). Controls 97 (52 per cent).  
 Dead, 176 . . . . . Fed 93 (52 per cent). Controls 83 (48 per cent).

resistance to lack of oxygen. The experiments also support Mathews' theory of protoplasmic respiration, that any substances which can readily unite with nascent hydrogen, and thus act as depolarizers, can replace atmospheric oxygen and permit respiration to go on in the absence of air.

Howell<sup>1</sup> has observed that cane sugar and dextrose will produce recovery of contractions after the so-called "sodium chloride arrest" in strips of heart muscle. He says: "After a heart strip has given its typical series of contractions in NaCl and the stage of the so-called exhaustion is reached, immersion in isotonic solutions of sugar gives usually a more or less definite series of contractions lasting for about an hour." The immersion of a fresh strip of heart muscle in the solutions of sugar gave no contractions whatever.

Lingle<sup>2</sup> believes that the "sodium chloride arrest is probably due to a lack of oxygen in the salt solutions," and finds that hydrogen peroxide and oxygen gas will produce recovery of contractions after sodium chloride exhaustion.

Martin<sup>3</sup> also mentions the fact of the recovery of muscle strips after sodium chloride arrest by the removal of the strip to an isotonic cane sugar solution, and also shows that the sugar solution has no effect on fresh muscle. Its effect is produced only after previous exhaustion.

In a more recent paper Martin<sup>4</sup> has carefully studied the influence of oxygen upon the activity of heart muscle strips. He finds that under conditions of deficiency of oxygen or in the presence of a moderate oxygen supply the sodium chloride arrest is probably chiefly due to lack of oxygen, and that "after exhaustion in an oxygen-free bath an excellent and long-continued recovery could be obtained by a thorough oxygenation of the solution or by transferring the strip to a moist chamber."

The above facts may be explained on the basis of Mathews' theory of respiration and in accordance with the experiments described in this paper. The sodium chloride arrest is acknowledged to be due in part at least to lack of oxygen, and the action of sugar solutions in the recovery from the exhaustion is simply their effect in acting as depolarizers and enabling the muscle to carry on respiration for a

<sup>1</sup> HOWELL: This journal, 1901, vi, p. 181.

<sup>2</sup> LINGLE: *Ibid.*, 1902, viii, p. 75.

<sup>3</sup> MARTIN: *Ibid.*, 1904, xi, p. 103.

<sup>4</sup> MARTIN: *Ibid.*, 1906, xv, p. 303.

time in the absence of oxygen. Placing the exhausted muscle in the sugar solutions has the same effect as placing them in a moist chamber in contact with the air or in an oxygenated solution. This explanation that carbohydrates act as depolarizers also indicates that the rôle of carbohydrates as the source of energy in muscular contraction may be somewhat different from that ordinarily accepted.

Among the plants, Diakonow<sup>1</sup> has found that "*Penicillium* and *Aspergillus* are killed or severely injured by the withdrawal of oxygen for a single hour, but both live a little longer when provided with sugar," which fact is also easily explained on the basis of the above theory.

#### IV. RESISTANCE TO LACK OF OXYGEN IN FUNDULUS EMBRYOS.

It is a commonly accepted fact that the embryo has a greater power of resistance in general than the fully developed animal. Little is known, however, as to whether this power of resistance decreases steadily with the progress of development of the embryo, or whether there are sudden changes in resistance in the transition from one stage of development to another. Loeb<sup>2</sup> has studied the relative sensitiveness of *Fundulus* embryos in various stages of development to lack of oxygen. He finds that the embryo is more sensitive to lack of oxygen the older it is, and that the sensitiveness increases more rapidly at first than later. Eggs just fertilized would continue their development after they had been in an oxygen vacuum for four days. Twenty-four hours after fertilization they would resist a lack of oxygen only forty-eight hours, while two-day-old and three-day-old embryos lost their power of development after thirty-two hours and twenty-two hours respectively. Young fish just hatched were still less resistant.

As it has been found that the average resistance to lack of oxygen in the adult *Fundulus* is about three and a half hours,<sup>3</sup> it seemed advisable to determine whether the decrease in power of resistance from twenty-four hours in the three-day-old embryo to three and a half hours in the adult was a constant decrease or not, and to determine if possible some of the causes of the decrease.

<sup>1</sup> DIAKONOW: The original papers were not available. Cited from PFEFFER'S *Physiology of plants*, translated by A. J. EWART, i, p. 536.

<sup>2</sup> LOEB: *Archiv für die gesammte Physiologie*, 1894, lv. p. 530.

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

The results given in the following experiments show that the decrease in power of resistance to lack of oxygen is not continuous but exhibits sudden changes. The resistance of an embryo about ready to hatch (*i. e.*, twelve to fifteen days after fertilization) is practically the same as that of a three-day-old embryo. There is a sudden decrease in resistance of nearly one-half at the time of hatching and

TABLE VII.  
LENGTH OF LIFE OF FUNDULUS EMBRYOS IN LACK OF OXYGEN.

Age of embryos.	Minimum.	Maximum.	Average of all experiments.
3-15 days after fertilization	hrs. min. 12 30	hrs. min. 17 30	hrs. min. 16 33
Just hatched . . . . .	7 45	9 30	8 15
Removed from egg membrane 12-16 days after fertilization. } 12-24 hours after hatching	7 15	11 00	8 52
36-48 hours after hatching	3 45	7 30	5 02
4 days after hatching . .	2 45	4 30	3 25
7 days after hatching . .	2 15	2 45	2 30
	50	2 10	1 14

following that a rather steady decrease until about forty-eight hours after hatching, when the power of resistance is no greater than that of the adult. This sudden increase in sensitiveness to lack of oxygen seems to be connected with the loss of the perivitelline fluid at the time of hatching, and the absorption of the yolk sac which takes place during the first thirty-six hours after hatching.

In these experiments the oxygen was removed by hydrogen gas, which was prepared in the usual manner in a Kipp apparatus. The gas was thoroughly washed through sodium hydrate solution, alkaline potassium permanganate solution, and distilled water. For observation the embryos were placed in paraffin cells in an Englemann gas chamber. As the circulation in the *Fundulus* embryo is already well established by the third day, the cessation of the heart contractions was in every case taken as the deathpoint. Table VII gives the results of these experiments.

No constant change in resistance to lack of oxygen could be detected in the embryos from the third day up to the time of hatching. All the differences shown in the different experiments were well

within the limits of individual variation in resistance. Loeb's observations showed that from after fertilization to the third day there was a constant decrease in power of resistance; but it seems that from this point on, after the circulation is well established in the embryo, there is practically no decrease. The general average of resistance to lack of oxygen during this period is given as about sixteen and a half hours.

At the time of hatching, however, a sudden change occurs. The resistance of the embryos just hatched is not more than half that of the embryo within the membrane. In many experiments with embryos twelve to sixteen days old and still within the egg membrane, the stimulus of the withdrawal of the oxygen was sufficient to cause a number of the embryos to break out of the egg membrane, and thus there were in the gas chamber both embryos still within the egg membrane and those just hatched out. In every case the resistance of those embryos just hatched was approximately one-half that of the embryos of the same age, but still within the egg membrane. The general average of resistance of such embryos just hatched is given in the table as eight hours and fifteen minutes.

It was also found that embryos from twelve to sixteen days old could be artificially hatched by pricking and tearing the egg membrane apart. The embryos thus removed from the membrane had approximately the same average of resistance as the embryos hatched under more normal conditions, and many experiments were made with embryos artificially hatched in this manner. Their general average of resistance is given as eight hours and fifty-two minutes, which is approximately the same as those which were more normally hatched.

From the time of hatching there is a constant and rapid decrease in power of resistance to lack of oxygen. In embryos from twelve to twenty-four hours after hatching the general average of resistance was about five hours, and for embryos thirty-six to forty-eight hours after hatching about three and a half hours, which is practically the general average of resistance for the adult individuals.

Thus within forty-eight hours the resistance of the embryos has decreased from sixteen and a half hours, the general average of the unhatched embryos, to three and a half hours, which is the general average of the adult. After this period of sudden change in power of resistance there follows slow and continuous decrease, which was followed in our experiments until the seventh day after hatching. The general average for the fourth day after hatching was two and a

half hours; for the seventh day one hour and fourteen minutes. At this time in the aquarium the embryos were dying rapidly. It seems difficult to keep them for a longer period under the artificial conditions of even a well-balanced aquarium.

In explanation of the relative increase in sensitiveness of the fish embryos to lack of oxygen Loeb<sup>1</sup> assumes "that the cells which are formed from the egg cell during the first stages of cleavage are different chemically from the cells which are formed later, so that the latter go to pieces more easily in lack of oxygen than the former." No indication, however, is given as to what constitutes this chemical difference.

On the basis of the experiments given in the first part of this paper the following explanation is offered.

It may be assumed that the *Fundulus* egg is well provided with some substance or substances, presumably of a carbohydrate nature, which can act as depolarizers in the process of respiration, and thus enable the egg to carry on respiration for a time in the absence of air. This would make the egg very resistant to the withdrawal of oxygen. It may further be assumed that these carbohydrate substances are used up in the processes of development, and the embryo thus becomes less and less resistant as development progresses. This material seems to be used up very rapidly during the first three days of development, and but very slowly if at all after the establishment of the circulation until the time of hatching. At the moment of hatching there is lost the fluid between the embryo and the egg membrane. In the embryos which were removed by pricking the egg membrane this fluid was always seen to exude through the puncture. Presumably this happens when the embryo itself breaks the membrane. The loss of this perivitelline fluid would account for the very sudden decrease of resistance at the time of hatching. After hatching the embryo enters upon a much more active life than when within the membrane. At the time of hatching the yolk sac attached to the embryo is large and rounded. It is so heavy that the animal swims about with difficulty. During the first forty-eight hours after hatching, however, this yolk sac is rapidly absorbed and the contour of the embryo becomes smooth.

This period of absorption of the yolk sac and of greatly increased activity coincides with the period of rapid decrease in the resistance of the embryo to lack of oxygen. The greatly increased activity

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



rapidly uses up the supply of stored carbohydrate material, and the resistance to lack of oxygen is correspondingly decreased until it becomes no greater than in the adult. The slow decrease in resistance to lack of oxygen which follows this period, and which renders the embryo less resistant than the adult, is probably entirely due to the unfavorable conditions of aquarium life to which the embryos are subjected, in which the conditions of oxygen supply are very poor compared with natural conditions.

TABLE VIII.  
LENGTH OF LIFE IN LACK OF OXYGEN OF FUNDULUS EMBRYOS  
IN SUGAR SOLUTIONS.

Solution of sugar used.	Length of life of controls in ordi- nary sea water.	Length of life in sugar solution.	Increase.
	hrs. min.	hrs. min.	per cent.
10 c.c. sea water + 2 c.c. of 0.95 mol. lævulose . . . .	55	1 55	73
10 c.c. sea water + 2 c.c. of 0.95 mol. glucose . . . .	1 55	3 25	78
10 c.c. sea water + 2 c.c. of 0.95 mol. maltose . . . .	1 00	2 25	141
10 c.c. sea water + 2 c.c. of 0.95 mol. cane sugar . . . .	1 05	2 30	130
10 c.c. sea water + 2 c.c. of sat. sol.-lactose . . . .	55	58	0

V. EFFECT OF CARBOHYDRATES ON RESISTANCE TO LACK OF OXYGEN  
IN FUNDULUS EMBRYOS.

If the resistance to lack of oxygen of the *Fundulus* embryos is less in the later stages of development than in the earlier stages because of the loss of carbohydrate material, their resistance ought to be increased by supplying them with the necessary substances. Experiments show that this is the case.

Young *Fundulus* embryos seven and eight days old after hatching were placed in sea water to which a certain amount of isotonic (0.95 molecular) solution of the sugar whose effect was to be tested was added. They were left in this solution for several hours to allow time for the absorption of the sugar. The sugar could be absorbed either directly through the body wall or through the alimentary system after digestive processes. For experimentation three embryos were placed in an Englemann gas chamber along with three other embryos of the same age in ordinary sea water to serve as controls and the oxygen

replaced by hydrogen. The controls and the embryos in the sugar solution were placed in separate paraffin cells side by side, and hence were under the same conditions as regards temperature and lack of oxygen. As the heart beat in the embryos was plainly visible, the stoppage of the contractions was taken as the death point. Table VIII gives the length of life in the controls in ordinary sea water and the length of life in the different sugar solutions, together with the percentage of increase in each case.

The results amply confirm the experiments with the injection of these sugars given in the first part of this paper. Maltose again has a greater effect than either glucose or lævulose. Cane sugar is evidently inverted and absorbed through the digestive processes. Its effect is nearly as great as that of maltose. Lactose probably cannot be digested or absorbed.

It is to be noted in this connection that glucose and lævulose arising from splitting processes in digestion are much more effective in their action than the same sugars when chemically prepared outside the body.

#### SUMMARY.

1. Those carbohydrates which can be absorbed when injected peritoneally into *Fundulus heteroclitis* (*i. e.*, maltose, glucose, and lævulose) greatly increase their resistance to lack of oxygen. This is due to the fact that simple sugars can act as depolarizers in the processes of protoplasmic respiration, and thus enable respiration to be carried on to a certain extent in the absence of oxygen.

2. The decrease in resistance to lack of oxygen shown by *Fundulus* embryos in successive stages of development is due to the using up of material (probably of carbohydrate nature) stored in the egg. When embryos in later stages of development are supplied with carbohydrates which can be digested and absorbed, their length of life in lack of oxygen is greatly increased.

My thanks are due to the Director and Assistant Director of the Marine Biological Laboratory for the privilege of occupying a research room during the past summer.

I wish also to thank Professor A. P. Mathews for kind assistance and direction.

# THE EFFECT OF INJURIES OF THE BRAIN ON THE VASOMOTOR CENTRE.<sup>1</sup>

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## INTRODUCTION.

WE believe in common with many physiologists that the individual nerve cell groups lying within the cranium are peripheral with respect to the bulbar vasomotor centre. To this centre afferent impulses flow constantly from other parts of the brain. The interruption of these impulses exalts the reflex excitability of the vasomotor cells. Thus the rise in blood pressure on stimulation of the central end of the sciatic nerve is increased by severing the bulb from the brain. A further demonstration of the afferent connection between the brain and the bulbar centre is seen in the changes in blood pressure following stimulation of various areas of the brain, changes so marked that certain observers have taken them as evidence of additional vasomotor centres anterior to the classical centre in the bulb.

The normal afferent impulses from the brain to the vasomotor centre and the impulses excited by electrical stimulation as ordinarily employed, upon the cortex, for example, are obviously only of moderate intensity, yet they produce marked effects upon the vasomotor cells.

Impulses of moderate intensity are the portion of the fortunate. In cerebral injury or disease, we must believe, the cells whose function it is to safeguard the general blood pressure are beaten upon by streams of afferent impulses far more powerful than the placid currents of ordinary life. How do the vasomotor cells support these sudden strains? Are they overwhelmed by a summation of extraordinary impulses, or is the margin of strength great enough to meet an emergency so rare that prevision would seem almost wasteful? The

<sup>1</sup> Some of the experiments in this article were reported to the American Physiological Society, December 27, 1904 (This journal, 1905, xiii, pp. xxii-xxiii).

problem is of interest, because it bears on the general physiology of nerve cells and because cerebral injuries are frequent; at the operating table life itself may depend on the reactions of the vaso-motor centre to afferent impulses. In the present investigation we have attempted to solve this problem by determining whether the work done upon the centre by afferent impulses of uniform strength is lessened by coincident impulses discharged from gross mechanical injuries of the brain.

#### METHOD.

The animals employed were rabbits and cats. They were invariably etherized. Tracheotomy was performed, and cannulas were placed in the carotid artery and, when necessary, in the jugular vein. The blood pressure was recorded by a simple form of Hürthle membrane manometer, which was frequently calibrated with a mercury column. Afferent impulses were obtained by the electrical stimulation of the central end of the depressor, sciatic, and branches of the brachial plexus in or near the axilla. For convenience, these branches will be called in the text the brachial nerve. Precautions were taken to prepare the nerves with the least possible injury and to protect them against drying. The nerves were always severed and the proximal end lifted into air when the electrodes were applied. A constant current from a Daniell cell supplied the inductorium, and the secondary coil was kept at a uniform distance from the primary coil.

Before stimulating the brachial or sciatic nerves curare was injected. This was mixed with a considerable quantity of warm normal saline solution and placed in a long graduated pipette emptying into the rubber tube upon the venous cannula. The pipette was very slightly inclined and held at this angle by a clamp. Thus the very dilute solution of curare flowed very slowly into the vein. From time to time the effect of the drug was tested by stimulating the peripheral end of the cut brachial nerves. By these precautions it was possible to operate with the least injurious dose of curare. Efforts were made to have the curarization uniform, so that any possible error from its use might be avoided. Care was also used to equalize as far as possible the stimulating action of the ether. In most of the experiments the injury to the brain, by destroying the perception of pain, made an anæsthetic unnecessary except in the preliminary operation.

The injuries to the brain were as follows:

1. Concussion, often with fracture and intracranial hemorrhage, from blows on the head of the etherized animal.
2. Penetrating wounds, produced by driving a pointed instrument into the brain.
3. Removal of brain substance.
4. Increased intracranial pressure.

The penetrating wounds were made by forcing through the cranial wall a piece of steel about three inches long, three-eighths inch in diameter at the large end, and tapering almost to a point at the small end.

In the experiments with increased intracranial pressure, the skull was trephined and a metal tube fastened water tight into the opening by means of a screw ring. The tube bore on its lower end a thin-walled rubber bag which in the earlier experiments had a length of about 3 cm. and a diameter of about 2 cm. In later experiments these dimensions were reduced about one-third. The pressure bag was connected with a pressure bottle. As near as possible to the skull, a side branch led to a mercury manometer. Undoubtedly the pressure in this manometer was always somewhat higher than that within the cranium, but this difference was not important to the experiments in hand. In all protocols the blood pressure was measured from the atmospheric pressure line to the lowest point in the blood pressure curve. This point can of course be easily determined, whereas it would be very difficult to determine the mean or systolic pressure. Thus all readings are lower than if the mean or the systolic pressure had been used, and this is particularly to be noted with regard to the very numerous cases in which the usual difference between the systolic and the diastolic pressure was increased by a lessening of arterial tension not compensated by an equivalent lessening in the force or frequency of the ventricular stroke.

It often happens that afferent nerves are stimulated when the blood pressure is slowly but steadily falling or rising. Thus in record 5, Experiment 16, Table I, page 188, the carotid blood pressure was 132 mm. before, 200 mm. during, and 118 mm. Hg after stimulation of the brachial plexus nerve. Evidently the diastolic line from which the rise upon stimulation must be measured was falling during stimulation. If reckoned from the higher end of the diastolic line, the reflex rise would be too small; if reckoned from the lower end, too large. In this dilemma one-half the difference between the diastolic pressure at the beginning and at the end of stimulation was subtracted

from the beginning or over-high pressure. In the above instance, the difference between 132 mm., which is the diastolic pressure at the beginning, and 118 mm., the diastolic pressure at the ending of stimulation, is 14 mm., one-half of which, subtracted from 132, gives 125 mm. as the corrected diastolic value from which the rise in blood pressure was calculated. Such a correction was made in every case where the original difference exceeded 2 mm. Hg, except where the



FIGURE 1.—Experiment 1, October 12, 1904. Two-thirds the original size. Fall of 57 per cent in carotid blood pressure on stimulation of the central end of the depressor nerve in the rabbit, twenty-five minutes after the skull was fractured.

variation in diastolic pressure had some special explanation, for example, a second stimulus before the after effect of the first stimulus had entirely disappeared.

In the following text typical protocols will set forth the effect upon the vasomotor centre of each class of injuries to the brain.

#### CONCUSSION, FRACTURE, AND INTRACRANIAL HEMORRHAGE.

Four successful experiments were performed on rabbits and two upon cats.

*Experiment 1, October 12, 1904.*—An etherized rabbit was tracheotomized and the blood pressure written with a membrane manometer. Stimulation of the depressor nerve caused the blood pressure to fall from 63 to 35 mm. Hg (46 per cent). At 3 p. m. the skull received a heavy blow in the parietal region. The blood pressure fell to 33 mm., but gradually rose again, partly in consequence of the injection of normal saline solution. Artificial respiration was necessary. On stimulation of the depressor nerve, at 3.45 p. m. the pressure fell from 78 to 21 mm. Hg (73 per cent). At 3.55 p. m. depressor stimulation caused a fall of 57 per cent (Fig. 1). The pressure now gradually fell from 80 mm. to 50 mm., at which level depressor stimulation caused a fall of 41 per cent.

At the autopsy a venous clot was found beneath the skin, covering about 4 square centimetres in the parietal and occipital regions. The space between the cerebellum and the cerebrum was filled with a venous

clot, resting on the corpora quadrigemina. A fracture, 22 mm. in length, extended through the parietal and occipital bones.

In the next two experiments the sciatic as well as the depressor nerve was stimulated.

*Experiment 2, October 21, 1904.*—Stimulations of the depressor nerve in an etherized and sufficiently curarized rabbit caused an average fall of 59 per

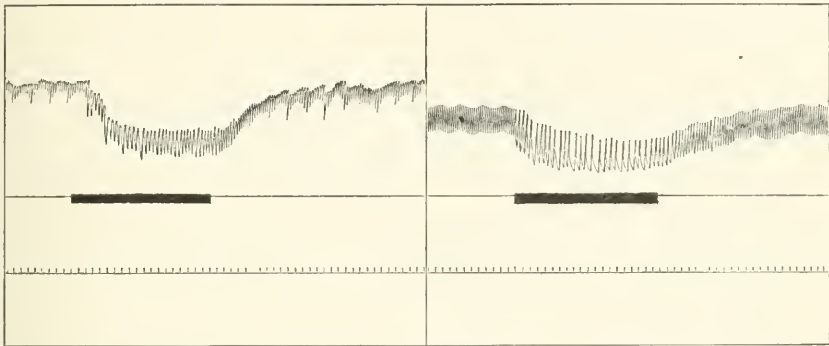


FIGURE 2.—Experiment 2, October 21, 1904. Depressor stimulation in the curarized rabbit before and after fracture with intracranial hemorrhage. Stimulation before fracture (left curve) lowered the carotid blood pressure 49 per cent; after fracture (right curve), the fall was 69 per cent. Time in seconds.

cent in the carotid blood pressure. Stimulation of the sciatic nerve caused a rise of about 40 per cent, but the readings were marred by cardiac irregularities. After a heavy blow on the skull, the pressure fell to 40 mm. Hg, but soon rose. At the 47 mm. level, depressor stimulation caused a fall of 59 per cent, at the 67 mm. level 69 per cent (Fig. 2). At the 74 mm. level the stimulation of the sciatic nerve caused a fall of 30 per cent, after which the pressure rose steadily, but the operation was interrupted before the rise was completed.

*Experiment 3, October 24, 1904.*—In an etherized and barely but sufficiently curarized rabbit, depressor stimulation lowered the carotid blood pressure from 85 to 52 mm. Hg (39 per cent), while stimulation of the sciatic caused the blood pressure to rise 48 per cent. A blow on the skull caused the blood pressure to fall from 100 to 30 mm., after which it gradually rose to 102 mm. On depressor stimulation it now fell 44 per cent. Stimulation of the sciatic nerve caused first a fall of 23 per cent and then a rise of 79 per cent above mean level, the pressure rising from 40 mm. to 98 mm. Hg. Upon a third blow, the pressure fell to 20 mm. Hg, and neither depressor nor sciatic stimulation gave any noticeable result.

The fourth experiment records an injury to the bulb itself.

*Experiment 4, October 26, 1904.*—Repeated stimulations of the depressor nerve in an etherized rabbit lowered the carotid blood pressure an average of 39 mm. Hg. A blow on the head caused a temporary fall from 105 to 42 mm. As the blood pressure rose, the depressor was stimulated three times, the average fall being 35 per cent. A second blow caused hemorrhage into the medulla, probably directly injuring the vasomotor apparatus. The pressure fell to 35 mm., but stimulation of the depressor caused, nevertheless, a fall of 24 per cent.

One of the cases of fracture in the cat was as follows :

*Experiment 21, March 24, 1905.*—The stimulation of the brachial and sciatic nerves in an etherized cat, sufficiently curarized to paralyze the motor nerves, caused an average rise of 27 per cent in the carotid blood pressure. After a heavy blow on the skull, the blood pressure fell from 140 to 35 mm. Hg. At this level the stimulation of the brachial and sciatic nerves had little effect, the pressure rise being only 3 mm. Hg. After an hour the carotid pressure rose spontaneously to 90 mm., and then to 130 mm.; at these levels, three stimulations caused an average rise of 30 per cent. The autopsy showed fracture in the left frontal region, extending to the base of the skull, and hemorrhage beneath the seat of fracture.

#### PENETRATING WOUNDS OF THE BRAIN; FRACTURE.

In two etherized cats a piece of steel was forced through the cranial walls into the brain. One protocol will suffice.

*Experiment 22, March 31, 1905.*—In an etherized and sufficiently curarized cat the stimulation of the sciatic nerve caused the carotid blood pressure to rise 21 per cent, and brachial stimulation caused a rise of 16 per cent. At 4 P. M. a pointed instrument was driven against the left frontal bone, and then against the right frontal bone, behind the zygoma. These wounds did not penetrate the skull, but the heart stopped beating for several seconds. Alternate sciatic and brachial stimulation gave with the sciatic a rise of 32, 33, 36, and 36 per cent, respectively, and with the brachial 42, 40, and 39 per cent.

At 4.25 P. M. the pointed instrument was driven through the right parietal bone, penetrating the skull to a distance of 3 cm. For a few seconds the heart stopped beating. Cerebral tissue escaped from the wound. Sciatic stimulation now caused a rise of 52, 48, and 50 per cent; brachial stimulation caused a rise of 61 per cent.

At 4.50 P. M. the same instrument was driven through the external auditory canal and the temporal bone into the base of the skull. Blood



escaped through the ear. The heart again stopped beating during a few seconds. Sciatic stimulation caused a rise of 66 per cent, and brachial stimulation a rise of 52 per cent (Fig. 3).

The blood pressure at the beginning of the experiment averaged 101 mm. Hg. At the end it averaged 97 mm.

The autopsy showed that the second wound (the first penetrating wound) caused a deep laceration of the brain substance. The third wound was a fracture of the base without laceration of the brain.

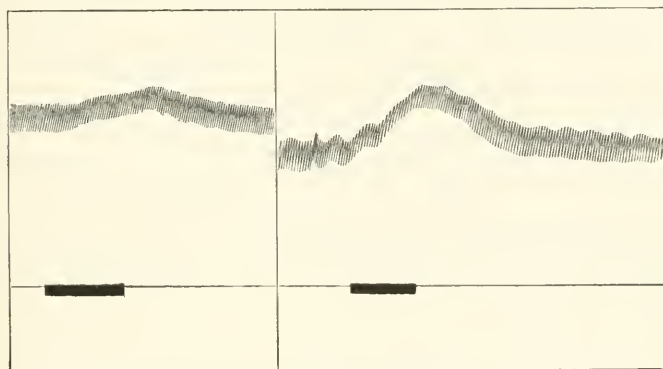


FIGURE 3.—Experiment 22, March 31, 1905. Carotid blood pressure in the cat upon brachial stimulation before and after a penetrating wound of the brain, with fracture of the base and intracranial hemorrhage. Before the injury brachial stimulation raised the blood pressure 16 per cent; after the injury, 52 per cent.

#### REMOVAL OF BRAIN SUBSTANCE.

The injury in the following experiment is in effect a lacerated wound of the cerebral hemispheres, though produced through a trephine hole instead of by a penetrating blow. The protocol is given verbatim.

*Experiment 16, March 29, 1905.*—At 3 P. M. a cat was etherized and cannulas placed in the trachea, the carotid artery, and the jugular vein. The right sciatic and left brachial nerves were exposed and severed. The secondary coil of the inductorium was placed at 8 cm.

4 P. M. 25 c.c. very dilute solution of curare in warm normal saline solution were allowed to flow very slowly into the jugular vein. Artificial respiration.

4.30 P. M. 25 c.c. more of the curare solution very slowly injected.

4.50 P. M. After four stimulations, in order to make sure that the nerves were in good condition, the apparatus satisfactory, and the curare effective, the records in Table I were made.

5.00 P. M. The skull was trephined, a small scoop introduced, and the cerebral lobes were extensively lacerated, considerable tissue being removed outright. There was some hemorrhage. The operation was finished at

TABLE I.

Hour.	Record number.	Nerve stimulated.	CAROTID BLOOD PRESSURE.			Increase.
			Before stimulation.	During stimulation.	After stimulation.	
p.m.			mm. Hg.	mm. Hg.	mm. Hg.	per cent.
4.50	5	Brachial	132 (125)	200	118	60
4.55	6	Sciatic	121	170	122	40
5.00	7	Brachial	124 (123)	190	121	54

5.15. The blood pressure fell to 60 mm., i. e., 50 per cent. The records in Table II. were now taken. At 5.37 P. M., 25 c.c. curare solution were slowly injected through the jugular vein.

TABLE II.

Hour.	Record number.	Nerve stimulated.	CAROTID BLOOD PRESSURE.			Rise.	Rectal temperature.
			Before stimulation.	During stimulation.	After stimulation.		
p.m.			mm. Hg.	mm. Hg.	mm. Hg.	per cent.	
5.20	{ 8	Sciatic	62 (64)	92	66	44	35.0°
	{ 9	Brachial	65 (61)	108	58	77	
5.35	{ 10	Sciatic	59	95	58	61	
	{ 11	Brachial	61 (57)	115	52	100	
5.45	{ 12	Sciatic	60	92	62	53	34.5°
	{ 13	Brachial	71 (65)	120	58	85	
5.50	{ 14	Sciatic	67 (66)	121	65	83	34.0°
	{ 15	Brachial	80 (73)	132	65	81	

*Autopsy.* — The hemispheres were thoroughly destroyed for a distance of 3 cm. from their anterior margins. Much brain tissue had been removed. In several places there were other deeper lacerations, the small scoop having entered the ventricles and gone as far as the base of the skull.

In the two following experiments the cerebral hemispheres of a rabbit and a cat were completely removed.

*Experiment 8, November 15, 1904.* — An etherized rabbit was tracheotomized and the carotid artery connected with a membrane manometer. The blood pressure was about 60 mm. Hg. Six stimulations of the depressor nerve caused an average fall of 34 per cent.

5.30 P. M. The cerebral hemispheres were completely removed. Rectal temperature, 38° C. The blood pressure fell to about 50 mm., but in half

TABLE III.

Hour.	Record number.	CAROTID BLOOD PRESSURE.			Fall.	Rectal temperature.
		Before stimulation.	During stimulation.	After stimulation.		
p. m.		mm. Hg	mm. Hg.	mm. Hg.	per cent.	
7.00	26	67	21	65	69	34.6°
7.30	27	67	22	65	67	34.4°
7.45	28	72	41	72	43	34.0°
8.00	29	67	24	65	64	33.8°
8.10	30	67	35	69	48	33.2°
8.20	31	65	30	65	54	33.0°
10.00	32	67	27	67	60	31.5°
10.30	33	60	40	62	33	30.7°
11.00	34	61 (64)	47	67	27	30.3°
11.00	35	67 (64)	40	60	38	30.3°
11.00	36	60 (63)	32	67	49	30.3°

an hour had risen to about 65 mm. Between 5.30 and 6.15 the depressor was stimulated nineteen times, with an average fall of 57 per cent. The subsequent record is shown in Table III.

Figure 4 shows the response to depressor stimulation at intervals throughout the experiment.

*Experiment 18, March 13, 1905.* — An etherized cat was tracheotomized and cannulas were inserted in the jugular vein and carotid artery. The right sciatic and left brachial nerves were exposed and severed.

4.15 P. M. 25 c.c. dilute curare solution were allowed to flow very slowly into the jugular vein.

4.30 P. M. Stimulation of the peripheral end of the brachial nerve indicated that the motor nerves were not completely paralyzed by the curare. 7 c.c. more were given.

Between 4.32 and 4.45 P. M. the following increases in carotid pressure

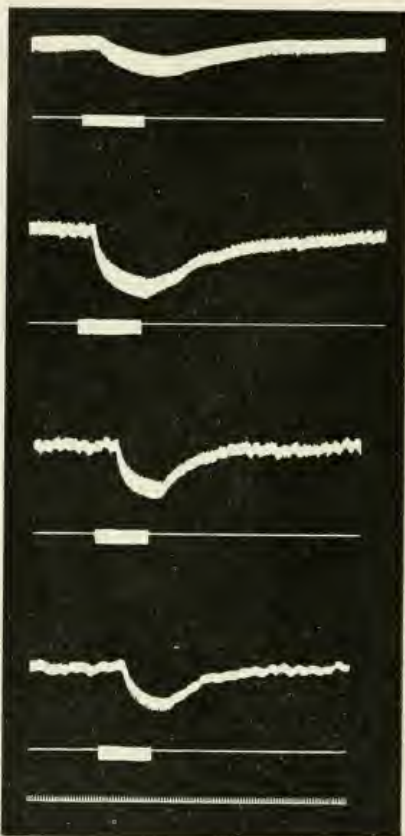


FIGURE 4. — Experiment 8, November 15, 1904. Carotid blood pressure upon depressor stimulation in the rabbit, before removal of the cerebral hemispheres, and one, four, and five and a half hours after removal. The blood pressure fell 34, 69, 60, and 49 per cent, respectively. The rectal temperature gradually sank from 38° to 30.3° C.

results in each case were unmistakable. The percentage fall of blood pressure upon stimulation of the depressor nerve and the percentage rise upon stimulation of the sciatic or a brachial nerve averaged greater after the cerebral injury than before. The comparisons in Table IV are instructive.

In each of the not exceptional cases in Table IV the absolute as

were obtained: sciatic, 27; brachial, 29; sciatic, 29; brachial, 33 per cent.

4.45 to 5.00 P. M. The cerebral hemispheres were removed.

5.05 P. M. The blood pressure has fallen from 159 to 53 mm. Hg. Stimulation of the sciatic nerve seeming to cause a reflex twitch, 25 c.c. curare solution were injected.

Between 5.10 and 6.30 P. M. the brachial was stimulated ten times, with an average rise of 59 per cent, and the sciatic nine times, with an average rise of 47 per cent (Fig. 5).

*Autopsy.* — The cerebral fossae were found to be emptied of their normal contents. A few lacerated remnants of brain tissue were seen on the floor of the cavity. Blood clots filled the spaces not occupied by the cotton which had been plugged into the opening in the skull to stay the hemorrhage caused by the removal of the cerebral hemispheres.

The cerebral hemispheres were removed in two other rabbits (Experiments 5 and 7) and in one cat (Experiment 17). In one of the rabbits part of the cerebellum was also removed. The

well as the percentage fall in blood pressure upon depressor stimulation was at least as great after the removal of the cerebral hemispheres as in the normal state.

TABLE IV.

Experiment.	Animal.	CAROTID BLOOD PRESSURE.				Fall in blood pressure upon depressor stimulation.		Nature of injury.
		Before injury.	After injury.	Upon depressor stimulation.		Before injury.	After injury.	
				Before injury.	After injury.			
		mm. Hg.	mm. Hg.	mm. Hg.	mm. Hg.	per cent.	per cent.	
5	Rabbit	68	70	53	32	22	54	Two blows on the skull, followed by removal of the cerebral hemispheres. Blood pressure fell at once to 25 mm. Hg, necessitating injection of warm Ringer's solution. Removal of cerebrum and part of cerebellum. Blood pressure fell to 27 mm. Restored by injection of Ringer's solution.
		48	45	35	33	27	27	
		62	65	41	32	34	51	
7	Rabbit	75	75	52	52	31	31	

INCREASED INTRACRANIAL PRESSURE.

The effect on the irritability of the vasomotor centre of increasing the intracranial pressure was studied in four rabbits and two cats. As the bulbar centres lie within the cranium and are therefore directly exposed to alterations in the intracranial pressure, the vasomotor apparatus must have suffered, together with the remainder of the brain, from the pressures employed. This difficulty would diminish or explain away a negative result, *i. e.*, a failure to change the blood pressure by the stimulation of afferent nerves, but it would increase the importance of a positive result, obtained necessarily from a centre working under conditions obviously unfavorable.

The following protocol illustrates the observations upon the rabbit.

*Experiment 9, November 21, 1904.* — An anæsthetized rabbit was tracheotomized and the carotid artery connected with a membrane manometer.

The central end of the depressor nerve was now stimulated four times,

lowering the blood pressure 23, 19, 24, and 24 per cent, respectively. The pressure bag was now placed in the cranium, through a trephine hole, and the intracranial pressure raised until the pressure-bottle manometer marked 95 mm. Hg. The carotid blood pressure rose from 67 to 87 mm. Hg. On stimulating the depressor nerve the blood pressure fell 50 per cent.

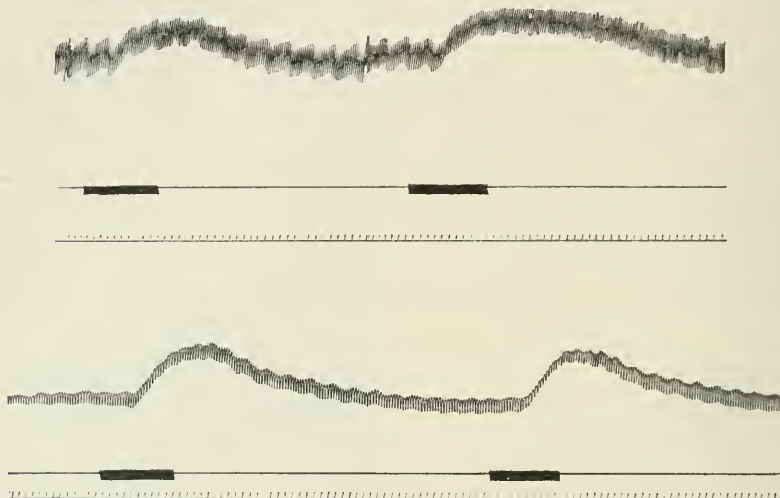


FIGURE 5.—Experiment 18, March 13, 1905. Carotid blood pressure upon stimulation of the sciatic and brachial nerves before and after the removal of the cerebral hemispheres in a curarized cat. In the upper tracing the rise upon stimulation of the sciatic (left black band) is from 159 to 212 mm. Hg (29 per cent), and the rise upon brachial stimulation (right band) is 33 per cent. In the lower tracing, taken after the removal of the cerebral hemispheres, sciatic stimulation (left band) raised the blood pressure from 85 to 150 mm. Hg (76 per cent), and brachial stimulation raised the blood pressure 87 per cent. The membrane manometer was more “damped” in the lower than in the upper curve.

The intracranial pressure was reduced until the pressure-bottle manometer stood at zero, and after an interval was increased until this manometer marked 115 mm. Hg. The carotid pressure rose to 95 mm. Hg. Depressor stimulation caused a fall of 32 per cent. As the stimulation ended, stertorous breathing began and was soon very marked. This labored breathing immediately ceased when the intracranial pressure was lowered to zero of the manometer scale, but the blood pressure remained at 100 mm. Hg. A third stimulation caused a fall of 28 per cent. The intracranial pressure was now increased until the pressure-bottle manometer read 85 mm. The blood pressure rose to 120 mm. Depressor stimulation reduced the blood pressure 28 per cent. Labored, stertorous breathing was again very marked, showing plainly on the blood-pressure curve (Fig. 6). The details of these stimulations are given in Table V.

**Autopsy.**— On removing the pressure apparatus it was noted that the pressure bag had formed in the cerebrum a cavity about 2.5 mm. deep, partly filled with clotted blood and serum.

TABLE V.

Intracranial pressure-bottle manometer.	CAROTID BLOOD PRESSURE.			Fall.	Remarks.
	Before stimulation of depressor.	During stimulation of depressor.	After stimulation of depressor.		
mm. Hg.	mm. Hg.	mm. Hg.	mm. Hg.	per cent.	
0	53 (52)	40	51	23	} Before rise in intracranial pressure.
0	53 (52)	42	50	19	
0	57 (59)	45	62	24	
0	68 (63)	48	58	24	
95	87 (84)*	42	80	50	} After rise in intracranial pressure
115	95 (91)	62	87	32	
0	100	72	98	28	
85	120 (109)†	78	98	28	

\* Before the increase in intracranial pressure the blood pressure was 67 mm. Hg.  
 † Before the increase in intracranial pressure the blood pressure was 98 mm. Hg.

Experiments 19 and 20 were performed on cats. Experiment 19 is sufficiently illustrated by Fig. 7. The protocol of Experiment 20 is as follows:

*Experiment 20, March 23, 1905.*— An etherized cat was tracheotomized, cannulas placed in the carotid artery and jugular vein, and the sciatic and brachial (median and ulnar) nerves exposed and severed. The pressure apparatus, provided with a bag 2 cm. in length and 1.5 cm. in diameter, was fixed in a trephine hole, the dura mater having been ruptured with scissors. At 3.40 P. M. 25 c.c. dilute curare solution were very slowly injected into the jugular vein and artificial respiration begun. At 3.50 P. M. the sciatic nerve was stimulated, causing the carotid pressure to rise 31 per cent. The intracranial pressure was now increased until the intracranial pressure-bottle manometer had risen from 0 to 120 mm. Hg. The blood pressure was increased thereby from 122 to 140 mm. Hg. On stimulation of the sciatic nerve, the blood pressure now rose 30 per cent. Further observations are made, but they are discarded because increasing the reading of the intracranial pressure-bottle manometer produced a suspiciously small increase in the blood pressure.

## DISCUSSION.

As stated in the Introduction to this paper, the immediate problem in the present investigation was whether the work done upon the vasomotor centre by afferent impulses of uniform strength is lessened

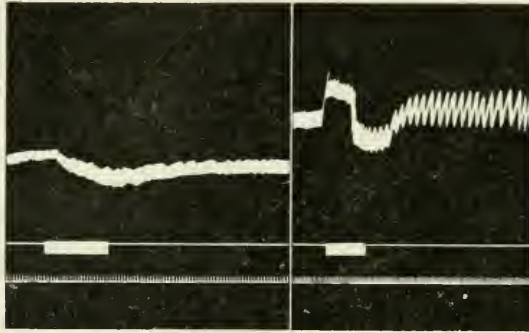


FIGURE 6. — Experiment 9, November 21, 1904. Carotid blood pressure in the rabbit, upon stimulation of the depressor nerve (1) with the intracranial pressure about normal, and (2) with the intracranial pressure raised until the pressure-bottle manometer records 85 mm. Hg. In curve 1 (left), without increase of intracranial pressure, the blood pressure was 68 mm. Depressor stimulation lowered it 24 per cent. In curve 2, with increased intracranial pressure, the blood pressure was 120 mm., and depressor stimulation lowered it 26 per cent. At this moment stertorous breathing set in, well shown in the tracing. The breathing became normal on reducing the intracranial pressure to its usual level.

by coincident impulses discharged from gross mechanical injuries of the brain. Before discussing this problem it will be profitable to consider a phenomenon which appeared as soon as the experiments began; namely, the sudden and great fall of blood pressure occasioned by blows on the skull. Table VI records five such injuries, the average reduction in blood pressure being 61 per cent.<sup>1</sup>

This remarkable descent of the blood pressure is not to be ascribed to the heart. Arrest of the heart was indeed observed to take place in Experiment 22, described in the section on penetrating wounds (page 186), but this arrest is very brief, and would not account for a change prolonged through many minutes. The cause of the latter must be sought in the bulbar vasomotor cells. On examining Table VI

<sup>1</sup> It must be remembered that some time necessarily elapses between the blow and the record of blood pressure. During this interval the pressure was probably lower than that recorded in Experiment 2, Table VI.



it will be seen that in Experiment 3, a and b, upon a rabbit, and in Experiment 21, upon a cat, the blood pressure fell each time about 70

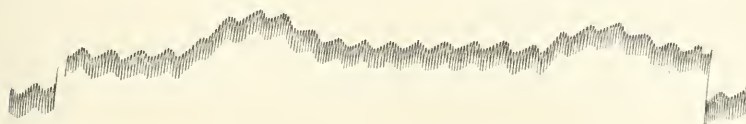


FIGURE 7. — Experiment 19, March 22, 1905. At the left of the tracing, the carotid blood pressure in the curarized cat stands at 70 mm. Hg. Upon raising the intracranial pressure, the blood pressure rises to 105 mm. On stimulation of the central end of the sciatic nerve (first black band) the blood pressure rises 33 per cent. On stimulation of one of the brachial nerves (second black band) the blood pressure rises 24 per cent. The intracranial pressure was now reduced to its level before stimulation, whereupon the blood pressure fell to 60 mm. Previous to these observations, while the pressure bag was in place, but the intracranial pressure not increased, the stimulation of the sciatic nerve increased the blood pressure 27 per cent.

per cent. In Experiments 1 and 2 the record was less, probably because the interval between the blow and the record was longer, thus allowing a greater rise towards the normal. In the five observations,

TABLE VI.

Experiment.	Animal.	Carotid blood pressure falls	Absolute fall.	Percentage fall.
		mm. Hg. from	mm. Hg.	per cent.
1	Rabbit	60 to 33	27	45
2	Rabbit	90 to 45	45	50
3 a	Rabbit	100 to 30	70	70
3 b	Rabbit	60 to 20	40	67
21	Cat	140 to 35	105	75

the level reached by the descending pressure averaged 33 mm. Hg. This is about the level to which the blood pressure sinks on section of the vasoconstrictor fibres in the spinal cord, as the following experiments show:

*Experiment January 28, 1907.*—The crural blood pressure in an etherized cat was recorded with a mercury manometer. On section of the spinal cord at the seventh cervical vertebra the blood pressure fell from 120 to 32 mm. Hg (73 per cent).

*Experiment January 29, 1907.*—The crural blood pressure in an etherized cat was recorded with a mercury manometer. On section of the spinal cord at the junction of the sixth and seventh cervical vertebræ, the blood pressure fell from 86 to 28 mm. Hg (67 per cent). The distal segment of the spinal cord was then destroyed by passing a large iron wire down the vertebral canal. The blood pressure rose temporarily, but soon sank to its former level. As it is difficult to be certain that the cord has been destroyed by pithing, the vertebral arches were now cut through and the fragments of the cord from the sixth cervical to the second lumbar vertebræ removed. The blood pressure still remained at 28 mm.

It seems probable, therefore, that the concussion produced by the blow on the skull threw out of function for a time the bulbar vasomotor centre, producing an effect equal in degree to the severing of the vasoconstrictor paths in the cord.

Unquestionably this condition is dangerous, though in most of our experiments the blood pressure returned shortly to its former level. Recent observations<sup>1</sup> have shown that if the blood pressure be lowered continuously, either by external hemorrhage or by filling the portal system and other large veins out of the arteries, a critical zone will be reached below which the usual afferent impulses cease to call forth a reflex from the vasomotor centre. The refusal of the vasomotor centre in these cases of hemorrhage or venous congestion is due to anæmia of the bulb, and the observations appear to indicate that the condition is usually self-perpetuating rather than self-correcting. In the present experiments the blood pressure was reduced almost or quite to this critical zone. Thus, in Experiment 3, October 24, 1904, described on page 185, a third blow on the head of a rabbit caused the blood pressure to fall from about 58 to 20 mm. Hg, at which level neither depressor nor sciatic stimulation had any effect. In Experiment 4, October 26, 1904, a blow on the head reduced the blood pressure to 35 mm., but depressor stimulation still caused a fall of 24 per cent. It is probable that in such injuries the critical zone is not seldom passed, and that death then takes place

<sup>1</sup> Presented by Dr. PORTER at the meeting of the American Physiological Society, December 29, 1906.

unless the bulbar cells are promptly supplied with an oxygenated and sufficiently isotonic liquid.

The chances of reaction before the critical zone is reached are apparently greater where the fall in blood pressure is due to a passing cause, such as the concussion from a blow on the skull, than where the fall is due to a continued stream of afferent impulses from some injured peripheral field. Moreover, the continued local dilatation of the blood vessels often present in such injured peripheral fields would hydraulically hinder reaction, by maintaining the bulbar anæmia, especially where the vascular area was large, as in injuries involving the splanchnic vasoconstrictor fibres. Thus the recovery of vascular tone in concussion of the brain should in the main be more easily secured than in prolonged injuries to large vascular areas, or in cases in which a persistent stream of afferent impulses enters an abnormally irritable centre.

It must not be supposed that the immediate fall of blood pressure following a blow on the skull is due to inhibition, provided this much abused word be understood as the arrest or depression of function by means of nerve impulses. Injuries culminating in the gross removal of the brain anterior to the bulb did not materially lessen the reflex power of the bulbar vasomotor cells. Indeed, such injuries usually increased this reflex power. Similar observations have been made in the case of the respiratory centre. Extensive injuries very near the respiratory cells may not stop the respiration, although these injuries probably pour into the respiratory cells many strong afferent impulses. The immediate fall in blood pressure following a blow on the skull is probably to be explained by the excessive mechanical vibration of the vasomotor cells. The vasomotor apparatus may be compared to a sensitive short-arm balance, oscillating slightly about a position of equilibrium and quickly responding to variations on either its afferent or efferent side. Mechanisms thus highly tuned are easily disturbed by mechanical shocks.

We are now in a position to answer the problem with which this investigation began. The work done upon the normal vasomotor centre by afferent impulses of uniform strength is not lessened by coincident impulses discharged from gross mechanical injuries of the brain. The measurements of the reflexes in cases of concussion with fracture and intracranial hemorrhage, in penetrating wounds, in the removal of brain substance, and in augmented intracranial pressure, permit no other conclusion.

In many of the injuries to the brain the reflex power of the vasomotor centre was not merely preserved, but noticeably increased. This increase we must now consider.

Since the investigation here presented was begun, repeated efforts have been made in this laboratory to obtain a significant, enduring fall of blood pressure by the stimulation of afferent nerves in normal animals, but wholly without success. The stimulating procedure may indeed cause the local dilatation of a great vascular area, thus hydraulically lowering the blood pressure, as when the intestines are roughly handled; but such experiments are of course useless as evidence of the depressing effect of afferent impulses upon the vasomotor centre. Excluding these errors of method, in which a local mechanical effect prevents all access to the problem in hand, there is as yet no evidence that the stimulation of afferent nerves in the normal animal will cause a prolonged reduction of blood pressure.

The results are very different in animals in which the irritability of the vasomotor apparatus has been increased by the removal of checks long endured, as when the pons is cut across to sever the bulb from the remainder of the brain. The increase in the vasomotor reflexes after this operation, the experiments of Langendorff, Wertheimer, Marckwald, Porter, and others upon the respiratory reflexes after the separation of the bulb from the brain and from the spinal cord, the experiments of Goltz and Ewald upon dogs with parts of the spinal cord separated from the brain, and other well-known observations, show that an increase of irritability in bulbar and spinal mechanisms after their separation from the brain is a general phenomenon. The loss of some of the accustomed afferent impulses disturbs the poise of these bulbar and spinal mechanisms. In their altered state afferent impulses ordinarily of but little power may occasion extraordinary reflexes. These disproportionate discharges are often harmless enough, as in the prolonged scratch reflex witnessed by Goltz and Ewald in their dog with "shortened" spinal cord; but if the reflex centre, the irritability of which is thus augmented, is charged with a duty like the maintenance of the blood pressure, grave consequences may result. Separation from the brain undoubtedly explains the increased reflexes in some of the present experiments.

Another explanation must, however, be sought for the rise of reflex power in the cases of fracture, intracranial hemorrhage, penetrating wounds, and augmented intracranial pressure, which form a large proportion of our experiments. That the irritability of the vaso-

motor centre was increased in these experiments is certain; that the brain substance was not anatomically removed is also certain; that the cerebral hemispheres were not functionally removed, for example, by an increase of intracranial pressure, seems probable. There remain two possible explanations. Either the increased reflex was due to an increased irritability from some chemical stimulus, or it was due to the combined operation of concurrent impulses, which increased the irritability of the centre to a point at which the moderate stimulation of the ordinary afferent nerves provoked the irritated centre to powerful discharges, capable of greatly changing the blood pressure. In the present instance, the chemical hypothesis has little in its favor, and we must conclude that our results in these injuries in which the bulb was not separated from the brain are best explained by an increase in the irritability of the vasomotor centre due to concurrent stimuli. It is hoped that experiments now making will bring further evidence of such double stimulations.

Meanwhile, it seems clear that the dangers to be apprehended from the vasomotor centre in cerebral injuries are, first, the profound fall in blood pressure caused by the concussion, and, second, an increased irritability through which very ordinary afferent impulses might cause violent changes in the blood pressure. The vasomotor cells are not exhausted. They support these rare emergencies with extraordinary success. Measured by the present investigation, the first, so far as we are aware, that has approached this question quantitatively, their power continues substantially unimpaired so long as they are fed.



## OBSERVATIONS ON NITROGENOUS METABOLISM IN MAN AFTER REMOVAL OF THE SPLEEN.

BY LAFAYETTE B. MENDEL AND ROBERT BANKS GIBSON.

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

**D**ESPITE a goodly number of experimental researches continued over many years, it cannot be said that the rôle of the spleen in the bodily functions has received any noteworthy elucidation. By some it has been made responsible for certain hæmopoietic activities which other investigators have in turn denied. The relation of the spleen to certain factors in the production of immunity has been debated; and attempts have been made to associate this organ with certain phases of the digestive cycle, without convincing results. The possibility that the spleen may exert some noticeable influence upon the general metabolic changes in the body has occupied the attention of a few observers. A brief review of the published studies on the connection of the spleen with metabolism may serve as an introduction to the experiments which we have recorded in a case of splenectomy in man.

Noël Paton<sup>1</sup> has studied the elimination of nitrogen (in the form of urea and ammonium compounds) and of phosphorus in bitches before and after splenectomy. The conclusion derived from his experiments is that "there is no essential difference in the course or nature of the metabolism either during fasting or after feeding with the ordinary proteids of flesh, with vegetable food such as oatmeal, or with food rich in nucleins, such as thymus. There is a more rapid excretion of water after a meal, probably indicating a more rapid absorption." Less satisfactory experiments by Nicolas and Dumoulin,<sup>2</sup> leading to contradictory or equally negative results, need not be detailed here.

The observed formation of uric acid in spleen pulp under favorable conditions, as well as certain statements regarding an increased

<sup>1</sup> NOËL PATON: *Journal of physiology*, 1900, xxv, p. 443.

<sup>2</sup> NICOLAS and DUMOULIN: *Journal de physiologie*, 1903, v, p. 859.

elimination of uric acid in cases of splenic hypertrophy and lienal leucæmia, have led to the assumption of a relationship between the spleen and uric acid formation in man. Mendel and Jackson<sup>1</sup> made a direct study of this subject in animals after extirpation of the spleen. In splenectomized dogs and cats the feeding of both purin-free and purin-yielding foods was attended by an excretion of uric acid and allantoin such as follows in the normal animals. In starvation endogenous uric acid was eliminated as usual. It thus became evident that the spleen is by no means the chief organ involved in uric acid production in the living body, if indeed it normally plays any part whatever in this process. Lo Monaco<sup>2</sup> has also reported the excretion of 0.4–0.6 gm. of uric acid per day by a splenectomized man. These facts no longer appear unexpected, since the series of purin-converting enzymes leading to a formation of uric acid has been found to have a wide-spread distribution throughout the body in other organs and tissues than the spleen.<sup>3</sup>

Two other recorded cases of splenectomy in man show little, if any, deviation from the course of nitrogenous metabolism characteristic for normal man under comparable conditions. Von Moraczewski<sup>4</sup> made a few analyses of the urine of a man of fifty-one years, seven months after removal of the spleen. The patient was in a febrile condition (pneumonia fibrinosa, temp. 40°) during most of the period of observation, rendering matters of diet and other control somewhat difficult. The ingested food was apparently purin-free. Von Moraczewski states that the urine showed no noteworthy variation in composition from what is commonly found in fevers. The distribution of the nitrogen was reported as follows: urea-N, 70–78 per cent; ammonia-N, 3.5–6 per cent. During the fever the elimination of chlorides was small, and as the temperature fell, the ammonia, urea, and uric acid likewise diminished, the chloride and sodium tending to increase in the urine. The elimination of phosphorus and calcium varied from the usual in a way apparently associated with marked changes in the leucocytes.

The most valuable record has been published by Umber.<sup>5</sup> The

<sup>1</sup> MENDEL and JACKSON: *American journal of physiology*, 1900, iv, p. 163.

<sup>2</sup> LO MONACO: *Abstract in Schmidt's Jahrbücher*, 1896, cclii, p. 109.

<sup>3</sup> Cf. MENDEL: *The formation of uric acid*, *Journal of the American Medical Association*, March, 1906; also the *Harvey Lectures*, 1905–1906, p. 195.

<sup>4</sup> VON MORACZEWSKI: *Berliner klinische Wochenschrift*, 1903, xl, p. 1002.

<sup>5</sup> UMBER: *Zeitschrift für klinische Medizin*, 1904, lv, p. 289.



data are of especial interest because careful observations were made both before and after the operation. A diagnosis of typical Banti's disease was established. The patient, a boy of fifteen years, was pale and icteric, showing a hard splenic tumor. The extirpated spleen weighed 1300 gm. The liver was observed to be enlarged and showed signs of incipient cirrhotic alterations. As a result of the operation the icterus disappeared, the liver resumed its normal size, and the previous anæmia rapidly disappeared. It was thus apparent that the participation of the liver had been a secondary and incipient influence. The second metabolism study included a twelve-day period twenty-four days after the operation. The diet was purin-free and analyzed. In the urine total nitrogen, nitrogen precipitable by phosphotungstic acid, urea, ammonia, amino-acid nitrogen, purins, chlorides, and phosphates were estimated quantitatively, as well as the nitrogen content of the fæces. The analytical data show no pronounced variations in the distribution of the urinary constituents which can be associated with splenic functions. Prior to the operation it was difficult, as in cases of fever, to attain nitrogen-equilibrium except with very large proteid intake. After the extirpation (and exclusion of the pathogenetic factors) N-equilibrium and favorable balances were readily obtained. Noteworthy in contrast with our case are the data regarding the endogenous purin output. Before the operation this showed marked and sudden fluctuations from 0.1 gm. to 0.26 gm. purin-N per day, which Umber associates with periodic destruction of corpuscles and discharge of their purins. In terms of the total nitrogen in the urine the endogenous purin-N varied in different dietary periods as follows :

Before the operation . . .	1.38, 1.08, 1.17, 1.22 per cent
After splenectomy . . .	1.06 per cent.

The urea-N remained at about 90 per cent of the total-N,—a normal figure.

Before the publication of Umber's careful observations an opportunity was afforded to us to supplement our earlier experiments on animals by the study of the metabolism of a young man after extirpation of the spleen.<sup>1</sup>

<sup>1</sup> A brief report of the results of this study was presented to the American Physiological Society in 1903. See American journal of physiology, 1903-1904, x, p. xxix.

**History.**<sup>1</sup>— The patient, E. A., a baker twenty-six years of age, was admitted to the New Haven Hospital September 25, 1903. The family history was without interest and the patient's own history indicated attacks of malaria, whooping-cough, scarlet fever, measles, and rheumatism in earlier years. He was accustomed to moderate use of alcohol. At the age of twelve he was kicked in the abdomen just above the symphysis pubis by a colt. The present illness began a year ago, when he first noticed that his clothes were becoming small about the waist. He had malaria in October, 1902, but continued to work until one month ago, having previously contracted gonorrhoea, as a result of which a urethral discharge still persisted. A month ago he began to complain of severe darting pains from his back through to the front of the abdomen and downward. They came on at night usually, and were relieved by morphine. The patient spoke of his trouble as a tumor, locating it in the left umbilical region, and was most comfortable when lying on his side. He has lost about twenty pounds.

A physical examination showed a normal color of the skin, with slightly yellow sclera, in a somewhat emaciated patient. The abdomen, markedly distended and somewhat tender, was the seat of an irregular tumor mass situated mainly on the left side and extending up as high as the fifth rib. It was firm, easily outlined, freely movable, and not tender. There was considerable fluid in the abdominal cavity. A blood examination failed to show any plasmodium. The differential leucocyte counts and other data regarding the blood will be found in a tabular summary below.

October 3 the patient went home, but returned to the hospital on October 5, without indicating any pain or abnormal temperature.

October 8 an operation was performed by Drs. Sanford and Carmalt. An incision, six inches long, was made from the ensiform cartilage down to the left of the umbilicus. Ascitic fluid escaped from the exposed peritoneal cavity. The spleen was found to be enlarged and adherent on its convex surface. The opening in the abdomen was enlarged, the vessels of the spleen ligated, and the organ removed. The wound was closed with drainage. The spleen, measuring 21 cm. × 13 cm. × 9 cm., weighed 1300 grams. The pathologist's report noted diffuse chronic hyperplasia; no pigmentation; organizing thrombi. No primary cause for the enlargement could be given from the examination.

October 18, the stitches were removed, the patient being comfortable, with a temperature of 99.8° F.

October 28, he had a severe coughing attack and expectorated a large amount of somewhat bloody fluid. Temperature 103.8° F. This

<sup>1</sup> We are especially indebted to Professor W. H. Carmalt, M.D., for the opportunity to study this case and for the clinical records here presented.

condition continued on October 29 (temperature 104.4° F.) and the possibility of a rupture of a pleurisy into a bronchus was suggested at a consultation. This condition gradually improved, with a brief coughing attack, etc. (temperature 100.2° F.) on November 6. By November 15 the wound was entirely closed, and on November 18 the patient was up in a chair feeling well. November 29 the wound was nearly healed, and on December 12 the patient left the hospital entirely healed. He was seen at work, and apparently well, by Dr. Carmalt nearly two years after the operation.

The examinations of the blood made at various periods are tabulated for comparison (see table on blood statistics).

The metabolism experiments consisted in a study of the composition of the urine collected in daily periods for a considerable number of days. During part of the time the character of the diet was specially regulated. The analytical methods used were as follows :

Total nitrogen was determined by the Kjeldahl-Gunning process ; urea, by the Mörner-Sjöquist method ; uric acid, by the Folin-Hopkins method ; ammonia, by the earlier procedure of Folin ;<sup>1</sup> phosphoric acid, chlorine and total acidity (with phenolphthalein) by the familiar titration methods ; sulphates by the usual gravimetric process. All analyses were made in duplicate.

The data are summarized in a series of tables in which the records are grouped into a number of periods corresponding to experimental conditions specifically noted (see table on urine analysis).

With respect to the diet it is to be noted in a general way that during most of the time the regular light hospital fare, consisting of eggs, milk, toast, jellies, and occasionally a little meat, was furnished. Speaking broadly, it may be considered as a diet poor in purin-yielding foods, except when these were specially prescribed in the experiments. Inasmuch as our attention was particularly directed to the question of purin metabolism, it will be noted that a purin-free dietary was carefully selected during some of the periods in order to note the output of endogenous uric acid.

**Period I.** — The first experiments were begun on October 25, seventeen days after the operation, at a time when the wound was rapidly healing. The temperature of the patient was elevated during this

<sup>1</sup> FOLIN: *Zeitschrift für physiologische Chemie*, 1901, xxxii, p. 575. Subsequent experience has shown that the results obtained by this method may be somewhat too low. Cf. FOLIN: *Ibid.*, 1902, xxxvii, p. 161.

## BLOOD STATISTICS FROM THE HOSPITAL RECORDS.

Date. 1903.	Erythro- cytes.	Hæmoglobin.	Leucocytes.	Differential leucocyte counts.					
				Polymor- phonuclear.	Small mononu- clear.	Large mononu- clear.	Eosino- philes.	Baso- philes.	Degenera- tive.
	per cmm.	per cent.	per cmm.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Sept. 23	4,200,000	70	6,200	....	....	....	....	....	....
29	....	....	....	53.9	32.8	9.	4.	0.3	....
30 Oct. 9 <sup>1</sup>	4,395,000	78	4,000	67.4	21.2	7.6	0.9	0.4	2.7
10	2,848,000	60	50,000	....	....	....	....	....	....
11	3,552,000	60	46,000	....	....	....	....	....	....
12	3,225,000	65	26,000	89.4	1.8	8.2	0.6	....	....
13	....	....	....	84.0	3.8	11.4	0.4	....	....
14	....	....	....	80.1	4.8	14.0	....	....	....
16	....	....	....	81.8	4.2	12.8	1.2	....	....
16	3,988,000	70	17,200	....	....	....	....	....	....
17	4,470,000	75	16,700	....	....	....	....	....	....
20	....	....	....	79.8	6.8	10.0	1.4	0.4	1.6
23	....	....	....	79.5	5.1	11.9	2.3	....	....
24	....	....	....	75.9	7.0	14.8	1.5	0.7	....
26	....	....	13,500	....	....	....	....	....	....
29 Nov.	....	....	20,000	75.0	14.6	8.4	1.9	....	....
1	....	....	13,000	80.3	10.8	6.0	3.0	....	....
8	....	....	....	67.7	24.0	6.1	2.1	....	....
9	....	....	13,400	....	....	....	....	....	....
11	....	....	14,400	82.9	10.3	5.8	0.8	....	....
19	....	....	9,000	....	....	....	....	....	....
21	....	....	10,000	....	....	....	....	....	....
26	....	....	9,400	....	....	....	....	....	....
30 Dec.	....	....	12,000	....	....	....	....	....	....
5	....	....	11,000	....	....	....	....	....	....

<sup>1</sup> Operation October 8.

entire period, and symptoms of pneumonia appeared at this time. The leucocyte count rose to 13,500 on October 26, and 20,000 on the 29th. A pulmonary abscess resulting from mechanical irritation incident to the operation was suggested. Medication was confined to administration of strychnine in small doses, an expectorant and a mild laxative when required. During the last two or three days the fever was very slight. In some respects this case shows a parallelism at this period with that reported by Von Moraczewski.

The urines were acid to litmus, and usually showed a small sediment of urates or uric acid, with a few leucocytes. Proteids and sugar were always absent. There was at first pronounced urobilinuria, the pigment being readily identified spectroscopically in the deeply colored reddish brown urines. It was learned that the patient had noted the dark color of the urine before the spleen began to trouble him severely. The urobilin may have been hepatogenic in origin and referable to the liver complications; although with the present divergence of opinion regarding the formation of urobilin our statement must be made with reserve. At a later period the urobilinuria disappeared, although the chromogen could still be detected in the urine. In Umber's patient the icterus speedily disappeared after the splenectomy, thus indicating an indirect or secondary involvement only of the liver.

The Jaffé-Obermayer test gave slight indications of indican. In splenectomized dogs an increased elimination of indican has been reported.<sup>1</sup> The rôle of the diet in this connection is too frequently overlooked. The ethereal sulphates were estimated in composite samples.

The variable figures for the total nitrogen elimination are associated with the dietetic habits and appetite of the patient. The distribution of nitrogen corresponds fairly well with the normal standards established by Folin's splendid researches.<sup>2</sup> The absolute and relative quantities of uric acid nitrogen are, however, noticeably higher than one would expect from a diet generally poor in purins. This will be emphasized in a later series.

The almost complete disappearance of chlorides from the urine corresponds with the well-known retention of chloride in febrile disease. This is so frequently associated with a deficiency of salt in

<sup>1</sup> MARCANTONIO: Abstract in *Jahresbericht für Tierchemie*, 1900, xxx p. 912.

<sup>2</sup> FOLIN: *American journal of physiology*, 1905, xiii, p. 66.

## SUMMARY OF URINE ANALYSES.

Date.	Volume.	Specific gravity.	Total N.	Urea N.	Uric Acid N.	Ammonia NH <sub>3</sub> .	P <sub>2</sub> O <sub>5</sub> .	Cl	Acidity as 1% acid.	Etheral SO <sub>2</sub> Daily Averages.	Distribution of Nitrogen.		
											Urea N.	Uric Acid N.	Ammonia N.
1903 Oct. 25	c.c. 750	1.020	gm. 12.50	gm. 10.60	gm. 0.689	gm. 0.77	gm. 2.24	gm. 2.9	c.c. 600	gm. 0.26	per cent. 84.90	per cent. 1.84	per cent. 5.10
" 26	765	1.022	13.83	11.34	0.757	0.70	2.13	3.2	565	0.26	82.00	1.81	4.16
" 27	625	1.021	10.14	8.76	0.615	0.68	1.53	3.2	450		86.40	2.13	5.52
" 28	680	.....	11.10	.....	.....	.....	.....	.....	.....		.....	.....	.....
" 29	500	1.021	12.06	10.41	0.930	1.12	2.53	1.7	560		86.48	2.51	7.64
" 30	440	1.023	9.75	8.61	0.696	0.71	1.47	0.2	340		88.30	2.38	5.99
" 31	410	1.025	9.36	8.04	0.637	0.90	1.14	0.4	300	0.23	85.90	2.85	7.91
Nov. 1	628	.....	13.95	12.15	1.059	1.20	2.18	1.8	480		87.09	2.53	7.08
" 2	500	1.023	10.63	9.00	0.722	1.10	2.28	1.5	470		84.26	2.25	8.47
" 3	600	1.026	11.16	9.21	0.839	1.24	1.74	3.7	430		82.56	2.51	9.15
" 4	340	1.026	6.48	5.52	0.560	0.73	0.81	4.7	210		85.18	2.88	9.27
" 5	950	1.022	9.21	7.44	0.685	1.07	0.98	1.3	205	0.23	80.78	2.47	9.56
" 6	330	1.019	4.23	3.30	0.382	0.70	1.10	4.8	215		77.65	2.93	13.63
" 7	910	1.021	10.50	8.64	0.837	1.39	2.02	3.7	450		82.28	2.65	10.90

II. METABOLISM AFTER THYMUS FEEDING.

Nov. 8	1180	1.018	10.48	8.43	1.180	1.24	2.09	3.7	472	....	80.44	3.75	9.74
" 9	1135	1.015	8.07	6.26	1.336	1.28	2.06	5.8	341	....	77.56	5.52	13.04
" 10	820	1.021	8.22	6.50	1.255	0.99	1.86	4.0	340	....	79.06	5.09	8.15

III. METABOLISM DURING A PURIN-FREE DIETARY.

Nov. 11	620	1.021	7.26	6.12	0.604	0.52	1.79	1.8	280	} 0.15	84.28	2.78	5.90
" 12	980	1.019	10.08	8.49	0.708	1.02	2.12	1.8	500		84.26	2.34	8.33
" 13	700	1.013	6.36	5.28	0.593	0.83	1.65	2.1	360		83.00	3.10	10.07
" 14 <sup>1</sup>	1235	1.018	10.49	8.82	0.921	1.01	2.08	4.1	416	87.24	3.05	8.24	
" 29	urine lost.	....	....	....	....	....	....	....	....	....	....	....	....
" 30	1735	1.016	10.83	8.95	0.921	1.32	1.96	....	486	82.62	2.84	10.03	
Dec. 1	urine lost.	....	....	....	....	....	....	....	....	....	....	....	....
" 2	1477	1.017	9.54	7.65	0.740	1.14	2.15	....	465	80.20	2.59	9.83	
" 3	2060	1.014	10.38	8.65	0.783	1.04	1.97	....	453	83.34	2.52	8.24	
" 4	2035	1.018	15.63	13.55	0.992	1.68	2.70	....	630	86.68	2.10	8.85	
" 5	1160	1.022	9.05	6.90	0.678	1.14	1.76	....	406	76.23	2.50	10.37	

IV. METABOLISM DURING A MIXED DIET.

" 10	1500	1.019	11.34	9.63	0.840	0.85	1.83	....	360	} 0.33	84.92	2.47	6.17
" 11	1925	1.025	16.29	13.98	1.074	1.18	2.31	....	540		85.92	2.20	5.95

<sup>1</sup> A small piece of beefsteak was given by mistake at 5 P. M.

the diet (usually milk and egg) that further comment on the significance of this finding is unnecessary.<sup>1</sup>

**Period II.** — During three days sweetbreads were added to the diet in order to study the capacity of the spleenless organism to metabolize exogenous purins. These experiments accordingly supplement the earlier investigation of one of us<sup>2</sup> on splenectomized dogs. At this time the general improvement of the patient was quite rapid and the urobilinuria was decreasing. The leucocyte count averaged 14,000 per cmm. The ingestion of the nucleoprotein food elicited the characteristic increase in uric acid output, as in the case of dogs, giving renewed evidence that the spleen is not the organ primarily involved in the metabolic oxidation of purins to uric acid.

**Period III.** — The patient was next put upon a purin-free régime consisting of eggs, milk, wheat bread, etc., in order to allow a study of the elimination of endogenous uric acid. Leucocyte counts during the period ranged between 9,000 and 12,000 per cmm. The wound had entirely healed. The strict purin-free diet was taken in two series of days, being begun on November 29 in the second series. On November 14 a piece of beefsteak was allowed by mistake. The urine of December 1 was accidentally lost.

Experience in our laboratory has shown that less than three days on a purin-free diet suffice to permit an elimination of all the exogenous purins still retained and afford strictly endogenous data thereafter.<sup>3</sup> The noteworthy feature of the figures here presented is the uniformly high endogenous output of uric acid. The average figures ordinarily quoted for an adult man vary between 300 and 400 mgms. per diem. We are inclined to associate the relatively high uric acid output, so constantly observed, with the disordered hepatic functions. It is assumed by many physiologists that uricolysis — the destruction of uric acid formed in intermediary metabolism — is primarily effected in the liver. When this organ is excluded, an increased output (presumably due to diminished destruction) is usually observed.<sup>4</sup> Corresponding with this, high uric acid figures have been obtained in cases of hepatic impairment, such as cirrhosis, atrophy, etc. In the

<sup>1</sup> Cf. SOLLMANN and HOFMANN: American journal of the medical sciences, February, 1905.

<sup>2</sup> Cf. MENDEL and JACKSON: American journal of physiology, 1900, iv, p. 163.

<sup>3</sup> A résumé of the subject of endogenous purins will be found in MENDEL: The Harvey Lectures, *Loc. cit.*

<sup>4</sup> Cf., for example, SWEET and LEVENE: Proceedings of the Society for Experimental Biology, 1906, iv, p. 14.



present case the urobilinuria as well as the inspection of the liver at the time of the operation lent probability to such an explanation.

**Period IV.** — Before the discharge of the patient from the hospital the urine of two days on a mixed diet was analyzed for comparison with Period I. Urobilinogen could still be detected. The patient appeared to be in good health. The urinary data are quite comparable with those of Period I, obtained under similar conditions of diet.

#### HOURLY EXCRETION OF URIC ACID AND TOTAL NITROGEN.

Various observers have noted that the curve of endogenous uric acid output from hour to hour tends to attain a level. After purin-containing meals an increased hourly output of uric acid is induced in a comparatively short period, giving rise to a typical curve for the individual.<sup>1</sup> Soetbeer<sup>2</sup> has noted that in certain disorders the curve may show great irregularity in contrast with that obtained from healthy persons. Hopkins and Hope found that the curves of the postprandial elimination of urea and uric acid are not exactly parallel. The uric acid reaches its maximum usually in four to five hours and then falls again, the urea showing a somewhat slower rise to the maximum. In view of the high uric acid figures obtained with our patient we have made a study of the hourly elimination in order to compare the rate of excretion with that pertaining in normal individuals. No noteworthy variation was observed.

*Protocol of December 8.* — A very light purin-free supper was taken at 5 P. M. on December 7. The collection of urine was begun at 6 o'clock on the morning of the following day and continued hourly. No nourishment was allowed until 11 A. M. At that hour a fairly bountiful meal, consisting of roast beef in good quantity, corn, potatoes, bread, milk, and custard, was served. Water was allowed sparingly during the afternoon. Uric acid determinations were made by the Hopkins method.

#### COMPOSITION OF THE URINE.

	Hours.	Volume. c.c.	Nitrogen. gm.	Uric acid. mgm.
A. M.	6 to 7	34	0.26	38
"	7 " 8	44	0.41	58
"	8 " 9	57	0.43	54

<sup>1</sup> Cf. HOPKINS and HOPE: *Journal of physiology*, 1898, xxiii, p. 271.

<sup>2</sup> SOETBEER: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 25.

COMPOSITION OF THE URINE (*continued*).

	Hours.	Volume c.c.	Nitrogen.	Uric acid.
A. M.	9 to 10	92	0.40	46
"	10 " 11	65	0.39	50
Meal at 11.				
"	11 " 12	30	0.28	45
P. M.	12 " 1	42	0.52	71
"	1 " 2	47	0.54	67
"	2 " 3	80	0.75	75
"	3 " 4	83	0.70	71
"	4 " 5	66	0.62	61
"	5 " 6	62	0.59	56

The result of the study of this case may be summarized by the statement that, aside from the incidental details already attributed to other factors, *the analytical data fail to indicate any striking variations from the normal distribution of urinary components which can be associated with the exclusion of the functions of the spleen.*

## THE DETERMINATION OF WATER IN PROTEINS.

BY FRANCIS G. BENEDICT AND CHARLOTTE R. MANNING.

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**I**N a previous paper<sup>1</sup> we discussed in considerable detail the methods of drying food materials and physiological preparations in high vacua.

While the evidence secured on some of the materials of animal nature, such as beef, implied that the water could be removed by desiccation in about two weeks, some of the vegetable materials persistently held water for weeks, if not, indeed, months. The majority of food materials studied were combinations of protein, fat, and carbohydrate, and hence little evidence was at hand to show what material held water so persistently.

The recent observations of Maquenne<sup>2</sup> would seem to indicate that extreme difficulty is experienced in determining the water of starch, and although it is highly probable that the greater proportion of the water so persistently retained in vegetable food materials is retained by virtue of the presence of starch in the material, it seemed desirable to continue this investigation and include a study of the pure proteins.

It was our good fortune to have an abundant supply of animal and vegetable proteins, the animal proteins being furnished by Prof. Wm. J. Gies of Columbia University, and the vegetable proteins by Dr. T. B. Osborne of the Connecticut Agricultural Experiment Station, New Haven, Conn.

Since it is the common custom of almost all investigators to determine the moisture content of animal or vegetable proteins by drying to constant weight in an air bath at 100°-110°, it is of considerable importance to note the efficiency of this method of desiccation.

**Experiments with animal proteins.**—The series of animal proteins used in the experiments published in the earlier report were likewise

<sup>1</sup> BENEDICT and MANNING: This journal, 1905, xiii, p. 310.

<sup>2</sup> MAQUENNE: Comptes rendus, 1905, cxli, p. 609.

subjected to a comparative test to note the relative efficiency of the method of determining moisture by desiccation in high vacua and by heating in an air bath at 100°. In all cases duplicate samples (2 grams) of the air-dry material were weighed in aluminum dishes, 52 mm. in diameter, 15 mm. high, and provided with closely fitting covers. These dishes were used in practically all of the previous work. The materials were then placed in a vacuum desiccator in a high vacuum, obtained by the sulphuric acid-ether method.<sup>1</sup>

The results of this series of experiments are recorded in Table I.

TABLE I.  
COMPARATIVE DESICCATION OF ANIMAL PROTEINS (PER CENT OF WATER).

Material.	High Vacuum Room Temperature.			In air bath at 100° for 5 hours.	High vacuum for 2 weeks.
	1st week.	2nd week.	4th week.		
Collagen and Elastin, Femur II. . . . .	10.61	10.64	10.60	10.30	10.34
Collagen and Elastin, Rib IV. . . . .	11.42	11.50	11.52	10.23	11.59
Ossein, I Femur . . . .	11.66	11.74	11.77	10.75	11.80
Gelatin, B Femur . . .	11.85	11.90	11.95	10.73	11.97
Collagen and Elastin, Femur III. . . . .	12.29	12.43	12.47	10.86	12.41
Collagen, Tendon C. . .	12.98	13.09	13.10	12.23	13.14
Elastin, Ligament D 2nd.	7.13	7.24	7.23	6.76	7.37
Ossein, V Femur . . . .	9.95	10.16	10.25	9.87	10.41

The dishes were weighed at the end of one, two, and four weeks respectively. After the last weighing they were placed in an air bath at 100° and dried five hours. After cooling, they were weighed and again placed in a high vacuum. The final weighing was made after two weeks' desiccation in high vacuum. A special analytical balance with carefully calibrated weights was set aside for use in this investigation in order to maintain constant conditions of weighing.

An inspection of the figures in the first three columns shows that at the end of two weeks the substances were nearly anhydrous, there being but a slight further loss during the second week. There was

<sup>1</sup> BENEDICT and MANNING: American chemical journal, 1902, xxvii, p. 340; see also This journal, 1905, xiii, pp. 318 *et seq.*

no measurable loss in the two weeks following, and these observations are fully in accord with those reported earlier to the effect that the animal materials were completely deprived of their moisture by desiccation in high vacua at the end of two weeks.

The most striking observation of these experiments was the fact that when these materials, already anhydrous, were placed in an air bath and dried for five hours, they all gained in weight, as was shown by the apparent lower per cent of water as determined. The gains amounted to from .30 to 1.61 per cent of water, thus showing that the anhydrous material was prone to absorb moisture out of the air in the air bath even at a temperature of 100°.

A subsequent desiccation of this material in a high vacuum for two weeks showed that the loss in weight was almost exactly comparable to the gain when placed in the air bath. The final percentage of water as determined in these compounds was essentially the same as it was after desiccation in a high vacuum for about four weeks. This indicates that with compounds of this nature there is no material oxidation or volatilization of substances when heated in hot air for five hours.

**Experiments with vegetable proteins.**—The investigations here reported were made in two series. In the first series a number of vegetable proteins were dried under essentially the conditions outlined for the experiments on animal proteins cited above. Duplicate samples (1 or 2 grams of substance) were placed in a vacuum desiccator and dried in a high vacuum for nine weeks. At the end of this period the dishes containing the substances were weighed and then placed in an air bath and dried for ten and a half hours at 110°. Subsequently all dishes were placed in a high vacuum and weighed at the end of three weeks. The results are given in detail in Table II.

In all cases the covers were immediately placed on the dishes after removing from the desiccator, and other weighings were made with the covers on. Previous experience has shown that with the form of aluminum dish and cover here used, anhydrous material may be allowed to stand even outside of a desiccator for several hours without any appreciable increase in weight.

A previous series of observations had shown that pure edestin had become practically anhydrous in three weeks.<sup>1</sup> Consequently it was

<sup>1</sup> See also results given in Table III, p. 217.

assumed that at the end of nine weeks these compounds would have reached constancy with regard to the water content.

Comparing the results obtained after the final heating at  $110^{\circ}$ , it is seen that as with the animal proteins all the materials had gained in weight, the smallest gain was .50 per cent and the largest 1.15. On subsequent desiccation in a high vacuum for three weeks, the loss in weight corresponded in almost every instance to the original

TABLE II.

COMPARATIVE DESICCATION OF VEGETABLE PROTEINS IN A HIGH VACUUM (AT ROOM TEMPERATURE) AND AN AIR BATH AT  $110^{\circ}$  C. (PER CENT OF WATER).

Materials.	High vacuum for 9 weeks.	In air bath at $110^{\circ}$ for $10\frac{1}{2}$ hours.	High vacuum for 3 weeks.
Edestin (hempseed) . . . .	10.98	9.83	10.97
Edestin (No. 1) . . . . .	11.19	10.10	11.13
Legumin (vetch) . . . . .	11.31	10.69	11.34
Conglutin (yellow lupin) . .	10.82	10.32	10.82

water content of the substance after desiccation for nine weeks. Thus it appears clear that with vegetable proteins as with animal proteins there is a tendency for the material dried in high vacuum to absorb moisture even from air at a temperature of  $110^{\circ}$ , and consequently the method of drying these substances which involves their remaining in an atmosphere at  $110^{\circ}$  is unsatisfactory. The return to the original degree of desiccation on placing in the desiccator after heating in hot air indicates that here likewise there was no noticeable change in weight due to oxidation or volatilization as the result of heating at  $110^{\circ}$  for some ten or eleven hours.

The results obtained with the four proteins recorded in Table II justified a more extended study of the effect of desiccation in an air bath when compared with that in a high vacuum. Furthermore, data regarding the rate of drying in a high vacuum as well as the influence of heat on the anhydrous material were also desired. Consequently a series of vegetable proteins were subjected to a similar treatment. Duplicate samples were placed in a high vacuum and weighed at the end of two and twelve weeks respectively. At the end of this time they were weighed and then placed in an air bath at  $110^{\circ}$  for ten and a half hours. They were then removed from the air bath, weighed

and placed in a high vacuum for eight weeks. During the heating in the air bath they were weighed twice, but no material alteration in weight could be observed after five and a half hours. The results are recorded in Table III.

It is seen that the proteins, with but few exceptions, all reached constant moisture condition after about two weeks. The samples

TABLE III.

COMPARATIVE DESICCATION OF VEGETABLE PROTEINS (PER CENT OF WATER).

Name.	High Vacuum.		In air bath at 110° for 10½ hours.	High vacuum for 8 weeks.
	2 weeks.	12 weeks.		
Amandin (almond) . . . . .	9.70	9.70	9.32	9.99
Corylin (hazel nut) . . . . .	8.76	8.81	8.16	8.99
Excelsin Brazil nut . . . . .	7.23	7.24	6.76	7.54
Edestin No. 1 (hemp-seed) . . .	10.19	10.20	9.37	10.28
Edestin No. 2 (hemp-seed) . . .	9.08	9.10	8.76	9.28
Edestin No. 3 (hemp-seed) . . .	8.82	9.00	8.74	9.23
Vignin (cow pea A) . . . . .	7.93	8.06	8.43	8.82
Vignin (cow pea B) . . . . .	8.83	9.24	8.43	8.97
Glycinin (Soy bean) . . . . .	8.78	8.76	7.94	9.05
Legumin (lentil) . . . . .	9.01	9.07	8.29	9.13
Legumin (horse bean) . . . . .	8.77	8.83	7.92	8.91
Legumin (vetch) . . . . .	8.95	9.10	8.82	9.30
Globulin (cotton seed) . . . . .	9.04	9.09	8.37	9.40
Phaseolin A (Adzuki bean) . . .	7.52	7.68	7.05	7.81
Phaseolin B (Adzuki bean) . . .	9.24	9.36	8.56	9.58
Phaseolin (kidney bean) . . . . .	8.53	8.63	8.31	8.81
Conglutin No. 1 (yellow lupin) .	7.15	7.14	6.62	7.42
Conglutin No. 2 (yellow lupin) .	7.50	7.44	7.94	8.63
Glutenin (wheat) . . . . .	9.72	9.62	9.46	9.77
Hordein (barley) . . . . .	9.59	9.62	9.46	9.77
Glycinin (Soy bean) . . . . .	8.18	8.14	8.10	8.40

showing the greatest loss in weight in the ten weeks between the two weighings were: edestin No. 3, 0.18 per cent; vignin A, 0.13 per cent; vignin B, 0.41 per cent; legumin (vetch), 0.15 per cent, and phaseolin B, 0.12 per cent. These alterations in weight, especially in vignin B, are of interest in subsequent comparison of the desiccation after heating at 110°.

On placing the dishes containing the desiccated material in the air bath and heating, all the materials, with the exception of conglutin No. 2, gained in weight, as shown by the diminished apparent water content. The gains amounted to from 0.04 per cent to 0.91 per cent.

The samples were then placed in a high vacuum for two weeks, after which they were weighed. During this period all the samples lost in weight materially and the percentages of water were correspondingly increased. The gains amounted to from 0.30 per cent to 1.09 per cent.

Contrary to the results as recorded in Table II, there is in all but one case (vignin B) an increase in the per cent of water at the end of the final desiccation as compared to the per cent found at the end of twelve weeks' drying *in vacuo*.

In some instances the increase is very marked; vignin A, +0.76 per cent; glycinin (Soy bean), +0.29 per cent; globulin (cotton seed), +0.31 per cent; and conglutin No. 2, +1.19 per cent. This last sample was the only one showing a loss in weight after heating at 110° C.

The sample vignin B obviously retains persistently water that may have been absorbed, for it is seen that this sample showed the largest loss of water in the desiccation *in vacuo* between the second and twelfth week. Even after the preliminary desiccation this material absorbed water from the air in the air bath, a portion of which it retained even after two weeks *in vacuo*.

From these results it would appear that the heat of the air bath resulted in a loss in weight. This loss may be water or volatilized organic material. It seems incredible that these materials could remain over sulphuric acid in a vacuum of less than one millimetre of mercury for twelve weeks and still retain moisture, and yet no other explanation of these losses in weight is at hand.

**Influence of the nature of the container.** — The hygroscopic nature of these vegetable proteins should determine the nature of the container in which such material should be weighed when making moisture determinations. Many investigators are accustomed to use watch



glasses, glass-stoppered weighing bottles, or small glass specimen tubes which can be corked, save at the moment of weighing. Obviously, the longer the material is exposed to the moist atmosphere, the more rapid and the greater will be the amount of water absorbed. Using pure edestin as a typical vegetable protein, a series of experiments was made in which the edestin was dried in the air bath at 110° and subsequently desiccated in a high vacuum. Six samples each were weighed in three kinds of containers, aluminum dishes, specimen tubes, and glass-stoppered weighing bottles. The results are recorded in Table IV.

The amount of moisture lost by desiccation in the air bath is with the aluminum dishes practically unchanged after five hours. With the specimen tubes and bottles there is a noticeable loss in the average after the second heating of three and a half hours, while the fourth heating results in no material change. Thus far then it would seem that the aluminum dishes are the least advantageous for use in weighing vegetable proteins when drying in an air bath. The glass-stoppered weighing bottles seem to give the highest per cent of water, and these are therefore to be recommended in place of the specimen tubes.

On subsequent desiccation the material in all the containers lost water, indeed, in considerable quantities. At the end of seven days the percentage of water as determined in the glass-stoppered weighing bottles was still slightly greater than in either the dishes or the specimen tubes. At the end of fourteen days there was still a slight advantage in favor of the weighing bottles, which advantage was not lost at the weighing at the end of five weeks.

Here again is exhibited the inability to expel all the water from vegetable proteins when heated in an air bath. The average error in this sample of edestin is with the aluminum dishes 0.86 per cent; with the specimen tubes 0.93 per cent, and with the glass-stoppered weighing bottles 0.50 per cent under the most advantageous manipulation, therefore it was possible to abstract one-half of one per cent more water by desiccation in a high vacuum after the sample had been dried (?) at 110°.

**Basis for analysis.** — The hygroscopic nature of the animal and vegetable proteins makes it practically impossible to make analyses on weighed portions of the anhydrous material, consequently accurate determinations of water are essential in reporting the results of analyses. The usual custom of investigators has been to deter-

TABLE IV.  
 DESICCATION OF EDESTIN IN AIR BATH AT 110° AND SUBSEQUENTLY IN A HIGH VACUUM. INFLUENCE OF NATURE OF CONTAINER FOR THE SAMPLE (PER CENT OF WATER).

No.	Dishes.					No.	Tubes.					No.	Weighing bottles.							
	110° 5 +3½ hrs.	110° +4 hrs.	Des. 7 days.	Des. 14 days.	Des. 5 wks.		110° 5 hrs.	110° +3½ hrs.	110° +4 hrs.	Des. 7 days.	Des. 14 days.		Des. 5 wks.	110° 5 hrs.	110° +3½ hrs.	110° +4 hrs.	Des. 7 days.	Des. 14 days.	Des. 5 wks.	
34	6.79	7.57	6.82	8.00	8.15	8.05	1	7.03	6.93	7.00	8.09	8.24	8.21	1	7.84	7.49	7.40	8.22	8.35	8.29
64	7.26	7.18	7.36	7.98	8.11	8.03	2	6.59	7.29	7.18	8.09	8.22	8.18	2	7.98	7.98	7.87	8.32	8.42	8.37
65	7.72	6.93	7.58	8.07	8.22	8.14	3	6.92	7.61	7.46	8.17	8.27	8.23	3	6.93	8.16	8.15	8.35	8.47	8.43
66	7.25	7.38	6.89	7.98	8.10	8.01	4	7.03	7.51	7.37	8.32	8.41	8.37	*	.....	.....	.....	.....	.....	.....
67	7.12	7.21	7.33	8.00	8.14	8.06	5	7.11	7.48	7.47	8.18	8.30	8.23	*	.....	.....	.....	.....	.....	.....
68	6.49	6.41	7.19	7.96	8.06	7.99	6	7.22	7.41	7.37	8.19	8.27	8.23	6	7.64	8.11	8.05	8.32	8.42	8.39
Av.	7.10	7.11	7.19	8.00	8.13	8.05	Av.	6.98	7.37	7.31	8.17	8.29	8.24	Av.	7.60	7.93	7.87	8.30	8.41	8.37

\* Sample lost through accident.

mine the moisture by heating in an air bath at  $110^{\circ}$  C., and as the results in this paper show, the determinations thus made may readily be subject to an error of one per cent. Obviously an error of one per cent or more in the water determinations would result in an error of one-half of one per cent in the carbon determinations, and a measurable error in the determination of nitrogen. It can only be said that in the majority of researches on the animal or vegetable proteins, the carbon and nitrogen determinations are in large measure only used for comparative observations, and hence the absolute nitrogen or carbon content is not of as great importance. It is clear, however, that at least so far as these materials used in these investigations are concerned, it is impossible to determine the moisture content in them by drying in hot air at a temperature of  $100^{\circ}$ – $110^{\circ}$ .

The removal of the final traces of moisture which are persistently retained by the proteins when dried in an air bath at a temperature of  $110^{\circ}$  can be effected by subsequent desiccation in a high vacuum for two weeks. It is furthermore highly probable that the method reported by Maquenne<sup>1</sup> for drying starch, *i. e.*, heating these materials in a *current of dry* air at  $110^{\circ}$ , will yield results closely agreeing with those obtained in high vacuum. We have not determined experimentally the value of this method for drying proteins.

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

## EXTRASYSTOLES IN THE MAMMALIAN HEART.

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### INTRODUCTION.

THE investigations recorded in this paper were undertaken with the hope of throwing some light upon the site of origin of the cardiac impulse in the mammalian heart, and also to obtain, for physiological and clinical purposes, a further knowledge of the characteristics of mammalian extrasystoles arising in the great veins as well as of those arising in the auricles.

The investigations of Engelmann upon the frog's heart have shown that in an extrasystole arising from a stimulus to the sinus region, the extrasystole and its diastolic phase (compensatory pause) plus the preceding cardiac cycle, is shorter than the length of time required for two regular cardiac cycles. In extrasystoles arising from stimulation of the auricles or ventricle, this interval is, however, just equal to two regular cardiac cycles. The explanation of this given by Engelmann led him to conclude that by this method it is possible to differentiate between that portion of the heart that initiates the prevalent rhythm and other portions. Subsequent work has tended to support this view, and it was with the purpose of attempting to apply this method for the differentiation of autogenetic and contractile tissue in the mammalian heart that the present work was undertaken.

### METHODS.

The animals used were dogs and cats. For the extrasystole experiments the animals were anesthetized, placed upon artificial respiration, the thorax opened after ligation of the internal mammary arteries, and the pericardium slit open and sewed to the margin of the opening in the thoracic wall. The contractions of the right auricle and the right ventricle, in some experiments also the left auricle,

were recorded by air transmission by means of transmitting and recording tambours. The tambours employed were of the same size, and care was used to have the tubes connecting the transmitting with the recording tambours of exactly the same length. In our

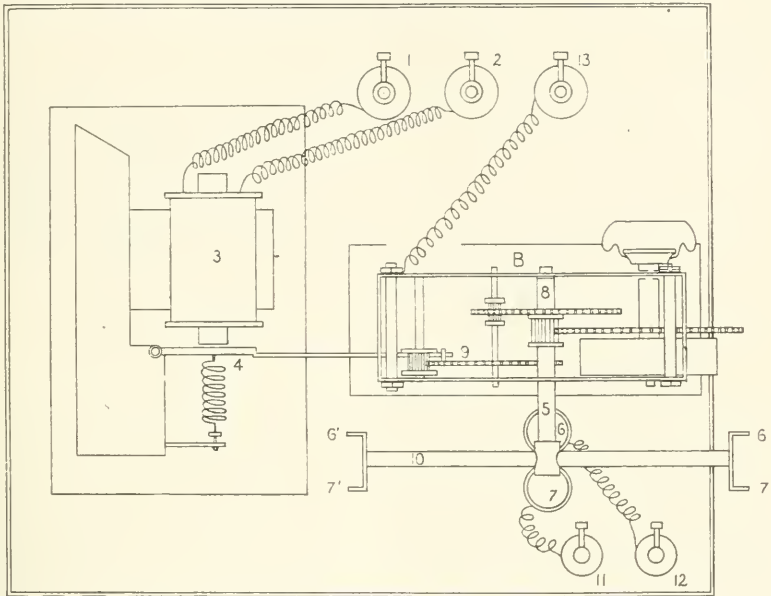


FIGURE 1.— Ground plan of apparatus for the production of extrasystoles.

early experiments extrasystoles were produced by mechanically touching or pinching certain areas, and by break induction shocks sent into the heart by means of a make and break key that recorded upon the drum and was manipulated by hand. In order, however, to make the comparisons that we wished, it soon became apparent that it was necessary to have some mechanism by means of which a series of stimuli at the same incidence in the cardiac cycle should be automatically transmitted to the heart. For this purpose we devised the apparatus which is shown in the following drawings (Figs. 1, 2, and 3).<sup>1</sup>

This apparatus consists of two parts: first, a clockwork released by an electromagnet and carrying an arm with platinum points which

<sup>1</sup> The somewhat similar apparatus devised by Rihl (*Zeitschrift für experimentelle Pathologie und Therapie*, 1906, ii, p. 533) suitable only for the production of continuous bigemini, could not be used for our purposes.

pass through two mercury cups on the base (Figs. 1 and 2); and secondly, an ordinary receiving tambour modified so as to close an electrical circuit with each down-stroke of the recording pen (Fig. 3). *M* (Fig. 3), is a steel cup containing mercury. The metal arm bearing this cup extends only to the hard rubber piece, *R*, the other end of

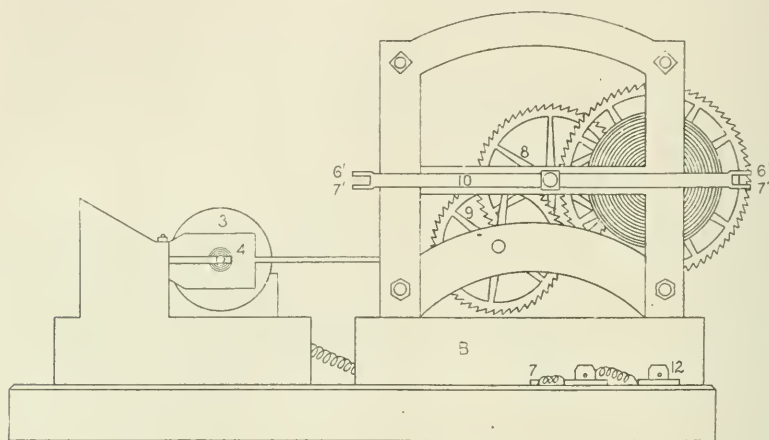


FIGURE 2.—Side elevation of apparatus for the production of extrasystoles.

which is connected with the tambour by a metal arm. One terminal wire, *A*, is soldered to the metal bar bearing the mercury cup, the other, *B*, to the metal arm carrying the writing lever. On the aluminum writing lever, about one third the distance from the end, is fastened a small platinum wire, which on the down-stroke of the lever dips into the mercury cup. The whole apparatus is of metal with the exception of the hard-rubber connection, *R*. This serves to insulate the two terminals from each other, the only possible connection being therefore by means of the mercury cup and the platinum wire connected with the writing arm.

This tambour is connected with the rubber tube leading to the transmitting tambour attached to the right auricle, and records the contractions of this chamber on the record. With each systole of the right auricle, the writing lever descends, and the platinum wire makes contact with the mercury in the cup. This closes the circuit through the wires *A* and *B*. These wires pass to the binding posts (1 and 2) of the apparatus shown in the first two figures. A battery is arranged in this circuit, so that with each systole of the right auricle the electro-magnet (3) is activated and attracts the metal

arm (4) in its field. *B* (Figs. 1 and 2) is a clockwork with the escapement removed, mounted on a base and bearing a metal arm which carries two platinum points on each end. These pass through the two metal cups (6 and 7) twice with each complete revolution of wheel 8 of the clock. Wheel 8 connects with wheel 9, and the latter is of such a size as to make exactly eight revolutions while wheel 8 makes one. At two opposite points on wheel 9 there are catches which are released or held by the metal bar (4) attached to the base of the electro-magnet, depending upon whether the latter

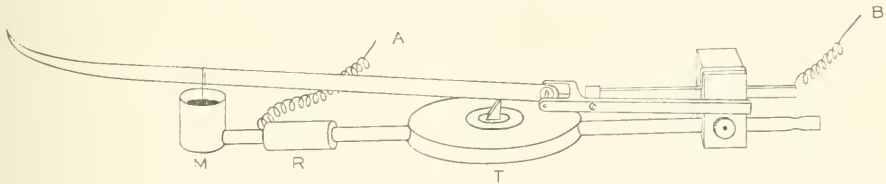


FIGURE 3.— Modified tambour for the production of extrasystoles.

attracts it or not. With each beat of the right auricle, therefore, the catch on wheel 9 is released, and the wheel makes one half of a revolution before being caught by the next catch. The large wheel 8 during this time and the arm that it bears (10) makes one sixteenth of a complete revolution. Thus, in sixteen releases of wheel 9, wheel 8 makes one complete revolution, and the platinum points on the arm (10) pass twice through the steel cups (6 and 7). These cups are filled with mercury and connected with the binding posts 11 and 12. The two platinum points on each end of the bar are set at a slight angle, so that one (7') enters one cup (7), and also leaves it before the other (6') enters and leaves the other cup (6).

Binding post 13 is connected with the metal framework of the clock, and there is a free electrical connection between it and the metal bar and the platinum points that it bears. Binding posts 12 and 13 are connected with the primary of a large DuBois Reymond coil, and a battery and a magneto-marking signal are interposed in the circuit. Binding posts 11 and 13 are connected with wires which interpose a short circuit in the secondary of this coil. From binding post 13, therefore, two wires lead, one going to one end of the primary, the other to one end of the secondary. From the secondary of the coil, finally, wires lead to a platinum stimulating electrode.

With the apparatus connected as described, the electrode is placed upon that portion of the heart which one wishes to stimulate. With

each beat of the right auricle, the bar 4 releases the catch, and the arm 10 makes one-sixteenth of a revolution. During every eighth beat its platinum points pass through the mercury cups. As they are set at a slight angle, the point 7' passes into the mercury of cup 7 before the other point (6') makes contact with the mercury of cup 6. The secondary is thus short circuited when the primary circuit is closed. When the primary is broken, however, by the platinum point 6' leaving the mercury of cup 6, point 7' has already cleared cup 7, so that the secondary is open and the breaking stimulus reaches the electrodes. By this means, the same principle as is employed in the Baltzer drum, only break shocks occur. Bar 10 is movable on the arm (5) of wheel 8, and may be so adjusted that the breaking of the primary may occur at any desired interval after release. The incidence of the stimulus after the previous auricular contraction is thus regulated, and if the clockwork is not run too long without re-winding, such incidence is constant for succeeding extrasystoles. A fine adjustment of the incidence is also possible by adjusting the writing lever of the tambour (Fig. 3) so that the platinum point that it carries will make contact with the mercury earlier or later in the auricular contraction. Interposed in the primary circuit of the coil is a magneto-signal pen which marks on the record. When platinum point 6' enters cup 6, this pen marks a down-stroke, and when it leaves cup 6, an up-stroke. The latter marks the instant of stimulation, and thus records graphically the incidence of the stimulus.

By means of this apparatus it was possible to produce a series of extrasystoles of the heart by break induction shocks thrown in at any desired phase of the cardiac cycle, and each extrasystole of a series would be separated from the last preceding and the next following extrasystole by seven normal cardiac cycles.

Series of extrasystoles were obtained with incidences of stimuli varying from the refractory period to late in the diastolic phase. The time on all records was recorded by a Jacquet chronograph marking one-fifth seconds. Careful starting marks were made, and the curves afterwards measured, and the results reduced to fractions of a second and tabulated.

A number of experiments upon excised hearts fed by oxygenated Ringer's solution were also performed, and the results, so far as they bear upon the present problem, will be reported here.

Extrasystoles were also produced by mechanical stimuli in some experiments.



PLACE OF ORIGIN OF THE CARDIAC IMPULSE AND THE  
PATH OF CONDUCTION.

Until quite recently, the view that the cardiac impulse in the mammalian heart has the same origin and course as in the heart of the cold-blooded animals has been almost free from attack. The classical description of the contraction arising at the mouths of the great veins, and thence sweeping over the auricles and ventricles, is to be found in almost all text-books of physiology. Within the last year much attention has been given by physiologists to that portion of the mammalian heart which represents the remains of the embryonic sinus, and which therefore corresponds anatomically with the sinus of the heart of the cold-blooded animals. Several years ago, Keith (1) concluded, from his anatomical studies, that although the embryonic sinus of the mammalian heart becomes in the adult a part of the auricle by the overgrowth of the musculature of the latter, nevertheless its separate functional activity may be inferred by the fact that when heart rigor is produced by a jet of steam upon the veins and auricles, the contractions occur in such a way as to separate the auricle into two more or less distinct chambers, one opening into the vena cava and the other into the ventricle. We have repeated this experiment of Keith's, and the result is fairly definite as he describes.<sup>1</sup> The musculature, however, of this sinus portion is quite indistinguishable from the rest of the auricle and communicates with it everywhere, interrupted only by what Keith describes as a very indifferently marked fibrous septum.

MacWilliam (2), and within the last year Adam (3), and Langendorff and Lehmann (4), have sought to give a physiological importance to this area. Adam mapped out an area which comprised that portion of the mouth of the superior vena cava and neighboring auricle within which the heart rate was affected by the application of heat and cold. Langendorff and Lehmann more recently have reported the effects of excision of this area. The result of such a procedure, according to these observers, is stoppage of the auricles and ventricles, the latter of which after a time again begin to beat at a

<sup>1</sup> Keith's results may, however, admit of a different explanation. At this point the auricular appendage begins to spread out from the main body of the auricle, and the fold seen in the auricular wall may be merely the one which would naturally occur at such an angle, with no relation whatever to the sinus

different or irregular rhythm. These experiments have been repeated by Erlanger (5), who obtains opposite results. This observer states that unless the cut is made sufficiently deep to include the auricular portion of the auriculo-ventricular bundle, the operation is without effect. In our work we have produced extrasystoles by stimulation of this area in a number of experiments, and so far as we are able to determine from our results, these extrasystoles differ in no way from extrasystoles arising somewhat further up on the superior vena cava, if that portion of the so-called sinus area is stimulated which is



FIGURE 4. — About two fifths the original size. Contractions of the two auricles in the excised heart of a dog. The scratch lines indicate corresponding points. Upper line, right auricle; middle line, left auricle; lower line, time in one-fifth second intervals.

included in the superior vena cava. Likewise there is no definite difference between extrasystoles arising from stimulation of the auricular portion of the sinus and from other portions of the auricle. We have considered it best therefore to designate all of our extrasystoles superior or inferior vena cava extrasystoles and auricular extrasystoles, and to avoid the term sinus extrasystole.

Fredericq, in 1901 (6), stated that in the heart of the dog the right auricular contraction precedes the left by an appreciable interval. In a recent paper (7), he repeats this conclusion, and from this and other observations concludes that the normal cardiac impulse originates in the right auricle and thence spreads over the heart, to the left auricle, the mouths of the vena cavæ, and pulmonary veins. Fredericq's observations were made upon excised hearts. We have obtained a similar sequence between the right and left auricles in experiments upon excised hearts of dogs, and also the reverse, namely, the left auricle contracting before the right. Fig. 4 is a tracing from an excised heart in which the right auricular contraction precedes the left. The same heart may show either sequence.

As a result of our experiments upon hearts *in situ*, however, we are able to state that under normal conditions the contractions of the right and left auricles are separated by no appreciable interval. This may be readily seen in the tracings Figs. 9, 10, and 11. In no experiments

with hearts *in situ* have we been able to observe any difference in the time of contraction of the two auricles, and never has this been seen in excised hearts with vigorous contraction and a rate approaching the normal. It is probable that the difference between the time of contraction of these two chambers observed by Fredericq and ourselves occurs only in dying hearts or when the nutrition is abnormal, as in long experiments upon the excised organ.

In 1897 Engelmann (8) showed in the frog, that when an extrasystole of the heart was produced, the effect upon the cardiac rhythm was dependent upon the portion of the heart to which the extra stimulus was applied. If the stimulus was applied to the sinus or vena cava, the time from the beginning of the last regular systole preceding the extrasystole to the next regular systole following the extrasystole was always less than the interval comprising two regular cycles. Previously he (9) and others had shown that when the stimulus was applied to either the right or left auricle, or to the ventricle, this interval was always exactly equal to the interval comprising two regular cycles. These observations he explained by the cardiac impulse arising at the sinus, the auricles and ventricle during the extrasystole failing to respond to one of the regular impulses of the sinus; they were compelled to await, therefore, the arrival of the next impulse from this chamber before carrying out the next regular contraction. Engelmann speaks of the postextrasystolic pause, therefore, as compensatory, only when the time from the regular systole preceding the extrasystole to the regular systole following the extrasystole is equal to two cardiac cycles. Other writers speak of full and shortened compensatory pauses. The best nomenclature, however, seems to be that of Hering (10), who designates in one word the regular systole, the extrasystole, and the pause following it as a *bigeminus*, and then refers to *full* (*unverkürzte*) and *shortened* (*verkürzte*) *bigemini*. It is in this sense that the terms will be used by us in this paper.

As the result of his researches, Engelmann came to the conclusion that when an extrasystole arose in the portion of the heart that was initiating the prevalent rhythm, as for example the sinus in the cold-blooded heart, shortened bigemini were always present; whereas, when the extrasystole arose in a portion that was simply following a rhythm initiated elsewhere, the true full compensatory pause and full bigeminus occurred. This view has received additional support from the observations of others. Loven (11) had found that when the auricle in the cold-blooded heart was separated from the rest of the organ,

an extrasystole produced in the isolated auricle was followed by a pause equal only to the regular interval. Trendelenburg (12) showed that when the rate of the cold-blooded heart is slowed by cooling, it is possible to produce extrasystoles between the regular ventricular beats which are attended by shortened bigemini, because under these circumstances the regular impulses from the sinus are so far separated that the refractory period produced by the extrasystole is past by the time the next regular impulse reaches the auricle.<sup>1</sup> Erlanger (13) has shown, that although in ordinary ventricular extrasystoles occurring in the mammalian heart full ventricular bigemini are present, shortened ventricular bigemini occur in extrasystoles produced in a ventricle beating spontaneously during heart block. Langendorff and Lehmann (4), in their observations upon the effect of excising the sinus area in mammalian hearts, state that in this condition ventricular extrasystoles are attended by shortened bigemini, while before the operation such bigemini were full.

In extrasystoles arising from stimulation of the auricle of the mammalian heart, the bigeminus, however, is often shorter than double the normal cycle. Cushny and Matthews (14) have shown that this is especially true of extrasystoles arising from stimuli applied to the auricle soon after its regular contraction. Full bigemini may occur as a result of stimuli late in the diastolic phase of the previous contraction. Rihl (15) has also published auricular extrasystoles showing full bigemini. Extrasystoles arising, however, from ventricular stimulation are attended under normal conditions by full bigemini. These experimental conclusions have received clinical confirmation from the venous tracings of Mackenzie (16), Wenckebach (17), and others. Cushny and Matthews (14) state that the shortening of the bigeminus is more marked when the stimulus is applied to the great veins than when it is applied to the auricles.

Assuming that the impulse in the mammalian heart originates in the vein or sinus region, at least two explanations have been offered as to the cause of the premature contraction of the auricle in shortened bigemini arising from auricular stimulation. It has been supposed that an impulse as a result of the extrasystole passes from the auricles to the great veins and causes them to contract. This impulse returns

<sup>1</sup> Similar "Interpolated Extrasystoles" have been obtained by J. Rihl (15) upon the mammalian heart; but as the above explanation evidently holds for this group of extrasystoles and differentiates them from the extrasystoles followed by pauses, only the latter group will be considered in this paper.

to the auricles and causes a premature systole of these chambers (Cushny and Matthews). This reverse rhythm occurs more readily in the mammalian heart than in the cold-blooded heart, because in the former the "sinus" region is "fused" with the auricle (Wencke-

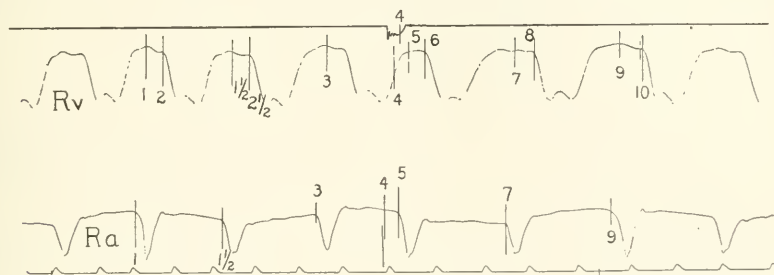


FIGURE 5. — Two thirds the original size. Full bigemini in an extrasystole arising from stimulation of the right auricle. Uppermost line, magneto-signal pen; second line from top, right ventricle; third line, right auricle; lowermost line, time in one-fifth second intervals. The occurrence of the stimulus is given by the up-stroke of the magneto-signal pen, and is marked on the record by 4. The incidence of the stimulus is the time from the tip of the previous auricular contraction to this point. The latent periods of the auricles and ventricles are the times from this line to the auricular and ventricular contractions respectively. The "Double Cardiac Cycle" is the time represented by the distance between the lines 1 and 3. "Regular Systole plus Extrasystole" is represented between the lines 3 and 7, and the "Extrasystolic Cycle" is represented between the lines 5 and 7. The shortening of the bigemini is the difference between the double cardiac cycle and regular systole plus extrasystole. The normal auriculo-ventricular conduction lies between the lines 1 and 2 and between the lines 3 and  $3\frac{1}{2}$  and 9 and 10; the extrasystolic auriculo-ventricular conduction between the lines 5 and 6. The numbers on all the figures (with the exception of Fig. 11) have the same reference as given for this figure.

bach) (18). Cushny and Matthews also suggest that possibly the irritability of the auricle may gradually increase until it culminates in a contraction independent of impulses coming from the great veins.

In our experiments we have obtained not only auricular extrasystoles with full bigemini, but also extrasystoles arising from stimulation of the superior and inferior vena cavæ in which the bigemini was equal to two regular cardiac cycles. Fig. 5 is an example of the former and Fig. 6 of the latter (superior vena cava). An examination of Tables I and II, in which the arrangement is such as to show the changes that occur with a gradual advancement of the incidence of the stimulus in the diastolic phase, shows that extrasystoles with full bigemini occur only when the stimulus giving rise to

TABLE I.  
 VARYING INCIDENCE OF STIMULUS.—SUPERIOR VENA CAVA.

Incidence of stimulus in diastolic phase of R. A.	Latent period.			Double cardiac cycle.	Regular systole + extrasystole.	Extrasystole of K. A.	Shortening of bigeminus.	Double cardiac cycle divided by extrasystolic cycle.	Auriculo-ventricular conduction.			Remarks.
	R. A.	L. A.	R. V.						Normal.	Extrasystole.	Difference.	
0.037	....	....	0.16	....	....	....	....	....	....	....	....	No extrasystole of auricle, but compensatory pause. Extrasystole of ventricle. No extrasystole.
0.038	....	....	....	....	....	....	....	....	....	....	....	No extrasystole of auricle, but compensatory pause. Extrasystole of ventricle. No extrasystole.
0.038	0.13	0.13	0.21	0.72	0.59	0.37	0.13	1.9	0.06	0.07	0.01	Strength of stimulus increased. Follows the preceding extrasystole. Extrasystole No. 212.
0.04	0.13	....	0.20	0.73	0.60	0.40	0.13	1.87	0.07	0.07	....	No extrasystole.
0.05	....	....	....	....	....	....	....	....	....	....	....	No extrasystole.
0.05	....	....	....	....	....	....	....	....	....	....	....	No extrasystole.
0.05	0.125	0.125	0.21	0.80	0.63	0.41	0.17	1.95	0.065	0.10	0.035	No extrasystole of auricle but compensatory pause. Same.
0.05	....	....	0.23	....	....	....	....	....	....	....	....	No extrasystole.
0.055	....	....	0.20	0.82	0.66	....	0.16	....	....	....	....	No extrasystole of auricle but compensatory pause. Same.
0.057	0.105	....	0.20	0.72	0.60	0.38	0.12	1.9	0.07	0.09	0.02	No extrasystole of auricle but compensatory pause. Same.
0.06	0.125	0.125	0.20	0.92	0.78	0.50	0.14	1.87	0.06	0.10	0.04	No extrasystole of auricle but compensatory pause. Same.
0.06	0.125	0.125	?	0.73	0.59	0.36	0.14	1.9	0.07	?	?	No extrasystole of auricle but compensatory pause. Same.
0.06	0.10	....	....	....	....	....	....	....	0.07	0.10	0.03	No extrasystole of auricle but compensatory pause. Same.
0.067	0.12	....	0.21	0.88	0.74	....	0.14	....	0.054	0.09	0.04	No extrasystole of auricle but compensatory pause. Same.

0.07	0.10	....	0.20	0.74	0.58	....	0.16	....	0.08	0.11	0.03
0.075	0.08	....	0.21	0.80	0.69	....	0.11	....	0.12	0.14	0.02
0.08	0.12	0.12	0.20	0.93	0.80	0.57	0.13	1.65	0.07	0.11	0.04
0.08	0.13	0.13	0.21	0.93	0.80	0.57	0.13	1.65	0.055	0.09	0.04
0.08	0.11	0.12	0.21	0.94	0.80	0.53	0.14	1.81	0.04	0.10	0.06
0.085	0.085	....	0.20	....	....	....	....	....	0.098	0.12	0.02
0.09	0.08	....	0.17	0.74	0.62	0.38	0.12	1.95	0.07	0.087	0.017
0.09	0.09	....	0.18	0.74	0.62	0.38	0.12	1.95	0.07	0.09	0.02
0.09	0.078	0.078	0.15	0.70	0.67	0.40	0.03	1.75	0.05	0.06	0.01
0.10	0.09	....	0.20	0.80	0.67	....	0.13	....	0.07	0.10	0.03
0.10	0.09	....	0.19	0.76	0.69	0.40	0.07	1.9	0.07	0.10	0.03
0.125	0.09	....	0.175	0.80	0.67	0.40	0.13	2.0	0.08	0.08	....
0.125	0.09	....	0.175	0.80	0.67	0.40	0.13	2.0	0.07	0.08	0.01
0.14	0.08	....	0.15	0.72	0.66	0.40	0.06	1.8	0.065	0.065	....
0.145	0.08	....	0.145	0.70	0.67	0.39	0.03	1.775	0.08	0.08	....
0.145	0.075	....	?	0.72	0.68	0.38	0.04	1.88	....	?	?
0.155	0.09	....	0.155	0.76	0.70	0.40	0.06	1.9	0.06	0.06	....
0.155	0.09	....	0.15	0.78	0.71	0.40	0.07	1.95	0.06	0.06	....
0.16	0.06	....	0.14	0.74	0.66	0.41	0.08	1.925	0.09	0.09	....
0.20	0.045	....	0.13	0.78	0.78	0.47	....	1.67	0.078	0.09	0.01
0.22	0.025	....	0.12	0.79	0.81	0.50	....	1.58	0.098	0.098	....

Full bigeminus.

Same.

TABLE II.  
VARYING INCIDENCE OF STIMULUS — RIGHT AURICLE.

Incidence of stimulus in diastolic phase of R. A.	Latent period.			Double cardiac cycle.	Regular systole + extra-systole.	Extra-systolic cycle of R. A.	Shortening of the bigeminalus.	Double cardiac cycle divided by extra-systolic cycle.	Auriculo-ventricular conduction.			Remarks.
	R. A.	L. A.	R. V.						Normal.	Extra-systole.	Difference.	
0.034	....	....	0.18	0.75	....	0.58	0.17	....	0.06	....	....	No extrasystole, but compensatory pause of auricle. Extrasystole of ventricle. Same.
0.035	....	....	0.18	0.60	....	0.54	0.06	....	0.07	....	....	Same.
0.04	0.105	0.125	0.21	0.77	0.59	0.42	0.18	1.83	0.05	0.10	0.05	After atropine.
0.04	0.10	0.12	0.19	0.77	0.59	0.42	0.18	1.83	0.05	0.10	0.05	Same.
0.045	0.10	0.125	0.20	0.76	0.61	0.42	0.15	1.81	0.05	0.10	0.05	Same.
0.045	....	....	0.18	0.71	....	0.58	0.13	....	0.08	....	....	Without atropine; no extrasystole of auricle, but compensatory pause. Same.
0.05	....	....	0.20	....	....	....	....	....	0.07	....	....	After atropine.
0.05	0.07	0.10	0.175	0.76	0.59	0.36	0.17	2.1	0.05	0.10	0.05	Same.
0.05	0.085	0.12	0.18	0.72	0.59	0.36	0.13	2.0	0.05	0.10	0.05	Same.
0.055	0.08	0.10	0.175	0.70	0.57	0.38	0.13	1.85	0.06	0.09	0.03	Same.
0.065	0.072	....	....	....	....	....	....	....	0.086	0.105	0.02	Same.
0.07	0.10	....	0.16	0.78	....	....	....	....	0.076	0.10	0.024	Same.
0.07	....	....	0.16	....	....	....	....	....	0.07	0.105	0.035	Same.



0.08	0.055	0.038	0.14	0.70	0.58	0.38	0.12	1.85	0.055	0.08	0.025	After atropine.
0.08	0.055	0.08	0.15	0.71	0.58	0.39	0.13	1.85	0.05	0.08	0.03	
0.085	0.07	....	0.17	0.74	0.60	0.40	0.14	1.85	0.07	0.09	0.02	
0.09	0.06	....	0.17	0.78	0.68	0.47	0.10	1.75	0.08	0.12	0.04	
0.09	0.08	....	0.17	0.72	0.60	0.38	0.12	1.88	0.07	0.09	0.02	
0.10	0.068	....	0.18	0.76	0.60	0.40	0.16	1.9	0.07	0.09	0.02	
0.11	0.07	....	0.16	0.79	0.61	0.37	0.18	2.13	0.06	0.08	0.02	
0.115	0.05	....	0.14	0.79	0.66	0.42	0.07	1.8	....	....	....	
0.12	0.07	....	0.18	0.76	0.69	0.42	0.07	1.8	....	....	....	
0.13	0.04	....	0.165	....	....	....	....	....	0.075	....	....	
0.14	0.08	....	0.15	0.72	0.66	0.40	0.06	1.8	0.065	0.065	....	
0.14	0.084	....	0.14	0.72	0.67	0.36	0.05	2.0	0.07	0.07	....	
0.15	0.055	....	0.133	0.80	....	....	....	....	0.08	0.08	....	
0.155	0.055	....	0.133	0.76	0.71	0.44	0.05	1.73	0.07	0.07	....	
0.16	0.035	....	....	0.76	0.70	0.45	0.06	1.68	0.09	0.09	....	
0.17	0.04	....	0.08	0.71	0.65	....	0.06	....	0.07	0.07	....	
0.20	0.06	....	0.15	0.82	0.78	0.45	0.04	1.82	0.08	0.085	0.005	
0.21	0.07	....	0.14	0.73	0.73	0.40	....	1.825	0.07	0.07	....	Full bigeminus.
0.235	?	....	0.15	0.87	0.89	0.45	....	1.85	....	....	....	Same.
0.24	....	....	....	0.86	0.86	0.46	....	1.85	0.09	0.09	....	Same.

the extrasystole occurs late in the diastolic phase, twenty hundredths of a second or more after the summit of the previous auricular contraction. All early auricular and venous extrasystoles, on the other hand, are accompanied by shortened bigemini. In Table III are two extrasystoles with full bigemini arising from mechanical stimulation, one from stimulation of the superior vena cava and one from stimulation of the right auricle. The incidence of stimulus is not given here,

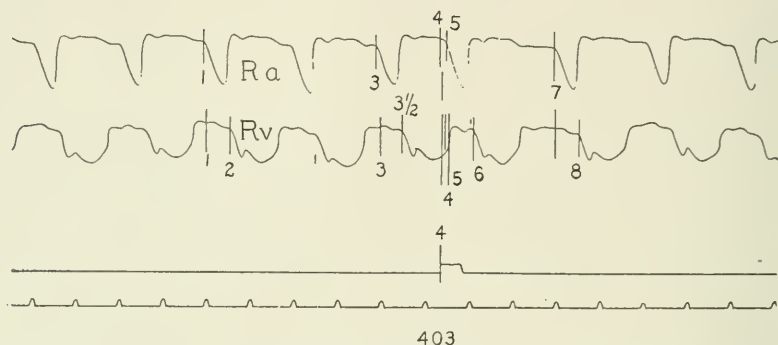


FIGURE 6. — Two thirds the original size. Full bigemini in an extrasystole arising from stimulation (break induction shock) of the superior vena cava. Uppermost line, right auricle; second line from top, right ventricle; third line, tracing of magneto-signal; lowermost line, time in one-fifth second intervals. In this tracing the rise of the curve of the magneto pen denotes the instant of stimulation. For explanation of numbers, see Fig. 5.

but from the first column of the table, which gives the incidence of the extrasystole after the summit of the previous auricular contraction (equal to incidence of stimulus plus latent period in the other tables), it may be seen that they are the latest extrasystoles recorded and occur near the end of the diastolic phase.

Thus the question of full and shortened venous and auricular bigemini in the mammalian heart depends upon the time of the extrasystole after the preceding regular contraction, and so far as our results show, it is impossible by this method to determine which portion of the heart initiates the cardiac impulse. Extrasystoles arising from all portions of the auricles and superior and inferior vena cavæ are similar so far as the question of full and shortened bigemini is concerned.

As to the origin and direction of the wave of contraction in the mammalian heart, our observations have led to nothing conclusive. We have observed the dying hearts of a large number of animals. In only three have we seen definite and unmistakable contractions of

TABLE III.  
MECHANICAL EXTRASYSTOLES. — SUPERIOR VENA CAVA AND RIGHT AURICLE.

Time of occurrence of extra-systole of R. A. after beginning of diastolic phase of R. A.	Double cardiac cycle.	Regular systole + extra-systole.	Extrasystolic cycle of R. A.	Shortening of bigeminus.	Double cardiac cycle divided by extrasystolic cycle.	Auriculo-ventricular conduction.			Remarks.
						Normal.	Extra-systole.	Difference.	
0.14	0.86	0.68	0.44	0.18	2.18	0.08	0.13	0.05	Superior vena cava.
0.15	0.78	0.66	0.45	0.12	1.74	0.08	0.14	0.06	Same.
0.185	1.00	0.90	0.57	0.10	1.75	0.08	0.11	0.03	Same.
0.28	0.80	0.78	0.50	0.02	1.60	0.08	0.09	0.01	Same.
0.29	0.98	0.98	0.57	....	1.725	0.08	0.08	....	Same. Full bigeminus.
0.175	0.82	0.76	0.53	0.06	1.55	0.07	0.13	0.06	Right auricle.
0.24	0.88	0.88	0.55	....	1.60	0.08	0.08	....	Right auricle. Full bigeminus.

the great veins, — one in the dying heart of a dog, the other two in the dying hearts of cats. In the first the vein and auricles seemed to be beating each with an independent rhythm, sometimes one preceding, sometimes the other. In one of the cats the auricles and ventricles had ceased to beat before the appreciable contractions of the great veins commenced, and did not again begin upon the occurrence of the latter. The contractions of the great veins, however, were very clear, and there was what seemed to be definitely 2 : 1, 3 : 1, and 4 : 1 rhythms at different times between the superior and inferior vena cava and the portions of the auricles in the neighborhood of their mouths. The contractions observed in the third cat were unimportant and also occurred after complete stoppage of the other chambers of the heart. In view of the large number of dying hearts observed by us and others, especially Hering, it is rather remarkable that if the great veins contract under normal conditions, such contractions in the dying heart should not be more frequently observed. Hering (19) reports several animals in which contractions of the great veins occurred, some of them without corresponding contractions of the auricles. August Hofmann (20), to explain the sudden halving of the pulse rate at the cessation of an attack of paroxysmal tachycardia, assumes a condition of sino-auricular block, and cases have been reported by Wenckebach (21), Hirschfelder (22), and Hewlett (23) in which there was an occasional interval between two auricular beats equal to two normal auricular cycles. The assumption has been in these cases that in this long interval in which the auricle did not beat, there was a sinus beat, which due to the sino-auricular condition did not reach the auricle. Recently Hering (24) has also reported similar tracings from animals, which however throw no further light upon the subject, as he does not state whether venous contractions could be observed in the long intervals.

Keith (1) has shown that there are definite muscular bands passing from the superior vena cava to the auricles, which might well serve as conducting paths. These are not collected into any one definite bundle, but there are several fairly well-defined bundles and in addition a rather diffuse muscular connection. One of these bundles, upon the anterior and mesial surface of the superior vena cava, apparently arises from the auricles and splits into two bands which embrace the vena cava, forming a sphincter-like band just above the mouth. This bundle is assumed by Wenckebach (21) to be the chief if not the sole path of impulses from the great veins to the auricles,

analogous in every way to the auriculo-ventricular bundle. In careful dissections of this region, especially in preparations macerated in Krehl's nitro-acid-glycerine mixture, it is evident that there are at least two additional muscular strands equally as large and anatomically as important passing between the vein and auricles, and there seems to be little reason to regard Wenckebach's bundle as the sole path for the impulse. The evidence that we have at present for sino-auricular block consists entirely in an eliminated auricular and ventricular contraction in an otherwise regular heart rhythm. The question as to whether the great veins in the mammalian heart contract at all under normal conditions is still entirely unsettled, and too much weight should not be given to such evidence as has been derived from dying hearts. The evidence in favor of sino-auricular block is not so valuable as it would be were this question settled in the affirmative.<sup>1</sup>

Our experiments have, however, shown that there is a physiological as well as anatomical connection between the great veins and auricles, and that an extrasystole arising in the former may be conducted to the auricles physiologically. This is shown in the first place by the fact that there is a definite veno-auricular interval (two hundredths of a second or longer) for such extrasystoles. If an incidence of stimulation is found which when applied to the auricles will fall just within the refractory phase and hence fail to give rise to an extrasystole, this same incidence, if the stimulus is now applied to the superior vena cava, will cause an extrasystole of the auricles, due to the fact that because of the time consumed for the conduction of the impulse from vein to auricle, the impulse reaches the latter after the refractory period. The same stimulus to cause an extrasystole when applied to the auricle must fall into this chamber two hundredths to three hundredths of a second later. If the conduction from the vein to auricle were simply electrical spread of stimulus, no appreciable conduction period would of course be present. The interval of veno-auricular conduction is further shown by the difference of the latent period of the auricle and ventricle in extrasystoles arising on one hand, from a stimulus to the vein, and on the other hand from a stimulus to the

<sup>1</sup> R. FISCHER (*Archiv für experimentelle Pathologie und Pharmacologie*, 1897, xxxviii, p. 228) finds that faradization of the heart (ventricles) in frogs causes acceleration with the occurrence of ventricular pauses equal to two regular beats. These tracings are similar to those obtained by Wenckebach, Hering, and others in the supposed condition of sino-auricular block.

auricle. In the latter case the latent period preceding the contractions of the auricles and ventricles is several hundredths of a second longer than in the former. This difference may be seen by a comparison of the latent periods in Tables I and II with the same incidence of stimuli, or in Table IV, where averages of a number of extrasystoles of approximately equal length are given. Furthermore, that the impulse arising from a stimulus to the vein is conducted physiologically to the auricles is shown by the fact that

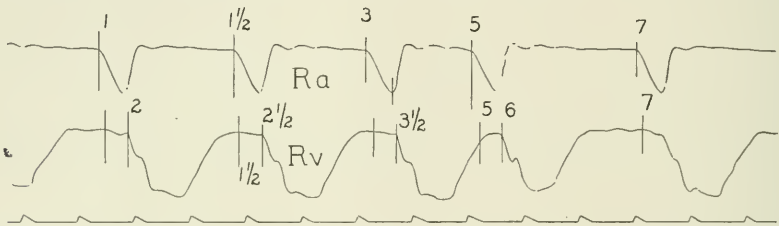


FIGURE 7.—Four fifths the original size. Extrasystole with full bigemini from mechanical stimulation of the superior vena cava. Upper line, right auricle; middle line, right ventricle; lower line, time in one-fifth second intervals. For explanation of numbers see Fig. 5.

such conduction does not occur if the wall of the vein close to the auricle is crushed in a clamp, continuity being, however, well preserved so as not to interfere with electrical conductivity. Under such conditions stimuli of any moderate strength or incidence applied just above the crushed area fail to give an extrasystole. Applied however below the crushed area, the same stimulus is effective and gives rise to an extrasystole. The difference in distance cannot be of any importance, for before the clamp was applied, stimulation much further up on the vein was effective in giving rise to an extrasystole. Finally, extrasystole of the auricle and ventricle may arise from mechanical stimulation of the superior vena cava, as is shown in Table III and Figure 7. It is therefore evident that while we have no direct evidence that the great veins do contract first, or even contract at all under normal conditions, there is a path or paths connecting the vein and auricles, which is capable of physiologically conducting an impulse arising in the great veins to the auricle.

#### CHARACTERISTICS OF MAMMALIAN EXTRASYSTOLES.

**Full and shortened bigemini.**—As has been stated above, auricular and venous extrasystoles with full bigemini occur only when the

TABLE IV.  
TABLE OF AVERAGES OF EXTRASYSTOLES WITH SAME INCIDENCE OF STIMULUS.

Incidence of stimulus in diastolic phase of R. A.	Latent period.			Double cardiac cycle.	Regular systole + extra-systole.	Extra-systolic cycle of R. A.	Shortening of bigeminus.	Double cardiac cycle divided by extra-systolic cycle.	Auriculo-ventricular conduction.			Remarks.
	R. A.	L. A.	R. V.						Normal.	Extra-systole.	Difference.	
0.063	0.106	0.106	0.205	0.81	0.68	0.45	0.13	1.825	0.069	0.108	0.039	Superior vena cava. Incidence, 0.04 to 0.08.
0.062	0.08	0.102	0.169	0.73	0.60	0.385	0.13	1.9	0.064	0.1135	0.049	Right auricle. Same.
0.108	0.088	....	0.18	0.79	0.685	0.40	0.105	1.97	0.07	0.084	0.014	Superior vena cava. Incidence 0.1 to 0.13.
0.112	0.06	....	0.16	0.775	0.64	0.41	0.13	1.825	0.072	0.091	0.019	Right auricle. Same.
0.178	0.074	....	0.14	0.75	0.71	0.42	0.04	1.775	0.077	0.079	0.002	Superior vena cava. Incidence 0.15 to 0.25.
0.183	0.053	....	0.13	0.78	0.75	0.444	0.03	1.775	0.081	0.081	....	Right auricle. Same.

stimulus and the resulting extrasystole are late in the diastolic phase of the previous contraction. An examination of column eight of Tables I and II will show that the shortening, that is to say, the difference between the length of the regular systole plus extrasystole and a double cardiac cycle, is in general greatest with the early extrasystoles, and, though not without exceptions, a fairly regular decrease of the shortening occurs as the incidence of the stimulus in the diastolic phase is advanced.

**Compensatory pause.**—In the seventh column of the tables is given the extrasystolic cycle, and, for comparison, in the ninth column is shown the result of dividing the length of the double cardiac cycle by the extrasystolic cycle. In certain cases the time from the beginning of the extrasystole to the end of its diastolic phase (extrasystolic cycle) is only equal to or less than a single cardiac cycle, and hence a "compensatory" pause does not exist. As a rule, however, the extrasystolic cycle is somewhat longer than a single cardiac cycle, and this is true for extrasystoles arising from stimulation of the superior vena cava as well as from stimulation of the auricle. Measurement of a large number of extrasystoles has failed to show any definite relation between the length of the extrasystole and its diastole and the amount of shortening of the bigeminus. There seems also to be no definite difference in this length in relation to the time of the extrasystole in the diastolic phase of the previous regular contraction. The quotient in column nine of the tables, obtained by dividing the double cardiac cycle by the extrasystolic cycle, is fairly constant, and shows no regular variation with the incidence of stimulus causing the extrasystole nor with the amount of shortening present. The diastolic phase of the extrasystole is usually designated as the compensatory pause. The length of systole in the very early extrasystoles is somewhat shorter than in later extrasystoles; but this difference is small, and its effect upon the comparison of the length of the compensatory pause by means of the length of the extrasystolic cycle may be neglected. We may therefore conclude that whatever may be the mechanism of production of the compensatory pause, its degree of action is not influenced by the length of the previous diastole of the heart.

Of considerable interest is the production of a compensatory pause in the auricle without preceding extrasystole by means of a stimulus occurring very early in the diastolic phase. Examples of this are given in Tables I and II and in Figure 8.



The auricle gives rise to no measurable or visible contraction, but a definite pause occurs. There is an extrasystole of the ventricles followed by a pause, and the auricle then again beats and normal cardiac cycles return. An incidence of stimulus slightly earlier causes no extrasystole and no pause; an incidence slightly later

results in an extrasystole of both auricles and ventricles. This same phenomenon has been observed by Meyer (25), by Cushny and Matthews (14), and recently by Schultz (26), the last observer in strips of cold-blooded hearts. Meyer considered the phenomenon of compensatory pause without preceding extrasystole to be a process of inhibition. Cushny and Matthews, however, observed it in atropinized hearts. Our results are negative so far as this point is concerned. We attempted

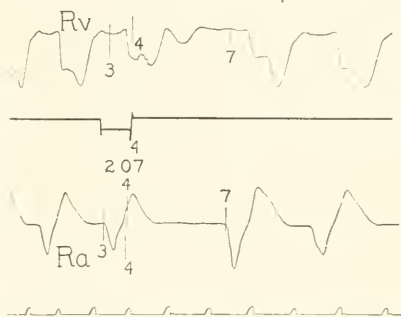


FIGURE 8.—Two thirds the original size  
Compensatory (?) pause of auricle without preceding extrasystole.

in a number of experiments to obtain this phenomenon after atropine by a careful adjustment of the incidence of stimulus, but without success. An incidence of stimulus which before atropinization gave a compensatory pause without extrasystole, always caused after the latter a definite extrasystole. There seem to be no other definite effects of atropine upon extrasystoles. The ordinary compensatory pauses are not affected in length. A number of extrasystoles after atropinization are given in Table II.

**Size of extrasystoles.**—The size of early extrasystoles is always less than a normal contraction. With our system of recording, it is impossible to say definitely as to when the normal size is reached, but it seems to be within the first third of the diastolic phase of the preceding contraction. The earliest extrasystoles are very small, and there is a rapid increase to normal as the incidence is advanced. We can draw no conclusions from our tracings as to the next regular systole following the extrasystole ("postextrasystolicsystole"). A recent paper by Rihl (27) tends to show that the increase in size of this beat that is usually observed does not occur in extrasystoles produced in heart strips, and that the cause of the increase observed in hearts *in situ* is therefore probably due to the greater filling of the auricles during the compensatory pause.

**Conduction intervals and latent periods.**—The interval consumed in veno-auricular conduction in extrasystoles, measured by the difference in latent period of the right auricle in vein stimulation and in auricular stimulation, varies between two hundredths and four hundredths of a second.

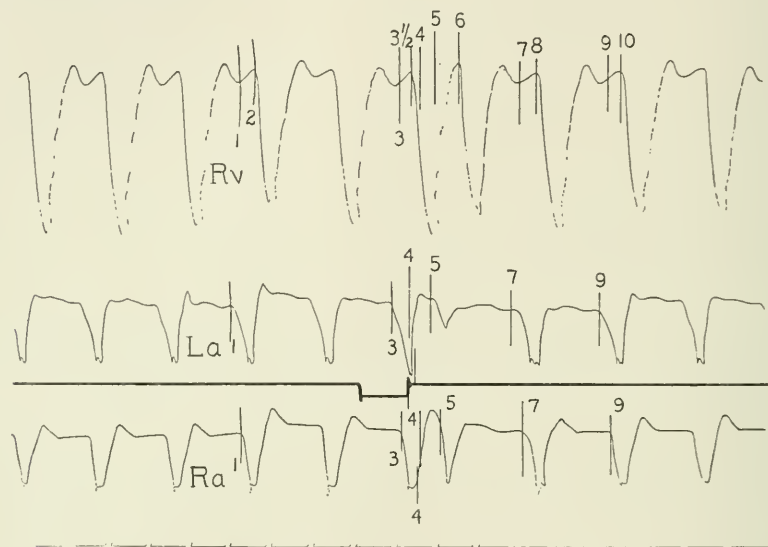


FIGURE 9.—Two thirds the original size. Early extrasystole with prolonged extrasystolic auriculo-ventricular conduction. Stimulus applied to the right auricle. Uppermost line, right ventricle; second line from top, left auricle; third, tracing of magneto-pen; fourth, right auricle; fifth, time in one-fifth second intervals. For explanation of numbers, see Fig. 5.

The normal auriculo-ventricular conduction in the dog's and cat's heart varies between five hundredths and ten hundredths of a second, with an average of about seventy-five thousandths. The A-V conduction in early extrasystoles shows a nearly constant lengthening. (The only exceptions to this rule in our experiments occurred in one dog with remarkably irritable heart.) This prolongation over the normal varies from one hundredth to six hundredths of a second. Though not without certain exceptions, it may be stated that the greatest increase in length of the A-V conduction occurs in the very early extrasystoles, and a gradual decrease of the prolongation occurs as the incidence of the stimulus is advanced in the diastolic phase. When the incidence of the stimulus falls twelve hundredths to fourteen hundredths of a second or later in the diastolic phase of the right

auricle, the extrasystolic A-V conduction is not prolonged over the normal. The results upon which these conclusions are based are given in the tables. Fig. 9 is an example of an early extrasystole with prolonged extrasystolic A-V conduction. Fig. 10 is an example of a late extrasystole with a normal A-V extrasystolic conduction.

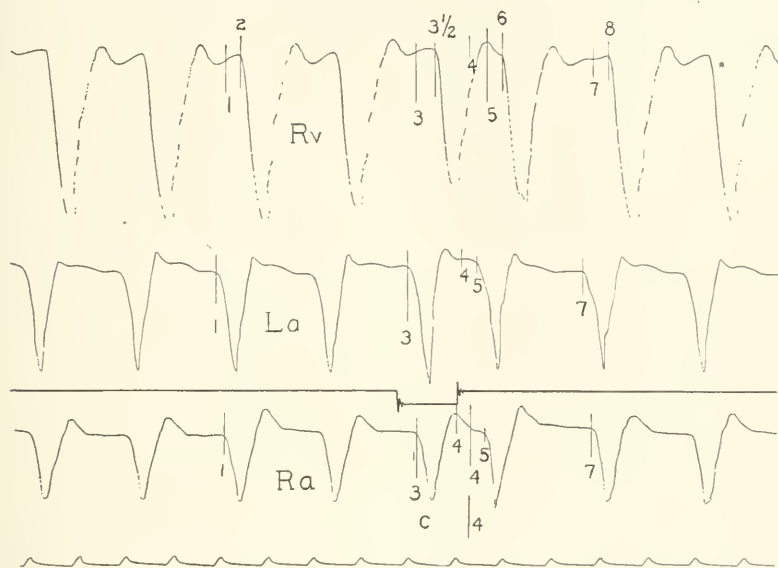


FIGURE 10.—About four fifths the original size. Late extrasystole with normal extrasystolic auriculo-ventricular conduction. Stimulus applied to right auricle. Curves as in Fig. 9. For explanation of numbers, see Fig. 5.

Fig. 11 shows a series of extrasystoles arising from a mechanical stimulus. The heart was not being electrically stimulated at this time, and the tracing of the magneto-pen is to be disregarded.

The A-V conduction (between the lines 2 and 3 on the tracing) increases with each extrasystole, and drops back to normal or nearly normal in the first subsequent regular systole.

In our opinion the lengthening of the A-V conduction in early extrasystoles is an expression of the lowering of the conductivity of the auriculo-ventricular bundle as the result of the passage of the previous cardiac impulse. The diastolic phase of the right auricle is from thirty hundredths to thirty-five hundredths of a second long in the dog's heart beating at normal rate, and hence it is apparent that the depression of conductivity passes off within the first third of the diastolic period. This lengthening of A-V conduction in extrasystoles

may be of some clinical importance. Such delayed conduction has been considered as definite evidence of permanent disturbance of auriculo-ventricular conductivity, whereas in many cases it may be due solely to the occurrence of extrasystoles. Before making a diagnosis of impaired conductivity, it is therefore of importance to observe whether the prolonged A-V intervals accompany regular or extrasystoles, as in the latter case it may be of no clinical significance.

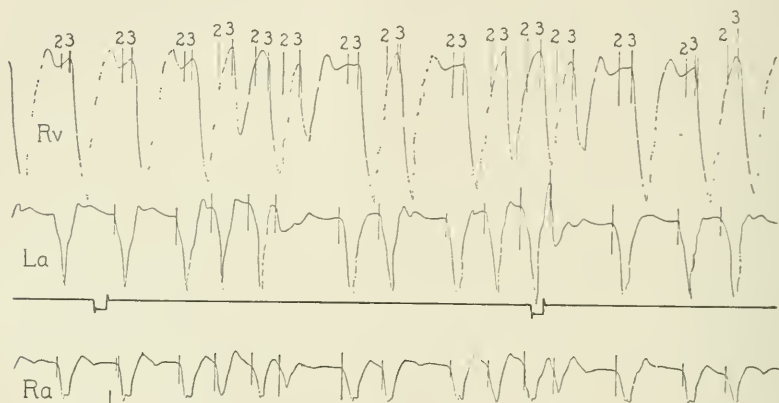


FIGURE 11. — One half the original size. Series of extrasystoles from mechanical stimulation of the right auricle. Shows prolonged auriculo-ventricular conduction accompanying the extrasystoles. Curves as in Fig. 9. The A-V conduction periods lie between the lines 2 and 3.

Mackenzie (28), v. Tabara (29), and Hewlett (30) have shown that digitalis diminishes conductivity, and hence is contraindicated in conditions in which this function is already impaired. According to our evidence, such contraindication is only present with a regularly beating heart or with extrasystoles occurring late in diastole.

The latent period of the auricle to stimuli applied directly to it, varies from five hundredths to twelve hundredths of a second, and this value is greatest in the very early extrasystoles and decreases as the incidence is advanced in the diastolic phase. This may be seen in the second column of Table II. This difference is also apparent in the latent period of the ventricle upon auricular stimulation. We have here, however, two factors acting in the same direction as the incidence of the stimulus advances: first, the decrease in the latent period of the auricle, and, second, the gradual decrease of the prolongation of the A-V conduction. The difference in the latent period

of the auricle in vein stimulation and in stimuli applied directly to it, has been brought out above. In stimuli applied to the vein, the contractions of the two auricles, so far as we can determine from our records, is simultaneous. In stimuli applied to either auricle it is often possible to observe a small difference in the time of contraction of the two auricles, that is, a short interval of *interauricular* conduction.

**Refractory period.** — With submaximal stimuli the earliest stimulus which will cause an extrasystole is about four hundredths of a second after the beginning of the diastolic phase of the right auricle, measured from the tip of the previous auricular contraction. The incidence necessary is less when the stimulus is applied to the superior or inferior vena cava than when it is applied to the auricle, due to the interval of conduction from vein to auricle. In other words, an incidence of stimulus, which if thrown into the auricle would fall within the refractory period, may, if thrown into the vein, reach the auricle after the refractory period has passed. This suggests the possibility that the refractory period of the vein, if such is present, passes off before that of the auricle, but with our present knowledge it is dangerous to carry this speculation too far.

The refractory period may be decreased by increase of the strength of stimulus. An example of this is given in the second and third extrasystoles of Table I. Similar observations have been made by Schultz (31) in hearts of cold-blooded animals. This observer recognizes two divisions, — a variable refractory period which may be made to disappear with increase of stimuli, and an absolute refractory period which remains constant towards all electrical stimuli.

#### CONCLUSIONS.

1. The rule formulated by Engelmann for the cold-blooded heart to differentiate between autogenetic and contractile heart tissue by means of extrasystoles, has yielded only negative results in our hands when applied to the mammalian heart. We are unable to determine by this method that portion of the mammalian heart from which the impulse arises. The so-called sinus region of the dog's and cat's heart shows no difference in this respect from other portions of the auricles and great veins.

2. Extrasystoles of the mammalian heart show full or shortened bigemini according as the extrasystole falls late or early in the

diastolic phase of the previous contraction. This applies to extrasystoles arising from stimulation of the great veins as well as to those from stimulation of the auricles.

3. There is a physiological as well as anatomical path connecting the superior vena cava with the two auricles, which conducts an impulse arising in the former to the latter according to physiological laws.

4. The auriculo-ventricular conduction is prolonged in early extrasystoles of auricular and venous origin, and becomes normal in extrasystoles occurring after the first third of the diastolic period.

5. The two auricles of the mammalian heart under normal conditions contract simultaneously. In dying hearts either the one or the other may precede.

6. Atropinization has no definite effect upon ordinary extrasystoles. We were unable, however, to obtain the phenomenon of compensatory pause without preceding extrasystole in these experiments.

7. The length of the compensatory pause in extrasystoles of auricular and venous origin is quite constant and does not seem to vary with the incidence of the extrasystole or with the region from which the extrasystole arises.

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## CONCERNING THE NEUTRALITY OF PROTOPLASM.

BY L. J. HENDERSON AND O. F. BLACK.

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

IT has been pointed out by one of us<sup>1</sup> that the phosphates of protoplasm must be concerned in the neutralization of acid produced by or introduced into the cell, and that they should be able to accomplish this result with very little change in hydrogen ionization even when considerable amounts of acid have to be taken care of. As a result of this idea it has seemed desirable, both because of the normal production of acid substances in metabolism, in particular sulphuric acid and phosphoric acid, and because of the great importance of the pathological production of acid substances, to endeavor to study the changes in equilibrium which occur in protoplasm upon the addition of acid. Clearly it is necessary to proceed by slow degrees in such an investigation, commencing with a system composed of a few only of the constituents of protoplasm, those which for theoretical reasons seem to be probably most concerned in the matter, and after the simpler equilibrium has been made clear, by the addition one by one of other substances, to determine their effect. In the end it should be possible to test conclusions with some such preparation as the expressed juice of muscle.

For several reasons an aqueous solution made up of mixtures of phosphoric acid, carbonic acid, and sodium or potassium hydroxide in varying amounts, seems a suitable subject of study for the purpose of collecting preliminary information, and it has been employed in the present investigation.

The results of the investigation indicate that in the presence of free carbonic acid it is impossible to obtain a solution which contains less than one molecule of mono-sodium or mono-potassium phosphate for every nine molecules of di-sodium or di-potassium phosphate, and on the other hand that sodium bi-carbonate cannot exist in consid-

<sup>1</sup> HENDERSON: This journal, 1906, xv, pp. 257-271.



erable amount in a solution containing more than one molecule of mono-sodium or mono-potassium phosphate for every molecule of di-sodium or di-potassium phosphate, but within these limits, or somewhat narrower ones so far as the phosphates are concerned, wide variation can occur. The presence of varying amounts of sodium sulphate seems to be without material effect upon the equilibrium. If in such systems the amount of base is relatively great, the amount of combined carbonic acid is also great, and little salt of the form  $\text{NaH}_2\text{PO}_4$  exists; if the amount of base is relatively small, little carbonic acid is taken up by the solution, and the amount of salt of the form  $\text{NaH}_2\text{PO}_4$  is relatively great. The tension of carbonic acid seems to have little effect upon the equilibrium. At room temperature all such mixtures give a faint pink color with rosolic acid corresponding to a nearly constant hydrogen ionization of  $1 \times 10^{-7}$ . According to the determinations of Salm<sup>1</sup> mixtures of mono- and di-sodium phosphates varying between the molecular proportions 5:5 and 1:9 possess a hydrogen ionization not less than about  $0.5 \times 10^{-7}$  and not more than about  $1 \times 10^{-7}$ .

Even without more accurate determinations than the present ones it seems certain that the hydrogen and hydroxyl ionization of protoplasm must be much more constant than that of blood plasma as measured by recent investigators;<sup>2</sup> indeed, variation of more than about  $5.0 \times 10^{-7}$ —that is to say, a variation in the mass of hydrogen ions of five parts in ten billion parts of protoplasm or an equivalent variation in hydroxyl ions—seems quite impossible in the presence of both free and combined carbonic acid and phosphates. Starting with such a mixture, consisting of relatively little phosphoric acid and relatively much base, the addition of strong acid produces no appreciable effect upon the hydrogen ionization of the solution until all of the combined carbonic acid has been driven off and a ratio of mono- to di-phosphate nearly 5:5 established. Thus, if a mixture in the ratio of one molecule of phosphoric acid and three molecules of sodium hydrate be saturated with carbonic acid, a precisely neutral solution results. To this solution may then be added 1.5 molecules of hydrochloric acid without appreciably upsetting the neutrality of the reaction. According to these conclusions the justness of the neutrality of protoplasm must far surpass the accuracy of adjustment of any other known equilibrium in the body, and at the same time

<sup>1</sup> SALM: *Zeitschrift für physikalische Chemie*, 1906, lvii, p. 471.

<sup>2</sup> See, for instance, SZILI: *Archiv für die gesammte Physiologie*, 1906, cxi, p. 82.

the system must be able to neutralize relatively enormous quantities of acid without becoming acid. On the other hand, the presence of an excess of carbonic acid must be sufficient absolutely to prevent even the slightest alkalinity if there were a tendency in that direction. Quite possibly the range of neutrality in the system is greater at body temperature than at 20°, and it may further be extended by the co-operation of other substances,— a possibility which is now being investigated in this laboratory.

Blood plasma containing almost no phosphate may present a somewhat different case to that here considered, and it is possible that in such a case much greater variation in hydrogen ionization may occur.

#### EXPERIMENTAL.

A series of preliminary experiments were made to determine the condition of equilibrium in dilute solutions of di-sodium and di-potassium phosphates in contact with carbonic acid of different tensions. The experiments were carried out by saturating solutions of the phosphates at room temperature with mixtures of carbonic acid and air carefully prepared, containing respectively 30, 60, and 100 per cent carbonic acid at a tension of 76 cm. of mercury. In the resulting solutions carbonic acid was estimated by treating 25 c.c. of the solution with acid and collecting the carbonic acid given off in an ordinary potash bulb according to the usual method of organic analysis. Another portion of the solution was tested with indicators according to the method of Salm.<sup>1</sup> In every case the final mixtures prepared at room temperature gave with rosolic acid the faint pink color which according to Salm corresponds to a hydrogen ionization of  $10^{-7}$ , and, as we have found in confirmation of Salm's determinations, corresponds to a mixture of mono- and di-sodium phosphates in the molecular ratio 4.5 : 5.5 and to mixtures varying not a little from this ratio in either direction. The preparations were then in all cases neutral; that is to say, they contained less than  $10^{-6}$  hydrogen ions and less than  $10^{-6}$  hydroxyl ions. The data of the preliminary experiments are collected in Table I.

Evidently in the above solutions an adjustment of equilibrium, varying little with carbonic acid tension, such that the solution contains somewhat more di-potassium or di-sodium phosphate than monosodium or mono-potassium phosphate is established. The above

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

results are, however, far from accurate, because of the great dilution of the solution and in particular because of doubt concerning the amount of dissolved carbonic acid. Slight variations in temperature also occurred, and these somewhat affect the accuracy of the results.

TABLE I.

Solution.	Tension CO <sub>2</sub> .	Wt. CO <sub>2</sub> found.	Dis-solved carbonic acid.	Com-bined carbonic acid.	Concentration NaHCO <sub>3</sub> .	Molecular ratio.				
						Na <sub>2</sub> HPO <sub>4</sub> :	NaH <sub>2</sub> P <sub>2</sub> O <sub>4</sub> :	NaHCO <sub>3</sub> .		
K <sub>2</sub> HPO <sub>4</sub>	cm. 22	0.031	0.013	0.018	$0.49 \times \frac{n}{10}$	51	:	49	:	49
"	44	(1) 0.037	0.025	0.012	$0.33 \times \frac{n}{10}$	67	:	33	:	33
"	"	(2) 0.035	0.025	0.010	$0.27 \times \frac{n}{10}$	73	:	27	:	27
"	76	0.057	0.042	0.015	$0.41 \times \frac{n}{10}$	59	:	41	:	41
Na <sub>2</sub> HPO <sub>4</sub>	22	0.025	0.013	0.012	$0.47 \times \frac{0.7n}{10}$	53	:	47	:	47
"	44	0.035	0.025	0.010	$0.39 \times \frac{0.7n}{10}$	61	:	39	:	39
"	76	0.052	0.042	0.010	$0.39 \times \frac{0.7n}{10}$	61	:	39	:	39

In the following experiments more concentrated solutions were employed, and at the same time the ratio of base to acid was varied by adding to a mixture of di-sodium phosphate and sodium bicarbonate varying amounts of sulphuric acid. In other respects the experiments were carried out as above. The experiments are tabulated below. In all cases except the last, in which combined carbonic acid was not present in considerable amount, the reaction was precisely neutral with rosolic acid.

Clearly, in the presence of relatively much base and relatively little acid, equilibrium is so adjusted that there is a relatively great amount of di-sodium phosphate compared with mono-sodium phosphate, while in the presence of less base and more acid there is present but little more of the di-sodium phosphate than of the mono-sodium phosphate. On the whole, however, the variation in the ratio is not great, it corresponds to neutral mixtures of pure phosphates which can still neutralize much acid without becoming acid in reaction, and the variation in the sodium bicarbonate content of the solutions is much greater.

An accurate study of this equilibrium is now being pursued in this laboratory; with the aid of the results to be gathered in the course of

this investigation it is hoped that the conditions of equilibrium in solutions made up of sodium hydrate, phosphoric acid, and carbonic acid at a temperature of 40° will be accurately established.

TABLE II.

Composition of solution in moles.	Wt. CO <sub>2</sub> found.	Dis-solved CO <sub>2</sub> .	Com-bined CO <sub>2</sub> .	Molecular ratio.						
				NaH <sub>2</sub> PO <sub>4</sub> : Na <sub>2</sub> HPO <sub>4</sub> : NaHCO <sub>3</sub> : Na <sub>2</sub> SO <sub>4</sub> .						
I. { Na 0.6 m PO <sub>4</sub> 0.2 m SO <sub>4</sub> 0.033 m	gm. (1) 0.205	gm. 0.021	gm. 0.184	} 26	:	174	:	160	:	67
	(2) 0.191	0.021	0.170							
	(3) 0.199	0.021	0.178							
II. { Na 0.6 m PO <sub>4</sub> 0.2 m SO <sub>4</sub> 0.067 m	(1) 0.160	0.021	0.139	} 48	:	152	:	114	:	133
	(2) 0.137	0.021	0.116							
	(3) 0.149	0.021	0.128							
III. { Na 0.6 m PO <sub>4</sub> 0.2 m SO <sub>4</sub> 0.1 m	....	....	....	} 48	:	152	:	48	:	200
	(1) 0.076	0.021	0.055							
IV. { Na 0.6 m PO <sub>4</sub> 0.2 m SO <sub>4</sub> 0.133 m	....	....	....	} 86	:	114	:	21	:	267
	(1) 0.045	0.021	0.024							
V. { Na 0.6 m PO <sub>4</sub> 0.2 m SO <sub>4</sub> 0.166 m	....	....	....	} .....	:	....	:	....	:	....
	0.025	....	....							
	....	....	....							

We are greatly indebted to the Proctor Fund for aid in this investigation.

## SUMMARY.

It is shown that in the presence of both free and combined carbonic acid at 20° mono- and di-sodium phosphates can exist only in molecular proportions varying between 1:9 and 5:5 approximately. All such solutions are precisely neutral, of hydrogen ionization  $1 \times 10^{-7}$  nearly, according to the method of Salm, and according to Salm's determinations of the hydrogen ionization of phosphate solutions the hydrogen or hydroxyl ionization in mixtures of mono- and di-sodium phosphates in such proportions cannot vary more than about  $5 \times 10^{-7}$ .

Accordingly protoplasm is extraordinarily safeguarded, by the presence of phosphates and carbonates in considerable amount, from

variation in hydrogen or hydroxyl ionization. This can hardly amount to more than five parts in ten billion parts of protoplasm. The occurrence of alkalinity is absolutely prevented by the presence of free carbonic acid, and the system can neutralize relatively enormous quantities of acid without losing its precise neutrality.

## THE MECHANISM OF EXPERIMENTAL GLYCOSURIA.

BY HUGH MCGUIGAN AND CLYDE BROOKS.

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THE glycosuria caused by lesions of the nervous system, decreased ability of the body to consume sugar, an increased production of sugar in the body, extirpation of the pancreas, the introduction of drugs, salts, or sugar, by anæsthetics, asphyxia, or other means, is not generally believed to have the same mechanism. It is thought by some that the mechanism may differ not only with different means of production, but also with the same drug when administered by different methods. We believe that the mechanism is essentially the same in each case.

Brown,<sup>1</sup> in 1903, showed that the glycosuria produced by the intravenous injection of sodium chloride could be inhibited by the intravenous injection of a small amount of calcium chloride. This work was corroborated by Fischer<sup>2</sup> and by McCallum.<sup>3</sup> Fischer observed that when sodium salts were injected directly into the arterial system the glycosuria was greater and more quickly recognized than when the injection was made into the venous system. From this he concluded that there were two factors involved in the mechanism: one, the action on the kidney by which the diuresis was induced; second, an influence on the diabetic centre in the bulb which caused the glycosuria. He believes that while the factors involved in the production of diabetes differ, the type is the same. Underhill and Closson<sup>4</sup> think that the deductions of Fischer are drawn from insufficient evidence. From a study of the sugar content of the blood they believe that the mechanism differs, depending upon whether the salt is introduced into the artery (axillary) or into the vein

<sup>1</sup> BROWN: This journal, 1904, x, p. 378.

<sup>2</sup> FISCHER: *Archiv für die gesammte Physiologie*, 1905, cix, p. 1.

<sup>3</sup> MCCALLUM: *University of California Publications*, 1904, cvi, p. 80.

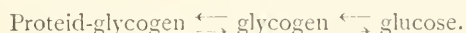
<sup>4</sup> UNDERHILL and CLOSSON: This journal, 1906, xv, p. 321.

(femoral). When injected into the vein, they think there is a probable increase in the permeability of the kidney, with polyuria and hypoglycæmia. When the injection is made into the artery, there is hyperglycæmia, but no polyuria. The increased sugar content of the blood they think may be referred to respiratory changes provoked by the introduction of the salt.

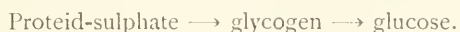
Brown, Fischer, McCallum, Underhill and Closson all give definite results to prove that calcium chloride has a marked influence in inhibiting glycosuria. Sollmann<sup>1</sup> has shown that calcium chloride will decrease the permeability of both the living and the dead kidney. Aside from these statements, no adequate explanation or theory of how calcium chloride inhibits glycosuria has been given.

It seems to us that a study of the physiological and chemical properties of the calcium salt offers a clue to the explanation of the mechanism. All the authors quoted agree that calcium salts will inhibit glycosuria. We have been able to confirm this result. There are several methods by which the inhibition may take place. The most probable are: (1) preventing an excessive sugar formation; (2) rendering the kidney impermeable to sugar; (3) forming a compound with the glycogen or sugar and so preventing the elimination of sugar by the kidneys; (4) stimulating the body to a greater consumption of sugar.

The glycogen of the liver, in all probability, exists in loose combination with colloid as proteid-glycogen. There is evidence that the sugar of the blood also exists in combination, as will be shown later. In ordinary metabolism the liberation of the glycogen may be represented<sup>2</sup> by the formula:



When salts causing glycosuria are injected, it is conceivable that the reaction is hastened, and using sodium sulphate as a type we get:



The action of the salt then is to increase the liberation of the glycogen, which is rapidly hydrolyzed to sugar with glycosuria as a result. The injection of the salt may also cause a simultaneous decrease in the oxidative energy of the organism, both factors leading

<sup>1</sup> SOLLMANN: This journal, 1906, xv, p. 324. *Ibid.*, 1905, xiii, p. 782.

<sup>2</sup> Suggestion of Professor A. P. MATHEWS.

to hyperglycæmia; but decrease in oxidation is not necessary for such a condition. So far we have been unable to demonstrate a decrease in the oxidative energy of the tissues of dogs that were highly glycosuric. Neither is hyperglycæmia necessary in the production of glycosuria, although it is usually found when glycosuria is demonstrable. In all the cases examined by us we found an increase in the sugar content of the blood. The real essential for glycosuria, however, is the presence of uncombined sugar in the blood. When this is present even in minute quantities, sugar passes into the urine. This may be easily and quickly shown if an injection of sugar solution is made into the renal artery. When this is done, sugar appears in the corresponding ureter before it appears in the other; but not all the sugar injected is excreted. This is in part due to the fact that the proteid of the blood, as a rule, retains the power to hold more sugar in combination than the amount that is normally found in it. Any salt that breaks down the proteid-glycogen compound will cause an increase in the sugar of the blood, and glycosuria. On the other hand, anything that prevents the breaking down of the compound, or that combines with glycogen and so renders its conversion into sugar less rapid, or anything that will combine with free sugar in the blood, will decrease or stop the elimination of sugar. Anything that will render the kidney less permeable may hinder somewhat the elimination of sugar, but the permeability of the kidney is of small import in the mechanism of experimental glycosuria, as will be shown in this paper.

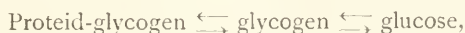
Calcium and some other salts combine with glycogen to form a compound  $\text{CaC}_{12}\text{H}_{18}\text{O}_{10}$ . This compound is broken down by  $\text{Na}_2\text{SO}_4$ ,  $\text{NaCl}$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{CO}_2$ , or even water; in fact, by any of the salts ordinarily used to produce glycosuria. The fact that  $\text{CO}_2$  will break down the glycogen compound will explain the glycosuria of asphyxia. The mechanism will also explain the results of Fischer and Underhill with sodium chloride. If the salt acts only on the proteid-glycogen compound without interfering with the respiration, less sugar will be formed than if the respiratory centre is also involved. If the centre is involved, we have the added effect of  $\text{NaCl}$  plus the effect of  $\text{CO}_2$ . This is more likely to be the case if the injection is made close to the respiratory centre. Calcium chloride will inhibit sugar formation, either by forming the compound  $\text{CaC}_{12}\text{H}_{18}\text{O}_{10}$ , or since calcium also combines with proteid it will more probably form a compound with both proteid and glycogen,  $\text{Ca} \begin{cases} \text{proteid} \\ \text{glycogen.} \end{cases}$  Neilson and



Terry<sup>1</sup> found that  $\text{CaCl}_2$  in certain concentration will inhibit the transformation of glycogen into sugar. They call attention to this fact as agreeing with the action of calcium salts in experimental glycosuria. In rather large quantities calcium salts do inhibit glycolysis. We find, however, that the small quantity that is ordinarily used to inhibit glycosuria will hasten rather than retard the action of ptyalin on either starch or glycogen. A larger amount will inhibit the action of ptyalin, but the concentration is more than is used to inhibit glycosuria. The mechanism, therefore, of the action of calcium in the inhibition of glycosuria is not due to its action on the diastatic enzymes. The more probable action is in retarding the elimination of glycogen from its compounds. This retardation is due to the affinity of calcium for glycogen and proteid. Calcium very probably combines also with free sugar in the same manner as it does with glycogen. Our experiments show that the excretion of sugar after the intravenous injection of calcium is less than if no calcium had been added, while the permeability of the kidney for sugar is unchanged.

#### EXPERIMENTS.

There is little to be gained by the determination of the amount of sugar in the blood. Most observers agree that there is an increase in the quantity. Hyperglycæmia, however, is not necessary for glycosuria. In the blood of several dogs which we examined we found an average of 3.1 parts reducing sugar per 1000 blood. If we assume that the formation of sugar takes place according to the formula



the questions to be answered are: 1. Does the glycosuria-producing salt act principally on the proteid-glycogen compound, liberating glycogen, or is its action of more importance in hastening the conversion of glycogen into glucose? 2. In the inhibition of glycosuria does the calcium chloride prevent the breaking of the proteid-glycogen compound, or does it inhibit the formation of glucose from the glycogen already free? 3. Is the action of calcium of most importance in decreasing the permeability of the kidney?

On hydrolysis glycogen yields a series of dextrans and maltose, as does starch. Either starch or dextrin, therefore, may be used to

<sup>1</sup> NEILSON and TERRY: This journal, 1905, xiv, p. 105.

determine the action of sodium sulphate or calcium chloride on the diastatic enzymes.

#### METHODS.

A 1 per cent starch paste was prepared by boiling 1 gm. of starch in water and making the volume 100 c.c. Saliva was collected, diluted one-half with water and filtered. The digestion was done in test tubes. In each case 5 c.c. of the starch paste was used. A known volume of the salt to be tested was added with water to make the volume 9 c.c. in each case; then 1 c.c. of the saliva was added, making the final volume 10 c.c. The time when digestion was complete was shown by the negative test with iodine. In cases where digestion was stopped before completion, the amount of sugar formed was estimated with Fehling's solution, using the starch iodine indicator as per method given in Sutton's Volumetric Analysis. The following table shows the results with sodium sulphate:

No. of tube.	$\text{Na}_2\text{SO}_4$ $\frac{m}{8}$ . c.c.	Volume of Fehling's solution reduced. c.c.
1.	0.2	6.2
2.	0.5	6.4
3.	2.0	6.3
4.	4.0	6.6
5.	1.0 gm. solid.	5.8
6.	control	6.0

The digestion was continued for five minutes at room temperature (23° C.). The action of the ferment was stopped by boiling in water. The titrations as given in the table are in harmony with others found, and can be taken as average results. They show that moderate or even large quantities of sodium sulphate accelerate the action of ptyalin on starch. The amount of acceleration varies with the amount of the salt used. Nasse<sup>1</sup> found a maximum acceleration when the concentration of the salt was about 4 per cent. The acceleration *per se* is probably not the cause of the glycosuria, as will be shown below.

**Action of calcium chloride on the diastatic enzymes.**—Starch was taken in the same amounts as before, and calcium chloride used instead of sodium sulphate.

<sup>1</sup> NASSE: Archiv für die gesammte Physiologie, 1875, xi. p. 155.

No. of tube.	CaCl <sub>2</sub> $\frac{m}{8}$ . c.c.	Volume of Fehling's solution reduced. c.c.
1.	0.5	1.4
2.	1.0	1.2
3.	2.0	0.8
4.	3.0	0.4
5.	control	1.8

Duplicates gave concordant results.

The action of calcium on a 1 per cent glycogen solution was determined in the same manner, using the following concentrations of the calcium salt :

No. of tube.	CaCl <sub>2</sub> $\frac{m}{8}$ c.c.	Volume of Fehling's solution reduced. c.c.	
I.	1.	0.2	2.25
	2.	0.5	1.80
	3.	1.0	1.80
	4.	2.0	1.30
	5.	3.0	1.0
	6.	3.0 $\frac{m}{4}$	trace
	7.	control	1.80
II.	1.	control	1.2
	2.	0.2	1.8
	3.	1.0	0.5
	4.	2.0	0.2
	5.	4.0	trace

The digestion in the first series was continued for five minutes, in the second series four minutes. Before titration the calcium was removed with sodium carbonate. Other trials gave similar results. It is seen from these that small amounts of calcium hasten, while larger amounts retard, digestion. It can hardly be said, however, that calcium chloride, in inhibiting glycosuria, does so by checking glycolysis, as the amount that will inhibit glycosuria will hasten glycolysis. The concentration used to inhibit glycosuria is about 25 c.c. of  $\frac{3}{8}$  Mol. made to 1000 c.c. with 0.9 per cent sodium chloride solution. Some other mechanism than the action on ferments must explain the action. Further, mixtures of calcium chloride and sodium sulphate will cause a more rapid digestion than will either salt by itself. This also speaks against the theory that the action of calcium is on the enzymes.

The pathology of experimental glycosuria is more probably due to changes in the protoplasmic activity of the cell. Proteid by itself, without the co-operation of ferments, has a marked influence on diastatic action.

**Influence of albumen on the digestion of glycogen.**—If the calcium-proteid-glycogen compound is broken down, we may ask what action the products would have on the formation of sugar from glycogen. As seen above, small amounts of calcium have an accelerating effect on the formation of sugar. A larger quantity has a retarding action. To test the action of proteid on glycolysis, egg white and blood serum were used. Egg white was mixed with an equal quantity of 1 per cent glycogen solution. The action of saliva on this solution was compared with its action on a one-half per cent pure glycogen solution of equal volume. After digestion had proceeded for a definite time, the action of the ferment was stopped by heating. The proteid was removed by basic lead acetate, and the excess of lead by sodium sulphate. The amount of sugar was determined by titration with Fehling's solution. In every case the solution which had contained the albumen showed a greater amount of sugar than the other. For example, after digestion had proceeded for three minutes 10 c.c. of each solution gave the following titration :

10 c.c. proteid-glycogen solution reduced	3.0 c.c. Fehling's solution.
10 c.c. glycogen " "	1.2 c.c. " "

After digestion for five minutes—

10 c.c. proteid-glycogen solution reduced	4.5 c.c. Fehling's solution.
10 c.c. glycogen " "	3.0 c.c. " "

All of the proteid was removed. So the difference was not due to the reducing property of the proteid. The small reducing substance of the egg white can also be neglected. Serum gave similar results.

Other observers have obtained similar results.<sup>1</sup>

Similar results were obtained when starch was used instead of glycogen. Proteids are thus seen to have an accelerating action on sugar formation. This action may be of the utmost importance in explaining the mechanism of glycosuria. Chemical changes in the cell protoplasm which neither injure the vitality of the enzymes nor

<sup>1</sup> LANGLEY and EVES: *Journal of physiology*, 1882, iv, p. 18. Also CHITTENDEN and SMITH, *HAMMERSTEN'S Text book of physiological chemistry* (MANDEL), 1906, p. 292.

change the permeability of the kidney, may be sufficient to explain the inability of the body to utilize sugar, and may also explain the mechanism of glycosuria. The presence of proteid also lessens the influence of calcium on enzymes, and sustains the claim that the action of calcium in glycosuria is not due to its action on enzymes.

**The influence of proteid on calcium in salivary digestion.**— To determine this action a 1 per cent starch paste was prepared as above. To a series of tubes containing 5 c.c. starch solution, 5 c.c. of distilled water was added. In a parallel series instead of water 5 c.c. of egg white was added. All were made to the same volume, and 1 c.c. of filtered saliva added. After digesting for five minutes at room temperature, the contents of the tubes were heated in boiling water to destroy the enzyme. The proteid and calcium was removed as above, and after removal of the excess of lead, titrated with Fehling's solution. Each set of tubes contained the following amounts of calcium chloride:

No. of tube.	$\frac{m}{g}$ CaCl c.c.	Amount of Fehling's solution reduced by 10 c.c. of	
		Starch solution. c.c.	Proteid starch solution. c.c.
1.	control.	0.6	1.4
2.	0.2	1.5	2.0
3.	0.5	1.3	2.2
4.	1.0	0.5	2.0
5.	2.0	trace.	1.5

From this it is seen that proteid protects the ptyalin from the action of calcium. Any concentration of calcium that has an inhibiting influence on starch paste alone, in the presence of proteid has an accelerating effect. The amount of calcium necessary to inhibit glycosuria is thus rendered inert by the proteid so far as inhibition of the enzyme is concerned. Any action on the diastatic enzymes, under these conditions, must be of an accelerating character. Its action therefore in glycosuria must be due to the compounds which it forms with glycogen and proteid. There is a probability also that calcium combines with free sugar to form a calcium-proteid glucosate. The results of the intravenous injection render this opinion tenable, for when sugar is injected intravenously its excretion in the urine is lessened by the injection of calcium. If, however, after the sugar has disappeared from the urine, a small quantity of sugar be injected into the renal artery, sugar can be demonstrated in the corresponding ureter with the first urine collected. However, all of the sugar

injected will not be eliminated. This shows that the normal blood has power to combine with some of the free sugar, and that the calcium salts apparently aid this process.<sup>1</sup> While the sugar is in the free state it easily and rapidly passes through the kidney. But the action of the calcium in preventing the elimination is not by changing the permeability of the kidney.

**Influence of calcium on the permeability of the kidney.** — Calcium chloride solutions injected intravenously decrease the flow of urine, and to that extent the permeability of the kidney is decreased. If sugar solution be now injected, its passage into the urine is apparently little changed. The following preliminary experiments bear out this assertion.

*Experiment 1.* — Dog. Female. Weight 4.5 kilos. Ether anæsthesia. In twenty minutes 40 c.c.  $\frac{m}{4}$  CaCl<sub>2</sub> was run into the jugular vein. A decrease in the flow of urine followed. A sugar solution (10 c.c. 2 per cent dextrose) was injected into the renal artery. The urine was flowing at the rate of 1 c.c. every five minutes and did not contain sugar. After injection of the sugar solution, when 1 c.c. of urine had run from the ureters, sugar was easily demonstrable in it.

It may be claimed that the amount of calcium chloride used was not sufficient to block the filtration of sugar through the kidney, and that if more had been used no sugar would have appeared. The amount used, however, was sufficient to cause a much smaller flow of urine, and as no glycosuric salt had been added, the action of the calcium was not lessened in that way. The next experiment precludes such objections.

*Experiment 2.* — Rabbit. Weight 2100 gm. Urethane anæsthesia.

Tmie.	Salt injected. c.c.	Urine. c.c.	Remarks.	
10.45	45 0.9% NaCl.	6.0	No sugar.	
11.12				
11.15				
11.28	25	5.0	No sugar.	
11.40	20	14.0	" "	
11.48	40 c.c. $\frac{m}{4}$ CaCl <sub>2</sub> to 260 c.c. 0.9% NaCl.	10.0	" "	
12.00		20	13.5	" "
12.20		40	40.0	" "
12.28		10	9.0	" "

<sup>1</sup> LOEWI (Archiv für experimental Pathologie und Pharmacologie, 1902, xlviii, p. 410) assumes that the sugar of the blood is normally in loose combination with colloid.

12.29	1	} 1% dextrose in 0.9% NaCl.		
12.32	..		5.0	Sugar.
12.38	..		....	"
12.40	2	} 40 c.c. $\frac{m}{4}$ CaCl <sub>2</sub> to 260 c.c.	4.0	Sugar, large amount.
12.57	15	} 0.9% NaCl.	2.0	" " "
1.20	25		23.0	"
1.34	3	$\frac{m}{4}$ CaCl <sub>2</sub>	3.0	
1.39	12	" "	5.0	Trace sugar.
1.44	10	" "	1.0	
2.04	20	" "	7.0	No sugar.
2.12	50	" "	10.0	(squeezed from bladder — no sugar)
2.15	10	1% dextrose in 0.9% NaCl.		
2.16		sugar in the urine in large quantity.		

All these solutions were run gradually into the jugular vein between the periods of time indicated. The very large amount of calcium chloride failed to stop the passage of sugar into the urine whenever sugar was present uncombined in the blood. The permeability of the kidney, therefore, is of small import in the mechanism of glycosuria. Similar results have been obtained on dogs, but so far we have not used as much calcium chloride per kilo as we have with the rabbits.

#### SUMMARY.

1. Experimental glycosuria is not due to increased ferment activity.
2. Proteids of all kinds accelerate the formation of sugar by the action of ptyalin.
3. If sugar is free in the blood, it passes into the urine readily. The normal sugar of the blood therefore must be in combination with a large molecule which prevents its passage through the kidney epithelium, or else the kidney epithelium has no selective action for this compound.
4. Calcium chloride will not prevent the passage of sugar through the kidney epithelium whenever free sugar exists in the blood.
5. The permeability of the kidney is of small import in the mechanism of experimental glycosuria.
6. The pathology of experimental glycosuria is very probably due to changes in the protoplasmic activity of the cell and is not related to ferment activity.

7. The probable mechanism of experimental glycosuria is an abnormal breaking down of proteid-glycogen compound. All salts that decompose this compound will cause glycosuria. Calcium chloride prevents the decomposition of this compound by the formation of a more stable compound, probably  $\text{Ca} \begin{cases} \text{proteid} \\ \text{glycogen.} \end{cases}$



## THE CAUSE OF THE TREPPE.

BY FREDERIC S. LEE.

[From the Department of Physiology of Columbia University, at the College of Physicians and Surgeons, New York.]

IT happens occasionally, even in an actively progressing science, when investigators are keenly watching every new discovery, ready to test it, explain it, and correlate it with other facts, that a phenomenon may be quoted for years, may be universally acknowledged to be striking, interesting, and important, and yet without falling into oblivion may quite fail to receive the attention that is due it from investigators. Such a phenomenon is the "treppe" or "staircase." In its literal sense this term signifies the fact that the repeated responses of a tissue to repeated and equal stimuli increase for a time in intensity. In a more general sense the phenomenon may be characterized as augmentation of activity resulting from previous activity. That this general fact is true of skeletal muscle seems to have been recognized at about the middle of the last century, for Ranke, writing in 1865, says: "Every one knows that the first twitch of a muscle is not its greatest. With stimuli uniform in strength the later contractions are stronger than the earlier ones—a phenomenon, the reason for which has heretofore been wholly obscure." So, too, in 1866 Marey called attention to the fact that in continued activity the height of the muscle curve at first increases, and then decreases. But to Bowditch is rightly given the credit for having first placed the phenomenon clearly before physiologists, for while studying in Ludwig's laboratory in 1871 the cardiac muscle of the frog, he found increased response to successive and equal stimuli so striking a characteristic of that muscle that he gave much time to its investigation, and moreover dignified it by the name "treppe"—by which it has ever since been known. Since Bowditch's work the treppe has been a recognized physiological phenomenon, and yet it has been investigated comparatively little. In

1875 Tiegel found it in the frog's skeletal muscle; in 1877 Rossbach and Harteneck in the skeletal muscle of various mammals; in the same year Romanes in the contractile tissue of the bell of the medusa. In 1878, in his experiments on the tetanus of frog's skeletal muscle, Minot gave considerable attention to the *treppe*. In 1879 Sewall, in an article entitled "On the Effects of Two Succeeding Stimuli upon Muscular Contraction," published an account of ingenious and clean-cut experiments, performed on the skeletal muscle of the frog with the aid of the pendulum myograph, many of which deal with augmentation of contraction. In 1880 Tigerstedt observed it in the contractions of a skeletal muscle when the nerve was stimulated mechanically, but he ascribed it to the nerve. In 1886, under von Frey's direction in Ludwig's laboratory, Buckmaster discovered an interesting parallelism between the *treppe* and tetanus. Waller (1897) found the *treppe* exhibited in frog's nerve when the electrical effect of excitation was studied. Stirling (1874) and Sherrington (1900) have pointed it out in the central nervous system of the frog and the dog respectively. These various investigators have contributed many interesting facts regarding the physical features of the phenomenon, such as its intensity and duration, its relations to the circulating blood and to the kind, intensity, rate, and direction of application of the stimulus, and its modifications by drugs, fatigue, and rest. But its cause has remained obscure, and few endeavors have been made experimentally to explain it. Ranke did indeed ascribe it to the action of minute quantities of acid, especially lactic acid, on the nervous system; Sewall, to an alteration of the elasticity of the muscle; and Tigerstedt, to an alteration of the elasticity of the nerve supplying the muscle. I find also that Waller has ascribed to carbon dioxide both the staircase which he observes in the electrical response of nerve to excitation and the staircase of muscle. But most investigators have been content to refer the *treppe* to an increased irritability of the acting tissue, caused by activity, and, following their lead, the writers of text-books have taken refuge in the same shibboleth. It may be allowed that increased irritability is present, but when we try to analyze the term "irritability," we find that, however convenient, it offers only a shallow and unsatisfactory refuge. We can, I think, develop the idea of causation somewhat further.

In searching for causes two possibilities at once present themselves: namely, that the acting tissue is benefited either from

chemical substances which are formed within it during katabolism, or from the heat that is at the same time disengaged. From what is known of the influence of external heat in increasing the activity of muscular and nervous tissues, the latter possibility seems not unreasonable, and while as yet I know of no decisive experiments in this direction, I am not prepared to deny to the heat of metabolism a rôle in the causation of the phenomenon. The first mentioned possibility, however, appears at first sight less plausible, from the widespread biological law that the products of metabolic activity are detrimental, rather than beneficial, to the protoplasm in which they are formed. Especially has this been supposed to be true of muscle, the tissue in which the *treppe* is best known, and where the depressing action of the katabolic or fatigue products was pointed out by Ranke long ago. But the universality of this law has been refuted by many recently accumulated facts, and it is in these very fatigue products that I believe that I have found the long-desired solution of the problem.

There is a very general consensus of opinion, based originally upon the work of Ranke, and supported by many later investigations, that the normal fatigue substances are at least three in number, namely, carbon dioxide, mono-potassium phosphate ( $\text{KH}_2\text{PO}_4$ ), and paralactic acid. We can probably accept this opinion as substantially correct in so far as it goes, although there is great need of a detailed investigation of muscular metabolism. It is doubtful whether all the normal fatigue substances have yet been recognized. Intermediate metabolic products may yet be found to play a much greater rôle in altering irritability than has heretofore been suspected. The discovery of a supposed fatigue toxin by Weichardt is still to be confirmed. For the present, however, I have accepted the three classic fatigue substances, and have been endeavoring to fill a gap in our knowledge by a careful study of their physiological action on skeletal muscle and their rôle in the production of fatigue.

I find that they act in ways essentially identical. Such action is, however, of two directly opposite modes, the appearance of the one or the other mode being dependent upon the quantity of the substance that is used and the duration of its activity. If used in what we may term moderate quantity, or in smaller quantity over a longer time, each substance is distinctly fatiguing, its action being characterized by a decrease in the irritability and the working power of the muscle, a lessened height to which the load is lifted, a decrease

in the total amount of work that is performed, and by other phenomena. I have discussed these results briefly in various preliminary papers, and am now preparing a more detailed account of them. The opposite action of the same substances is seen when they are employed in small quantity or in moderate quantity for a brief time. Instead of a diminution of activity, there is an augmentation, which is characterized by an increase in irritability and working power, an increase in the height to which the load is lifted, and an increase in the total amount of work performed — phenomena which are distinctive characteristics of the *treppe*. In the present paper I propose to deal only with this augmenting action of the substances in question.

#### METHOD.

My experiments were performed between the months of October and June inclusive. Both frogs and cats were employed. Before the preparation of the muscle the former were killed by pithing; the latter either were killed at once by decapitation, or were kept under the influence of ether throughout the experiment, and subsequently were killed without recovery. When ether was employed, it was found necessary to use great care in keeping the anesthesia uniform. A sudden increase in the amount of the ether respired causes a fall in the height of the muscle curves. The special muscles that were selected for study are the gastrocnemius and various other muscles of the frog, and the extensor longus digitorum and the tibialis anticus of the cat.

In some of the experiments with cats the muscle was left *in situ* with intact circulation; its two ends were exposed; and it was stimulated directly at regular intervals by break induction shocks, the resulting contractions being recorded graphically. While the record was in progress, the fatigue substance that was to be studied was added to the circulating blood, and its effect was manifested on the tracing.

• In other experiments, instead of maintaining the normal circulation, the animal was first killed and the two corresponding muscles of opposite legs were artificially irrigated, the one with an indifferent liquid, such as 0.7 per cent solution of sodium chloride for frogs, and for cats either 0.9 per cent solution of sodium chloride, or whipped blood, and the other muscle with the same indifferent liquid contain-

ing a known quantity of the fatigue substance. With frogs the two muscles were irrigated successively through a single cannula placed in the bulbus arteriosus, the leg whose irrigation was not desired being ligated for the time being. With cats irrigation was performed simultaneously in the corresponding muscles of opposite legs through cannulas placed in the two femoral arteries, and connected with pressure bottles containing the irrigation solutions warmed to body temperature. Either during the irrigation or immediately afterward, the two muscles, sometimes left *in situ* and sometimes excised, were similarly stimulated, and comparable graphic records were obtained.

The stimulus that was employed is the break induction shock regularly repeated. This was obtained usually through the mediation of a von Frey rheotome, two keys of which were placed in the primary and secondary circuits respectively of the inductorium and were so arranged as to allow the short-circuiting of the make shocks. The rheotome was kept in motion by an electric motor. The constancy of the stimulating current was insured by the use of a Grove cell. The muscle lever consisted of a piece of straw of light weight and 100 mm. in length. The muscle was attached to it 40 mm. from the axis, the weight only 4 mm. from the same point. A very delicate isotonic system was thus obtained. The muscle actually lifted only one-tenth of the weight that was attached to the lever. To insure a uniform abscissa the preparation was usually after-loaded.

The contractions of the muscle were recorded on the Baltzar or the Zimmerman clock kymograph. Usually the rate of the drum was very slow, and the single contractions appeared as vertical lines very close together. When, however, it was desired to expand the records into the customary muscle curves, the speed was greatly increased. At such times the stimulating shocks were mediated through keys similar to those of the rheotome, but attached to the kymograph itself, and opened automatically by its mechanism. Superposition of the muscle curves was effected by lowering the drum a definite distance between successive records.

In some experiments attempts were made to compare the total amounts of work performed by the two muscles by means of work-adders, but the impossibility of obtaining two work-adders capable of acting with entire uniformity rendered such attempts unsatisfactory.

Further details of the method will be mentioned in the later accounts of the experiments.

My thanks are due to two of my friends, Drs. Donald Gordon

and Edgar E. Stewart, for aid in the laboratory. Upon them has fallen a considerable portion of the task of performing the experiments, and they have done this with praiseworthy perseverance and efficiency.

#### RESULTS.

The results that have been obtained by the methods above outlined can best be presented with the aid of a series of figures which reproduce tracings from actual experiments. The experiments here quoted have been selected from a very large number, in which similar results have been observed. Figure 1 shows the effect of carbon dioxide.

*January 23, 1906.*—Frog: weight, 37 gm. One thigh was ligated temporarily. 15 c.c. of 0.7 per cent solution of sodium chloride were injected into the bulbus arteriosus during a period of five minutes. After fifteen minutes more had elapsed the irrigated gastrocnemius was excised and prepared for direct stimulation. Maximal stimuli, 29 per minute. Weight actually lifted, 5 gm. Every 50th contraction was recorded. After the record was complete the temporary ligature on the opposite leg was removed, and 15 c.c. of 0.7 per cent solution of sodium chloride, through which a vigorous stream of carbon dioxide had been passed for several minutes, was injected through the bulbus into the opposite leg. After a wait of fifteen minutes the gastrocnemius was removed, and a record as above was made, the muscle curves rising from the same abscissas as those of the first muscle. The tracing shows every 50th curve of each muscle, from the 1st to the 551st inclusive. The longer, or in the later contractions the lower, curves are those of the muscle under the influence of carbon dioxide.

It will be observed that the curves of the muscle under the influence of carbon dioxide, from the 1st to the 151st contraction inclusive, are higher than those of the normal muscle,—in other words, carbon dioxide exerts at first an augmenting action. From the two hundred and first contraction on, the fatiguing effect is manifest.

One of the readiest and surest means of demonstrating the augmenting action of carbon dioxide is the following: Etherize a cat. Expose the tendon of one extensor longus digitorum, and attach it to a muscle lever, after-weighted and arranged to record on a slowly moving drum. Attach electrodes to the two ends of the muscle, stimulate it at the rate of twenty-five per minute, and record each contraction. When the *treppe* is nearly or quite ended, clamp the

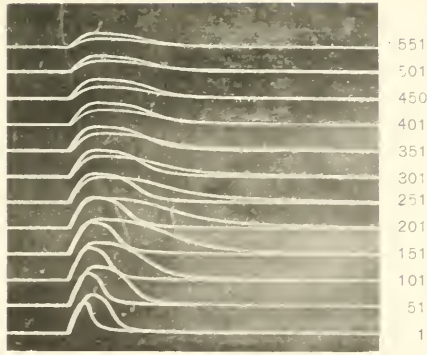


FIG. 1.

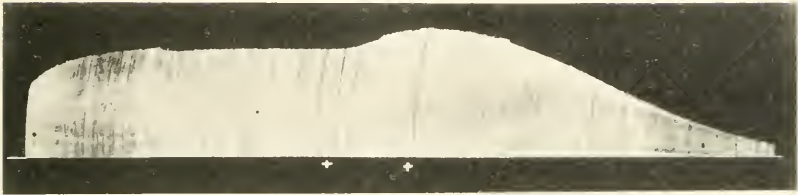


FIG. 2.

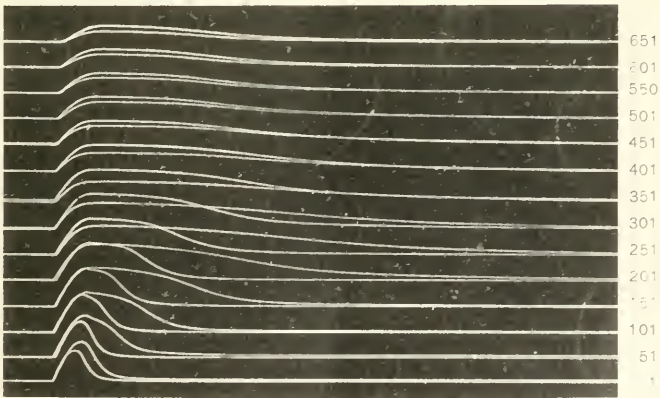


FIG. 3.

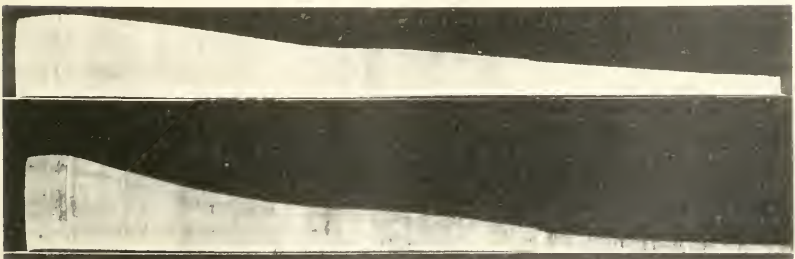


FIG. 4.





trachea and thus asphyxiate the animal. There will appear a marked rise in the height of the muscle curves,—in other words, a new *treppe*. Figure 2 shows the record of such an experiment. The first cross indicates the moment at which the trachea was clamped; the second cross, the moment at which the heart ceased to beat. There are two possible causes for the new *treppe*, absence of oxygen and accumulation of carbon dioxide. From such an experiment alone it is impossible to exclude the former, and from the analogy of the action of venous blood on the respiratory centre, it is perhaps not improbable that absence of oxygen is a real factor. The work of Zuntz and others, however, makes it probable that the carbon dioxide of the venous blood is a much more intense stimulus to the nerve cells of the respiratory centre than the absence of oxygen. By analogy, therefore, it seems altogether probable that the *treppe* in such an experiment as this is caused mainly by carbon dioxide.

Figure 3 shows the effect produced by sarcolactic acid.

*January 27, 1906.*—Frog: weight, 54 gm. One thigh was ligated temporarily. 15 c.c. of 0.7 per cent solution of sodium chloride were injected into the bulbus arteriosus during a period of three minutes. After eighteen minutes more had elapsed the irrigated gastrocnemius was excised and prepared for direct stimulation. Maximal stimuli: 29 per minute. Weight actually lifted, 5 gm. Every 50th contraction was recorded. After the record was complete the temporary ligature on the opposite leg was removed, and 15 c.c. of a 0.7 per cent solution of sodium chloride containing  $\frac{2}{3}$  paralactic acid were injected through the bulbus into the opposite leg. After a wait of eighteen minutes the gastrocnemius was removed, and a record as above was made, the muscle curves rising from the same abscissas as those of the first muscle. The tracing shows every 50th curve of each muscle, from the 1st to the 651st inclusive. The longer, or in the later contractions the lower, curves are those of the muscle under the influence of paralactic acid.

The augmenting action of paralactic acid is visible in the first one hundred and fifty-one contractions, after which the depressing action appears.

The augmenting effect of mono-potassium phosphate is shown in Fig. 4.

*November 10, 1906.*—A cat was killed by decapitation at 12.25 P. M. A cannula was placed at once in each femoral artery, and at 12.31 irrigation of the two legs was begun simultaneously under a constant pressure of

150 mm. Hg and a temperature of 38.5° C. The right leg was irrigated by the whipped blood of a bullock, the left leg by similar blood to which had been added pure mono-potassium phosphate in quantity to equal 1/150 of a grammolecular solution. The irrigation continued for five minutes. After its cessation the corresponding extensor longus digitorum muscles were rapidly excised, placed in moist chambers at room temperature, attached to exactly similar levers, weighted by similar weights, of which each muscle lifted 30 gm., and stimulated simultaneously by the same series of maximal break induction shocks, delivered at the rate of 29 in the minute, while each contraction was recorded on a slowly moving drum. The record began at 12.41 and ended at 1.08 P. M.

In the figure the upper tracing represents that of the normal muscle, the lower that of the muscle under the influence of mono-potassium phosphate. At the beginning of the record the contractions of the phosphate muscle were considerably greater than those of the normal muscle, and this advantage continued during one hundred and seven contractions, when the depressing action of the fatigue substance began to appear.

It usually happens that the augmenting action of the fatigue substance is present only in the early stages, and later gives place to the opposite effect, but occasionally augmentation continues throughout the experiment. This is well illustrated in Fig. 5.

*November 15, 1906.* — A cat was killed by decapitation at 10.06 A. M. A cannula was placed at once in each femoral artery, and at 10.11 irrigation of the two legs was begun simultaneously under a constant pressure of 150 mm. Hg, and a temperature of 38.5° C. The right leg was irrigated by the whipped blood of a bullock, the left leg by similar blood to which had been added pure mono-potassium phosphate in quantity to equal 1/150 of a grammolecular solution. The irrigation continued for three minutes. After its cessation the corresponding extensor longus digitorum muscles were rapidly excised, placed in moist chambers at room temperature, attached to exactly similar levers, weighted so as to lift 30 gm. each, and stimulated simultaneously by the same series of maximal break induction shocks at the rate of 29 in the minute, the contractions being recorded on a slowly moving drum. The record began at 10.20 and ended at 10.53 A. M.

In the figure the upper tracing represents that of the normal muscle; the lower, that of the muscle under the influence of the mono-potassium phosphate. At the beginning of the record the phosphate muscle is

seen to possess nearly twice the lifting power of the normal one, and it has the advantage of the latter even to the end. The augmenting action of the mono-potassium phosphate is very evident.

After this experiment was ended I made an attempt to determine how much mono-potassium phosphate the poisoned muscle had received.\* I measured the bulk of the muscle, the bulk of the whole leg, and the amount of liquid that had passed through the latter. On the supposition, which was, of course, not literally correct, that all portions of the leg had received equivalent amounts of the irrigating liquid, the figures showed that approximately 0.0005 gm. of mono-potassium phosphate had passed into the arteries of the muscle. This is a small amount; how much of it had actually been absorbed cannot, of course, be known, but the fraction could not have been great.

The augmenting action of mono-potassium phosphate may be easily demonstrated in the manner illustrated in Fig. 6.

A cat was etherized and tracheotomized. To insure regularity of subsequent respiration and etherization, artificial respiration was established. The right tibialis anticus was prepared for direct stimulation and the recording of its contractions; the crural and sciatic nerves of the right leg were cut; a cannula was placed in the left external jugular vein; the muscle was stimulated by maximal break induction shocks at the rate of 22 per minute. The weight actually lifted was 20 gm. While the record was in progress 10 c.c. of a 0.9 per cent solution of sodium chloride containing  $\frac{m}{15}$  mono-potassium phosphate were injected slowly into the jugular vein. The period of injection and the augmenting effect appear on the tracing.

In the experiments so far reported the fatigue substances either consisted wholly of or contained free acid, and since their effects are practically identical, the obvious inference is that they act by reason of their acid character. This is probably true. But the following experiment, which is illustrated in Fig. 7, shows that it is not necessarily the whole truth. A cat was treated in the same manner as in the preceding experiment. At the time indicated by the signal, 10 c.c. of a 0.9 per cent solution of sodium chloride containing  $m$  potassium paralactate were injected into the jugular vein. There ensued a marked rise in the height of the muscle curves. The solution here employed was a solution of the potassium salt, neutral to phenolphthalein, and yet the effect was the same as if the free acid had been injected. Such an experiment is justified by the possibility that paralactic acid exists in the muscle, not free, but in combination with potassium.

After this experiment was completed the skin of the animal was removed and the bulk of the whole body, measured by the displacement of water when submerged, was found to be 3445 c.c.; that of the tibialis anticus, 5 c.c. The proportion of the latter to the whole body was thus 1 : 689. The amount of the salt which was received by the single muscle must have been very small, yet the work performed was increased 19 per cent.

Is the augmenting effect of the fatigue substances due to their action on the muscle substance itself or its nervous supply? The *treppe* occurs in both curarized and non-curarized muscles. Ranke, who discovered that under the influence of small quantities of lactic acid the minimal stimulus of the muscle-nerve preparation was decreased, — in other words, that the irritability was increased, — was unable to observe the effect in curarized preparations. He therefore claimed that lactic acid acts on the nervous system alone, and he made this supposed fact an important part of his theory of the action of fatigue substances. I find myself unable to agree with Ranke in this. I have observed augmentation of activity after the administration of each of the fatigue substances in both curarized and non-curarized muscles. Curare, of course, depresses muscular irritability, and in harmony with this fact it has seemed to me that larger quantities of the fatigue substance are required to produce a given amount of augmentation in curarized than in non-curarized preparations. In curarized cats, for example, with maximal stimuli, after the normal *treppe* has been obtained, I have at times failed to observe the artificial *treppe* on stopping the respiration. It has appeared, however, when submaximal stimuli have been employed, and care has thus been taken to avoid the production of large quantities of the normal fatigue substances. I have not attempted to examine this question exhaustively, but can state that according to my experiments it is not necessary to assume that the fatigue substances exert their augmenting action through any portion of the nervous system.

If the chemical theory of the *treppe*, as here outlined, be true, a difference in the duration of the normal *treppe* is to be expected in muscles possessing an intact circulation of blood and in those from which the circulation has been excluded. Such a difference actually occurs, and is well illustrated in Fig. 8.

*December 22, 1906.* — At 12.25 P. M. a cat was etherized. The tibialis anticus muscles of the two legs were prepared for stimulation and the recording of contractions. At 1.19 both the external and internal iliac arteries of the

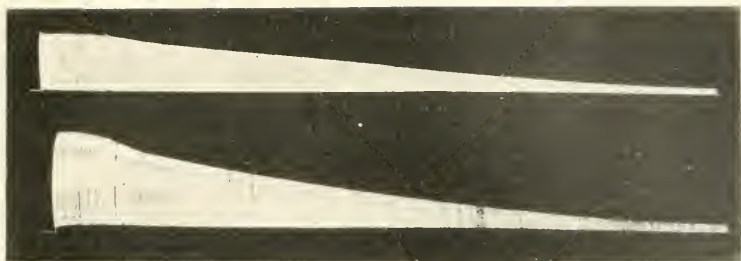


FIG. 5.



FIG. 6.



FIG. 7.



FIG. 8.



left leg were ligated, thus shutting off all blood supply on that side. At 1.23 the stimulation of both muscles by the same series of break shocks and the records of the contractions were begun. Maximal stimuli, 25 per minute. Weight actually lifted, 20 gm. The *treppe* was completed in the bloodless muscle after 260 contractions; in the opposite muscle after 383 contractions.

The result is what might have been expected. Where the circulation of blood is maintained, the fatigue substances are continually washed away, and hence accumulate only slowly. Their augmenting action therefore continues for a considerable time. In the muscle from which the circulation is excluded they accumulate rapidly; their augmenting action reaches its maximum early, and gives place early to depression. In experiments similarly performed on frogs the *treppe* developed also more rapidly in the muscle lacking a circulation. In an extreme case it continued in such a muscle for only five minutes, during which one hundred and twenty-five contractions were made, while on the opposite side, with the circulation continuing, the maximum was not reached until the end of twenty-eight minutes and after the muscle had made seven hundred contractions. In two other experiments the *treppe* of the bloodless muscle was ended after sixty-three and sixty-three contractions respectively, and of that possessing a circulation after one hundred and twenty-six and one hundred and three contractions. It is difficult to see how such differences can be explained on the theory of a simple alteration in elasticity: they are readily understood, however, on the chemical theory.

A fact which was pointed out by Tiegel is also capable of a similar explanation. Tiegel found that the *treppe* that accompanied sub-maximal stimulation continued for a longer time than when maximal stimulation was employed. This I would interpret on the assumption that during maximal stimulation a larger quantity of the fatigue substances is produced than during submaximal stimulation, and hence their augmenting action reaches its maximum earlier.

It is a well-known fact that even when there is no blood supply or other irrigating liquid, the fatiguing or depressing effect of previous muscular work gradually passes away, at least in part, when the muscle is allowed to rest. To a certain extent the muscle recovers its working power. Expressed in terms of the theory of fatigue, the toxic action of the fatigue substances gradually diminishes, and more or less disappears in the course of time. Various writers have pointed

out likewise that the augmenting effect of previous activity, while persisting for a time, continues in a diminishing ratio, and ultimately passes away. For example, Bowditch found for the muscle of the frog's heart fed with serum, that when the interval between successive single stimuli was increased by stages from four seconds up to sixty seconds, the resulting augmentation of the contractions became less and less, until at the higher interval it was slight or wanting. With mammalian muscle possessing its normal circulation, Rossbach and Harteneck found that the *treppe* disappeared when the interval between stimuli was increased to five seconds. Buckmaster, working with the curarized frog's gastrocnemius, showed that the after-effect of a stimulus continued for a certain time, but with diminishing intensity; with an interval of sixty seconds between two contractions, augmentation was scarcely noticeable. This observed transitory persistence with diminishing intensity and final passing away of augmentation is precisely what might be expected on the chemical theory of the nature of augmentation. Expressed in terms of this theory, the interpretation of the fact is that the fatigue substances accumulate, and if the interval between stimuli be brief, they exert a cumulative augmenting action; but their action diminishes with time, and if the interval between stimuli be long, augmentation may entirely fade out before opportunity is given for its expression in the next contraction. Thus the observed analogy between the disappearance of depression and that of augmentation may consistently be paralleled by an analogy in the modes of explanation of the two.

The chemical theory of the *treppe* may be extended to the explanation of the mysterious process known as summation of stimuli. As applied in its usual sense to contractile tissues, the term means the phenomenon wherein a stimulus, too weak to cause a contraction when applied singly, becomes efficient when repeated. A subminimal stimulus may thus pass the threshold and become minimal and even supraminimal. Summation of stimuli in this sense in contractile tissue was demonstrated clearly by Romanes in the contractile tissue of the bell of the medusa. In striated muscle it was observed by Richet in the claw muscles of the crayfish. It appears to be a widespread phenomenon of contractile protoplasm in both animals and plants. I shall not here enter into an extended discussion of the process, but its connection with the *treppe* is not to be denied. Romanes pointed out that the summation which he observed was



associated with the process of stimulation rather than contraction, and it is generally acknowledged that the chemical changes of muscular activity occur, in large part at least, during the period of the excitatory stage rather than during the subsequent mechanical stage. Gotschlich has found that stimuli too weak to effect contraction in muscle will yet cause the production of acid. The acid thus arising, it must be believed, increases irritability, and the subminimal stimulus is thus enabled to become effective. Summation of stimuli and treppe are thus similar processes, and in fact the former term is often applied by writers not only to the cumulative action of subminimal stimuli, but also to that of repeated submaximal or maximal stimuli,— in other words, to the treppe and treppe-like phenomena.

An interesting experiment by Sewall may here be quoted. He found that tetanization of a muscle in which the contractions were prevented by holding down the lever increased the irritability of the muscle to such an extent that subsequent maximal stimuli resulted in greater contractions than before the tetanizing stimulus had been applied. The tetanizing stimulus had evidently produced fatigue substances, and these had reacted on the muscle in their customary augmenting manner.

Thus far in the problem of the treppe I have worked with striated muscle only, and I can perhaps hardly be justified in extending my conclusions to other tissues. But the phenomenon is physically so similar in cardiac muscle, and apparently so in the central nervous system and the peripheral nerve, that it seems probable that the chemical phenomena in all these cases will ultimately be shown to be sufficiently analogous to afford an analogous explanation for all. This is obviously so with regard to cardiac muscle. With the central nervous system carbon dioxide is an undoubted product of activity, and there is no inherent improbability in its acting as a factor in making paths of conduction that have once been traversed more easy of subsequent passage. "Canalization," as Waller terms it, is a real phenomenon, and the establishment of a chemical process for it is by no means to be unexpected. The case of the nerve fibre is not so clear, because of the lack of evidence of metabolism within it; but the claims of Garten and Fröhlich to have demonstrated fatigue within nerves, and Waller's inference regarding the production of carbon dioxide therein, are suggestive of further insight into this difficult field of research. Waller does indeed go so far as to ascribe to

carbon dioxide the staircase in the electrical responses of nerve to excitation.

The facts here reported seem to emphasize anew and strikingly the great desirableness of a very careful and full investigation of the physiological actions on cells, tissues, and organs of the products of metabolism, both intermediate and final products. We seem not yet to realize how potent may be the influence of such substances. It is true that the important parts played by specific internal secretions, specific auto-intoxicants, and specific inorganic salts, are being recognized, but these are probably but isolated instances of a widespread principle, and we incur the charge of being unscientific if without experimental data we deny to even the humblest katabolic product a possible rôle as a physiological reagent. It seems to me that physiology is destined to make great progress along the lines here indicated.

#### CONCLUSIONS.

1. The physiological action on skeletal muscle of each of the commonly recognized fatigue substances, namely, carbon dioxide, paralactic acid, and mono-potassium phosphate, is of two opposite modes, the appearance of the one or the other mode being dependent upon the quantity of the substance that is present and the duration of its activity. If present in moderate quantity, or smaller quantity for a longer time, each substance is depressing or fatiguing. If present in small quantity, or moderate quantity for a brief time, it causes an augmentation of activity, which is characterized by an increase in irritability and working power, an increase in the height to which the load is lifted, and an increase in the total amount of work performed.

2. The augmenting action of fatigue substances is not due solely to free acid, since it is shown by the neutral potassium paralactate.

3. The augmenting action of fatigue substances occurs in both curarized and non-curarized muscles. Hence it is exerted upon the muscle protoplasm itself.

4. The *treppe* of skeletal muscle is due to the augmenting action on the muscle protoplasm of fatigue substances present in small quantity.

5. In a muscle from which the circulation of blood is excluded the *treppe* reaches its maximum earlier than in a muscle possessing a

circulation. This is due to the more rapid accumulation of fatigue substances.

6. Tiegel's discovery that the *treppe* accompanying maximal stimulation reaches its maximum earlier than with submaximal stimulation is explained as due to the larger quantity of fatigue substances produced in the former case, and the resulting earlier cessation of their augmenting effect.

7. Just as recovery from fatigue is explained by a diminution of the toxic action of fatigue substances, so the disappearance of the augmenting effect of previous activity may be explained by a diminution of the augmenting action of the same substances.

8. Summation of stimuli may be explained as a rise of irritability due to the augmenting action of fatigue substances.

9. Although the present research deals with skeletal muscle only, it seems not unlikely that the *treppe* of other muscle, the central nervous system, and peripheral nerve, will be ultimately explained by the chemical theory here presented.

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## CONCERNING GLYCOLYSIS.

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### INTRODUCTION.

THE mechanism of the destruction of d-glucose in the animal body, probably the chief source of energy for the muscle and for animal heat, has long been a matter of dispute and the subject of many theories. Since 1889, when Minkowski and von Mering published their investigations on pancreatic diabetes, the long-suspected rôle of the pancreas in this important reaction has been accepted as proved. These authors showed that the removal of the entire pancreas from a dog leads to a condition quite similar to severe diabetes in man, and ends in the death of the animal. However, even a small portion of the gland, remaining in the body quite disconnected from the alimentary canal, suffices almost completely to prevent the condition, otherwise established, so that the characteristic symptoms may be in this case slight and transient, or altogether absent. As a result of these and other investigations it is now generally believed that the pancreas supplies an internal secretion which is an essential factor in the normal destruction of glucose.

In 1903 O. Cohnheim<sup>2</sup> published the results of a series of experiments which materially advanced our knowledge of glycolysis and of the function of the pancreas therein. Mixing the expressed juice of muscle and of pancreas from a recently killed dog or cat, adding dextrose, toluol to prevent the growth of bacteria, and sodium bicarbonate to prevent acidity, he observed, after the mixtures had stood in the thermostat for a considerable time, a material diminution in the dextrose content as measured by the reduction of Pavy's solution. The expressed juice of pancreas unaided produced in Cohnheim's experiments no effect upon the power of reduction of such

<sup>1</sup> Carried out with the aid of a grant from the Elizabeth Thompson Fund.

<sup>2</sup> COHNHEIM: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 336.

a mixture. The expressed juice of muscle unaided produced a slight effect, but the calculated destruction of glucose in these experiments was greatly less than in the case of the co-operation of the two organs. Later Cohnheim<sup>1</sup> published a second communication confirming his original experiments and showing that the expressed juice of pancreas may be replaced by an alcoholic extract of boiled pancreas. The results with this extract were particularly satisfactory. This author experienced difficulty in carrying on the experiments when normal saline solution was used in preparing the muscle juice, and he believes that sodium chloride is very injurious to the active muscle substance. Difficulties also arose if large quantities of the pancreatic component, either as expressed juice or as alcoholic extract, were employed; accordingly he assumes an inhibitory action of pancreatic substance when present in large amounts. Results similar to Cohnheim's were reported at about the same time by R. Hirsch<sup>2</sup> from Hofmeister's Laboratory.

The results of Cohnheim have been questioned by Claus and Embden,<sup>3</sup> who reported several experiments in which no diminution in the power of reduction of the mixture was to be observed; while in those cases where they detected a diminished power of reduction they found bacteria present in the mixture and ascribed the diminution of glucose to their activity. They, however, used sodium chloride in preparing the muscle juice, and also large quantities of the juice of the pancreas, and these facts, Cohnheim believes, explain the negative results of their experiments. In a later article Claus and Embden<sup>4</sup> report further experiments. They maintain that bacterial action is the cause of the disappearance of glucose. Cohnheim, however, in his later publications<sup>5</sup> presents further evidence in support of his contention.

In addition to the work of these authors the presence of an "activating substance" in the pancreas has been shown by Arnheim and Rosenbaum,<sup>6</sup> by de Meyer,<sup>7</sup> and in particular by Miss Dewitt,<sup>8</sup> who

<sup>1</sup> COHNHEIM: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 401.

<sup>2</sup> HIRSCH, R.: *HOFMEISTER'S Beiträge*, 1905, v, p. 214.

<sup>3</sup> CLAUS and EMBDEN: *Ibid.*, 1905, vi, p. 214.

<sup>4</sup> *Ibid.*, 1905, vi, p. 343.

<sup>5</sup> COHNHEIM: *Zeitschrift für physiologische Chemie*, 1905, xliii, p. 547; 1905, xlvii, p. 253.

<sup>6</sup> ARNHEIM and ROSENBAUM: *Ibid.*, 1904, xl, p. 220.

<sup>7</sup> DE MEYER: *Archives internationales de physiologie*, 1905, ii, p. 131.

<sup>8</sup> DEWITT: *Journal of experimental medicine*, 1906, viii, p. 193.

obtained, as she believes, the active pancreatic component from the areas of Langerhans in pancreases the rest of which were degenerated so that they did not yield digestive ferments. De Meyer believes the pancreatic component to be in its action of the nature of an amboceptor.

The present investigation aims to test the results of Cohnheim's investigations and to extend our knowledge of this important physiological co-operation.

#### METHOD.

Muscle juice for the experiments was invariably obtained from a rabbit killed by bleeding. Immediately after death the muscles were removed, passed through a sausage machine, and mixed with sand, cold water, and phosphate solution<sup>1</sup> to preserve neutrality. From this mixture the muscle juice was obtained with the aid of a powerful hand press. To this preparation were then added glucose or another sugar and the pancreatic component in whatever form was chosen for the particular experiment. In certain series of experiments, however, the one component or the other alone was used. In every case the mixtures were shaken with toluol, and a layer of toluol was placed on top of them in test tubes. The reducing power of a sample was then measured with Pavy's solution, and the preparations, carefully stoppered with absorbent cotton, were placed in the thermostat for a varying period of time. At the end of the experiment the power of reduction was determined with Pavy's solution, and the amount of glucose which had disappeared was calculated.

The alcoholic extract of pancreas was prepared as follows: fresh pancreas, crushed and mixed with a small amount of water, was evaporated nearly to dryness on the water bath, and thoroughly extracted with ninety-five per cent alcohol. The alcoholic extracts were filtered through cloth, united and filtered through filter paper.

The following experimental data confirm Cohnheim's work, and essentially they are repetitions of it.

Of the four series of experiments the first test the action of the

<sup>1</sup> This solution consisted of nine parts di-sodium phosphate and one part mono-sodium phosphate; its strength was about ten per cent. Such a mixture of phosphates, devised by HENDERSON and WEBSTER (*Journal of medical research*, 1907, xvi), though almost absolutely neutral itself, can neutralize an amount of acid equivalent to its di-sodium phosphate content without becoming appreciably acid: see *American journal of physiology*, 1906, xv, p. 257.

juice of the pancreas upon glucose; the second, the action of muscle juice upon glucose; the third, the action of the two juices combined upon glucose. In Series Four the experiments of Series Three are

SERIES I.  
EXPRESSED JUICE OF PANCREAS + GLUCOSE.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
	gm.	hrs.	gm.		per cent.
1	2.176	24	2.180	-0.004	-0.2
2	3.242	16	3.237	0.005	0.2
3	2.786	20	2.754	0.032	1.1
4	2.916	32	2.899	0.017	0.6
5	3.115	24	3.116	-0.001	....
6	3.115	48	3.111	0.004	0.1

repeated, using alcoholic extract of boiled pancreas in place of the expressed juice of the gland.

It appears from the above tables that pancreatic expressed juice has no appreciable effect upon glucose (Series I). On the other hand, the expressed juice of muscle acting in neutral solution at 40° produces a constant and marked though rather slight disap-

SERIES II.  
EXPRESSED JUICE OF MUSCLE + GLUCOSE.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
	gm.	hrs.	gm.		per cent.
1	4.225	26	4.202	0.026	0.6
2	3.979	32	3.899	0.080	2.0
3	3.766	24	3.674	0.092	2.4
4	3.766	49	3.687	0.079	2.1
5	4.327	40	4.286	0.041	1.0

In experiments 1 and 2 sodium bicarbonate was used to preserve neutrality, in all other experiments the phosphate mixture was employed.



## SERIES III.

EXPRESSED JUICE OF MUSCLE + EXPRESSED JUICE OF PANCREAS + GLUCOSE.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
	gm.	hrs.	gm.		per cent
1	3.572	15	3.506	0.066	1.7
2	3.572	40	3.378	0.194	5.4
3	4.023	26	3.798	0.225	5.6
4	2.116	18	2.008	0.108	5.1
5	2.116	30	2.003	0.113	5.4
6	3.118	24	3.021	0.097	3.1

In Experiments 1 and 2, 25 c.c. of muscle juice and 10 c.c. of the juice of the pancreas were mixed. In Experiments 3, 4, and 5, 25 c.c. of muscle juice and 2 c.c. of the juice of the pancreas were mixed. In Experiment 6, 50 c.c. of muscle juice and 15 c.c. of the juice of the pancreas were mixed.

## SERIES IV.

ALCOHOLIC EXTRACT OF PANCREAS + EXPRESSED JUICE OF MUSCLE + GLUCOSE.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
	gm.	hrs.	gm.		per cent
1	5.000	24	3.472	1.528	30.6
2	5.000	48	3.571	1.429	28.6
3	4.625	24	4.250	0.375	8.1
4	4.625	48	4.025	0.600	13.0
5	4.625	72	3.787	0.838	18.1
6	3.562	24	2.637	0.925	26.0
7	2.312	48	1.950	0.362	15.6
8	2.500	24	2.225	0.275	11.0
9	2.500	48	2.150	0.350	14.0

In Experiments 1, 2, 6, 8, and 9, 25 c.c. of muscle juice and 5 c.c. of alcoholic pancreatic extract were mixed. In Experiments 3, 4, and 5, 25 c.c. of muscle juice and 2 c.c. of alcoholic pancreatic extract were mixed. In Experiment 7, 25 c.c. of muscle juice and 1 c.c. of alcoholic pancreatic extract were mixed.

pearance of glucose (Series II). When combined, however (Series III), these two juices are much more effective, under the conditions of the experiment, to produce a diminution in the power of reduction of the solution. Even more marked is the effect of muscle juice and alcoholic extract of pancreas in conjunction (Series IV). In this case a disappearance of nearly a third of the glucose has been noted. Experiments to determine the greatest possible destruction of glucose, both absolute and relative, will presently be undertaken in this

## SUMMARY OF FIRST EXPERIMENTS.

	Average destruc- tion in glucose. gm.	Percentage destruction. per cent.
Series I. Pancreas + glucose . . . . .	0.010	0.3
Series II. Muscle + glucose . . . . .	0.064	1.6
Series III. Pancreas + muscle + glucose . . . . .	0.134	4.4
Series IV. Alcoholic extract of pancreas + muscle + glucose . . . . .	0.742	18.3

laboratory. Variations in the amount of sugar which disappears, as for instance in Series IV, may well be due in great part to difference in the activity of the muscle juice obtained from different animals. In such experiments as these an autolytic formation of reducing substance is far from improbable; such may be the case in Series I, Experiment I, for instance.

Taken together, the above experiments strongly support Cohnheim's conclusions in so far as the disappearance of glucose is concerned. In these experiments bacterial action is hard to seek, for Series I presents consistently negative results, Series II consistently low results, Series III and IV consistently high results, though the experiments of the several series were not done consecutively. Moreover, the great quantity of toluol used in all the experiments is a strong safeguard against bacterial contamination. Finally, during the course of the investigation in six separate experiments cultures both aerobic and anaerobic were made after forty-eight hours, altogether twelve cultures. All but one of these proved sterile, this one showing a growth of *B. subtilis*. Further evidence against this contention of Embden will be presented later in this paper.

## SECOND EXPERIMENTS.

The following experimental data were collected to test the possibility of separating the active pancreatic constituent from some of the accompanying substances with phosphotungstic acid.

An aqueous solution of the alcoholic extract of boiled pancreas was prepared, and to this a saturated solution of phosphotungstic acid in five per cent sulphuric acid was added until no further precipitate occurred. The precipitate was washed with dilute phosphotungstic acid in sulphuric acid, ground in a mortar with barium hydrate, and repeatedly extracted with distilled water. The aqueous extracts were collected, filtered through filter paper, and to the filtrate sufficient sulphuric acid added to remove the barium quantitatively. The barium sulphate was filtered off, and the filtrate evaporated on the water bath to a thick brown paste. The filtrate from the phosphotungstic precipitate was treated with barium to remove the phosphotungstic acid, filtered, and the excess of barium removed by adding sulphuric acid, the barium sulphate filtered off, and the filtrate evaporated on the water bath.

Of the two series of experiments the first (Series V) test the action of the phosphotungstic acid precipitate, the second (Series VI) the action of substances not precipitable with phosphotungstic acid.

## SERIES V.

## PHOSPHOTUNGSTIC ACID PRECIPITATE + MUSCLE JUICE + GLUCOSE.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
	gm.	hrs.	gm.		per cent
1	2.187	24	1.450	0.437	20.0
2	3.636	20	3.378	0.258	7.1
3	3.906	20	3.125	0.781	20.0
4	3.675	20	2.975	0.700	19.0
5	3.675	20	2.450	1.225	33.3
6	1.325	20	1.096	0.229	17.3
7	1.412	40	1.323	0.089	6.3
8	1.500	40	1.350	0.150	10.0
9	2.906	40	2.350	0.456	15.7

It is clear, from a consideration of the above experiments, that the active pancreatic component is precipitated almost or quite completely with the aid of the phosphotungstic acid. Further efforts at separation and purification of the substance are now under way in this laboratory.

## THIRD EXPERIMENTS.

The following experiments were performed to determine the effect upon other sugars, a pentose, a hexose, and a di-saccharide, of muscle and pancreas in conjunction.

## SERIES VI.

FILTRATE FROM PHOSPHOTUNGSTIC ACID PRECIPITATE + MUSCLE JUICE + GLUCOSE.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
1	gm. 3.716	hrs. 24	gm. 3.718	-0.002	per cent. -0.1
2	3.911	26	3.897	0.014	0.4
3	3.212	24	3.203	0.009	0.3
4	3.214	26	3.216	-0.002	-0.1

## SERIES VII.

FRESH PANCREAS JUICE + MUSCLE JUICE + LEVULOSE.

No. of exp.	Original reduction in levulose.	Time.	Final reduction in levulose.	Calculated diminution of levulose.	Diminution of levulose.
1	gm. 3.967	hrs. 24	gm. 3.972	-0.005	per cent -0.1
2	2.785	24	2.777	0.008	0.3
Average . . . . .					0.11
ALCOHOLIC EXTRACT OF BOILED PANCREAS + LEVULOSE.					
1	3.217	26	3.202	0.015	0.5
2	3.217	36	3.197	0.020	0.7
3	2.783	24	2.797	-0.014	-0.5
4	2.783	40	2.695	0.088	3.2
Average . . . . .					0.97

SERIES VIII.

FRESH PANCREAS JUICE + MUSCLE JUICE + ARABINOSE.

No. of exp.	Original reduction in arabinose.	Time.	Final reduction in arabinose.	Calculated diminution of arabinose.	Diminution of arabinose.
1	gm. 3.314	hrs. 24	gm. 3.316	0.002	per cent -0.12
2	3.171	24	3.159	0.012	0.4
ALCOHOLIC EXTRACT OF BOILED PANCREAS + MUSCLE JUICE + ARABINOSE.					
1	2.911	24	2.903	0.008	...
2	2.911	40	2.913	-0.002	-0.1
3	3.112	24	3.100	0.012	0.4

SERIES IX.

FRESH PANCREAS JUICE + MUSCLE JUICE + LACTOSE.

No. of exp.	Original reduction in lactose.	Time.	Final reduction in lactose.	Calculated diminution of lactose.	Diminution of lactose.
1	gm. 4.112	hrs. 24	gm. 4.111	0.001	per cent 0.5
2	3.979	24	3.998	-0.019	-0.5
ALCOHOLIC EXTRACT OF BOILED PANCREAS + MUSCLE JUICE + LACTOSE.					
1	4.535	24	4.527	0.008	0.2
2	3.918	24	3.901	-0.017	-0.4
3	3.918	48	3.907	0.011	0.3

The results of the above experiments clearly indicate that Cohnheim's mechanism is designed to destroy glucose alone, and is more specific in its action than the yeasts and other carbohydrate attacking ferments. In the one experiment with positive result of the above series, when 3.2 per cent of the levulose content was destroyed, it

is possible that bacterial contamination occurred, or, in the long experiment, the ordinary mechanism of autolytic cleavage, which may well be different from the physiological co-operation of muscle and pancreas, may have produced the result; finally, it is possible that levulose is destroyed to a very slight extent by the physiological co-operation.

In any case these experiments indicate with certainty the occurrence of a highly specialized mechanism for the destruction of glucose in muscle, and accordingly they constitute one argument for the theory that glucose is the immediate source of muscular energy.

It remains to be pointed out that the negative outcome of these experiments, with one exception, argues very strongly against the contamination of the mixture here and elsewhere with micro-organisms.

#### FOURTH EXPERIMENTS.

The following experiments were carried out to test the action of trypsin upon the active muscle substance, in the hope that thus an explanation of the inhibitory action of a large quantity of the expressed juice of pancreas might be furnished.

Evidently some constituent of pancreatin and of the expressed juice of fresh pancreas has a deleterious effect upon the active muscle substance. Not improbably this substance is trypsin. These experiments offer a simple explanation of the greater activity of alcoholic extract of boiled pancreas, particularly because the alcoholic extract of boiled pancreas even in great amount seems to exert no inhibitory effect. It may, moreover, be pointed out that sodium bicarbonate used by previous workers is peculiarly favorable to the action of trypsin.

#### SUMMARY.

1. Confirming Cohnheim, it is shown that pancreas alone is incapable of destroying appreciable amounts of d-glucose; muscle alone can destroy small quantities of glucose; while small quantities of the expressed juice of pancreas mixed with muscle juice destroy considerable quantities of glucose. Even more effective in this co-operation than pancreas juice is the alcoholic extract of boiled pancreas.

2. The active pancreatic substance is completely precipitated by phosphotungstic acid.

## SERIES X.

ALCOHOLIC EXTRACT OF PANCREAS + GLUCOSE + MUSCLE JUICE DIGESTED IN THERMOSTAT FOR TWENTY-FOUR HOURS WITH PANCREATIN.

No. of exp.	Original reduction in glucose.	Time.	Final reduction in glucose.	Calculated diminution of glucose.	Diminution of glucose.
	gm.	hrs.	gm.		per cent
1	4.322	24	4.327	0.005	-0.1
ALCOHOLIC EXTRACT OF PANCREAS + GLUCOSE + MUSCLE JUICE.					
1	4.617	24	4.511	0.106	2.3
ALCOHOLIC EXTRACT OF PANCREAS + GLUCOSE + MUSCLE JUICE DIGESTED IN THERMOSTAT FOR TWELVE HOURS WITH PANCREATIN.					
1	3.944	24	3.939	0.005	0.1
2	4.111	24	4.007	0.104	2.5
FRESH PANCREAS JUICE + MUSCLE JUICE + GLUCOSE.					
1	2.787	24	2.700	0.087	3.1
2	2.302	24	2.226	0.076	3.3
3	3.011	24	2.920	0.090	3.0
4	3.117	24	3.106	0.011	0.4
5	3.304	24	3.311	-0.007	-0.2
<p>In Experiment 1, glucose was added immediately; in Experiment 2, after four hours; in Experiment 3, after eight hours; in Experiment 4, after sixteen hours; and in Experiment 5, after twenty-four hours of action in the thermostat of juice of fresh pancreas on muscle. In all cases the experiment was continued for twenty-four hours after the addition of glucose.</p>					

3. Under the same circumstances neither arabinose, nor lactose, nor levulose in material quantity is subject to the same destruction.

4. The action of bacteria to destroy glucose in these experiments is shown to have been absent by the failure of the mechanism with other sugars and by the proved sterility of the mixture.

5. Trypsin or another constituent of the pancreas has a harmful effect upon the active muscle substance, a fact which may perhaps account for the apparent inhibition sometimes observed.

6. In such experiments the use of a mixture of mono- and di-sodium phosphates to preserve neutrality is advantageous.



## HYDROLYSIS OF PHASEOLIN.<sup>1</sup>

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

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

**P**HASEOLIN is a globulin which forms nearly all of the protein substance of the white or kidney bean (*Phaseolus vulgaris*). This protein was formerly called legumin, and was described by Ritthausen<sup>2</sup> at first under this name. Subsequently<sup>3</sup> he showed that it differed very distinctly from the legumin of other leguminous seeds, but did not give it any distinctive name. One of us<sup>4</sup> later made an extensive study of the proteins of this seed which confirmed most of Ritthausen's observations and showed that preparations made under a great variety of conditions were of the same ultimate composition. No evidence was obtained which indicated that this globulin was not a definite protein substance, and it was therefore named phaseolin. A small amount of another protein, phaselin, was found in this seed, which was separated from the extracts by prolonged dialysis in distilled water or in alcohol or by heating. This protein differed in composition and properties from phaseolin, from which it could be separated, in consequence of its extreme solubility, in very dilute saline solutions.

Although the former study of this seed has shown that fractional precipitations of phaseolin were of uniform composition, we have thought it desirable to supplement the former work by fractional precipitations from ammonium sulphate solutions whereby we also hoped to obtain the phaseolin in a crystalline condition.

Crystalline products were, in fact, obtained, but the conditions of crystallization were so difficult to maintain that completely crystallized preparations were not secured.

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

<sup>2</sup> RITTHAUSEN: *Die Eiweisskoerper*, etc., Bonn, 1872.

<sup>3</sup> *Ibid.*: *Journal für praktische Chemie*, 1884, xxix, p. 452.

<sup>4</sup> OSBORNE: *Journal American Chemical Society*, 1894, xvi, pp. 633, 703, 757.

## FRACTIONAL PRECIPITATION OF PHASEOLIN.

This fractionation was conducted by extracting the finely ground beans with 10 per cent ammonium sulphate solution, filtering the extract perfectly clear, and saturating it with the same salt. The precipitate produced by saturation with ammonium sulphate was suspended in a little water and subjected to dialysis until a part of the salt had been removed and the protein dissolved by the resulting dilute saline solution. This solution was then filtered clear and dialyzed for four days, during which time a part of the phaseolin separated in octahedral crystals mixed with spheroidal forms and amorphous substance. This precipitate, A, was filtered out, and the solution, B, was returned to the dialyzer. Precipitate A was dissolved in 10 per cent ammonium sulphate solution and its clear solution dialyzed for four days. The precipitate again consisted of a mixture of relatively large well-formed crystals and non-crystalline forms. By suspending this precipitate, A, 1, in the solution from which it had separated, and decanting, after a brief subsidence, and repeating this process several times, it was possible to obtain a quantity of these crystals very nearly free from non-crystalline matter, which, when washed with water and alcohol, formed preparation 1, having the following composition :

C 52.49 ; H 6.89 ; N 15.88 ; S 0.27 per cent.

The substance that had been decanted from preparation 1 was filtered out, and the filtrate, A, a, dialyzed further. The substance filtered from this solution was washed with very dilute ammonium sulphate solution, then with dilute alcohol of gradually increased strength, and dried over sulphuric acid. It was then again dissolved in 10 per cent ammonium sulphate solution, and the part that had become insoluble on drying was filtered out. This weighed, when dry, 8.3 gm. The solution was dialyzed and yielded 12.5 gm. of preparation 2 which was partly crystalline, and gave the following results on analysis :

C 52.51 ; H 6.98 ; N 15.83 ; S 0.38 per cent.

The solution, A, a, after dialyzing for seven days, gave a precipitate containing some crystals which weighed 58 gm. This preparation, 3, had the following composition :

C 52.72 ; H 7.03 ; N 15.81 ; S 0.33 per cent.

Solution B, after four days' further dialysis, gave a precipitate which was filtered out, and the solution C returned to the dialyzer.

The precipitate was redissolved in 10 per cent ammonium sulphate solution and again dialyzed. The resulting precipitate, preparation 4, which was partly crystalline, weighed 67 gm. and had the following composition:

C 52.74; H 6.79; N 15.85; S 0.36 per cent.

Solution C, after dialyzing for seven days longer, gave a precipitate containing some crystals which formed preparation 5, weighing 22 gm. This was analyzed with the following results:

C 52.11; H 6.97; N 15.44; S 0.42 per cent.

The analyses of the fractions forming preparations 1-4 show that the globulin thus obtained has a constant composition. Preparation 5 contains less carbon and nitrogen, and is evidently mixed with a little phaseolin which was precipitated by the prolonged dialysis to which the solution had been subjected.

#### PREPARATION OF PHASEOLIN FOR HYDROLYSIS.

In preparing the large quantity of phaseolin required for hydrolysis the bean meal was extracted with about four parts of 2 per cent sodium chloride solution previously heated to 80°, in order to destroy any enzyme of the seed which might lead to decomposition of the phaseolin during subsequent dialysis. After agitating with the solution for about three hours, the extract was filtered on paper and the residue squeezed out in a press. The extract thus obtained was filtered perfectly clear through a dense felt of paper pulp and, protected by toluol, dialyzed in running water for four days, during which time most of the phaseolin was precipitated. The dialysis was not continued longer, in order to avoid contamination with the more soluble globulin. The precipitated phaseolin was collected on filters and then re-dissolved in 5 per cent sodium chloride brine, its solution filtered perfectly clear, and again dialyzed for four days. The precipitate of phaseolin which resulted was filtered out and washed thoroughly with water and alcohol, dehydrated with absolute alcohol, and dried over sulphuric acid. The preparation thus obtained was completely soluble in salt solution before washing with alcohol and drying, but after this treatment it was partly insoluble therein. It contained

0.74 per cent of ash and 10.45 per cent of moisture in the condition in which it was used for hydrolysis.

#### HYDROLYSIS OF PHASEOLIN.

Nine hundred and eighty grams of water and ash-free phaseolin were treated with a mixture of 1100 c.c. concentrated hydrochloric acid (sp. gr. = 1.2) and 1100 c.c. of water and heated at 100 degrees for eight hours. The solution was then boiled for twelve hours in a bath of oil.

After concentrating the hydrolysis solution under strongly reduced pressure very sharply, the thick syrup was esterified with alcohol and dry hydrochloric acid gas, as described by Fischer,<sup>1</sup> and the free esters liberated with sodium hydroxide and potassium carbonate.

After drying with potassium carbonate and anhydrous sodium sulphate, the ether was distilled off on the water bath and the residue subjected to fractional distillation.<sup>2</sup>

#### DISTILLATION A.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	75°	14 mm.	37.39 gm.
II	81°	4 "	53.76 "
III	113°	0.97 "	121.60 "
IV	150°	0.84 "	107.03 "
V	200°	0.74 "	44.25 "
Total			364.03 gm.

The undistilled residue weighed 147 gm.

The residue which remained after extracting the esters with ether was freed from inorganic salts and the thick syrup containing the hydrochlorides of the amino acids esterified as before.<sup>3</sup> As considerable ester was obtained, the aqueous layer was again freed from inorganic salts and the esterification repeated; but the yield of ester on this third treatment was small. The united esters from the second and third esterification were distilled together.

<sup>1</sup> FISCHER, E: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 151.

<sup>2</sup> In view of the large quantity of distillable ester yielded by phaseolin, a part of the ester obtained on the first was distilled with that from the subsequent esterifications.

<sup>3</sup> ABDERHALDEN, E.: Zeitschrift für physiologische Chemie, 1903, xxxvii, p. 484.

## DISTILLATION B.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	75°	12 mm.	10.94 gm.
II	90°	7 "	36.84 "
III	113°	0.97 "	36.67 "
IV	146°	0.97 "	44.37 "
V	200°	0.85 "	37.34 "
Total			166.16 gm.

The undistilled residue weighed 116 gm.

The fractions were worked up in the main according to the directions given from time to time by Emil Fischer and Emil Abderhalden and their collaborators.

**Fraction I:** This was saponified directly after collection by evaporating with strong hydrochloric acid, and the glycocoll separated as the hydrochloride of the ethyl-ester. After recrystallizing from alcohol, it melted at 144°.

*Chlorine*, 0.3014 gm. subst., gave 0.3095 gm. AgCl.

*Nitrogen*, 0.4183 gm. subst., required 4.21 c.c. 57 N-HCl.

Calculated for  $C_4H_{10}O_2NCl = N$  10.05; Cl 25.40 per cent.

Found . . . . = N 10.06; Cl 25.39 " "

The yield of glycocoll ester hydrochloride was 5.54 gm., equivalent to 2.98 gm. of glycocoll.

The remainder of the fraction consisted largely of alanine, of which there was obtained by fractional crystallization 4.81 gm.

Fraction.	Temp. of bath up to	Pressure.	Weight.
II } A	81°	4 mm.	53.76 gm.
} B	90°	7 "	36.84 "

The fraction was saponified by boiling with water for eight hours, when the alkaline reaction had ceased. The solution was then evaporated to dryness under reduced pressure and the proline extracted with boiling alcohol. The insoluble residue subjected to fractional crystallization yielded 22.69 gm. of leucine.

*Carbon and hydrogen*; 0.2607 gm. subst., gave 0.5227 gm. CO<sub>2</sub> and 0.2344 gm. H<sub>2</sub>O.

Calculated for  $C_6H_{13}O_2N = C$  54.89; H 10.01 per cent.

Found . . . . = C 54.68 H 9.99 " "

The substance decomposed at about 298°.

The fraction further yielded 10.20 gm. of substance having the general properties and percentage composition of amino-valerianic acid.

*Carbon and hydrogen*, 0.1671 gm. subst., gave 0.3144 gm. CO<sub>2</sub> and 0.1440 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.22 ; H 9.48 per cent.

Found . . . . = C 51.31 ; H 9.58 " "

**Specific rotation.** — 0.7153 gm. subst., dissolved in 17.94 c.c. of 20 per cent hydrochloric acid rotated in 2 dcm. tube 1.89° to the right at 20°.

$$(\alpha)_{D}^{20^{\circ}} = +23.74^{\circ}.$$

On again recrystallizing from dilute alcohol, a considerably higher rotation was found.

**Specific rotation.** — 0.8005 gm. subst., dissolved in 17.94 c.c. of 20 per cent hydrochloric acid rotated in 2 dcm. tube 2.2° to the right at 20°.

$$(\alpha)_{D}^{20^{\circ}} = +24.66^{\circ}.$$

E. Fischer and Dörpinghaus<sup>1</sup> obtained for their preparation from horn:

$$(\alpha)_{D}^{20^{\circ}} = +25.90^{\circ}.$$

And a similar preparation from glutenin<sup>2</sup> gave:

$$(\alpha)_{D}^{20^{\circ}} = +25.63^{\circ}.$$

As the amount of amino-valerianic acid in this fraction was comparatively large, it seemed worth while, by converting to a derivative, to compare it with that obtained by Fischer and Dörpinghaus from horn and with the synthetic of Slimmer.

The amino-valerianic acid was accordingly racemized by heating with baryta under pressure and again submitted to fractional crystallization. There were finally obtained 2.45 gm. of substance, which appeared under the microscope to be perfectly homogeneous.

<sup>1</sup> FISCHER and DÖRPINGHAUS: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 469.

<sup>2</sup> OSBORNE and CLAPP: *This journal*, 1906, xvii, p. 251.

*Carbon and hydrogen*, 0.1316 gm. subst., gave 0.2468 gm. CO<sub>2</sub> and 0.1160 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.22; H 9.48 per cent.

Found . . . . = C 51.15; H 9.79 " "

A portion was then converted into the phenylisocyanate derivative and the latter, by evaporating with hydrochloric acid, to the hydantoin. The phenylisocyanate derivative crystallized from water in well-developed hexagonal plates and melted constantly at 161° (corr.).

*Carbon and hydrogen*, 0.2371 gm. subst., gave 0.5315 gm. CO<sub>2</sub> and 0.1507 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>16</sub>O<sub>3</sub>N<sub>2</sub> = C 60.95; H 6.84 per cent.

Found . . . . = C 61.14; H 7.06 " "

The needles of the hydantoin melted constantly on repeated recrystallization from ether and petroleum-ether at 122.5° (corr.). While Fischer and Dörpinghaus<sup>1</sup> found for the phenylisocyanate derivative of their preparation from horn 162.5° (corr.) and 123° (corr.) for the hydantoin; while Slimmer<sup>2</sup> obtained in the case of the synthetic *α*-amino-iso-valerianic acid 163.5° (corr.) for the phenylisocyanate derivative and 124°–125° (corr.) for the hydantoin.

The remainder of the fraction consisted essentially of glycocoll and alanine, and for the isolation of the former it was found necessary to have recourse to the hydrochloride of the ethyl-ester, of which there was obtained 4.44 gm. of melting-point 144°, equivalent to 2.38 gm. of glycocoll. The filtrate from the glycocoll ester hydrochloride yielded 12.84 gm. of alanine, from which, after considerable difficulty, a preparation was obtained which decomposed above 290° and gave the following results on analysis:

*Carbon and hydrogen*, 0.2131 gm. subst., gave 0.3139 gm. CO<sub>2</sub> and 0.1520 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.40; H 7.93 per cent.

Found . . . . = C 40.17; H 7.92 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
III	113°	0.97 mm.	158.27 gm.

<sup>1</sup> FISCHER and DÖRPINGHAUS: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 470.

<sup>2</sup> SLIMMER, MAX D.: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 403.

This was saponified by boiling with water for seven hours, when the solution ceased to react alkaline to litmus. The solution was then evaporated to dryness under reduced pressure and the residue extracted with absolute alcohol to remove the proline. The insoluble portion was then fractionally crystallized from water, and yielded 71.09 gm. of leucine.

*Carbon and hydrogen*, 0.2824 gm. subst., gave 0.5674 gm.  $\text{CO}_2$  and 0.2523 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.2950 gm. subst., required 3.13 c.c. 5.7 N-HCl.

Calculated for  $\text{C}_6\text{H}_{10}\text{O}_2\text{N} = \text{C } 54.89$ ;  $\text{H } 10.01$ ;  $\text{N } 10.70$  per cent.

Found . . . . =  $\text{C } 54.80$ ;  $\text{H } 9.93$ ;  $\text{N } 10.61$  " "

The substance decomposed at about  $298^\circ$ .

The filtrate from the leucine contained about 9 gm. of amino acid, which appeared to consist, to some extent, of substances usually obtained in the higher fractions, among which aspartic acid was isolated as the copper salt. It seemed also to contain a not inappreciable quantity of valine; but efforts to isolate this substance, either as the free acid or in the form of the copper salt, failed.

The alcohol soluble substance of fraction II was united with that of fraction III. The solution was evaporated to dryness under strongly reduced pressure, and the dried residue extracted with boiling alcohol. On prolonged standing a considerable precipitate had separated, but its identity was not established. The filtrate was again evaporated to dryness, and the dried residue proved to be completely soluble in absolute alcohol.

For separating the racemic from the laevo proline the copper salt was employed. The part remaining undissolved in alcohol gave 7.31 gm. of racemic proline copper salt, equivalent to 5.13 gm. of proline.

*Water*, 0.2134 gm. air-dried subst., lost 0.0235 gm. at  $110^\circ$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2\text{Cu } 2 \text{ H}_2\text{O} = \text{H}_2\text{O } 11.00$  per cent.

Found . . . . . =  $\text{H}_2\text{O } 11.01$  " "

*Copper*, 0.1871 gm. subst., dried at  $110^\circ$ , gave 0.0505 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2\text{Cu} = \text{Cu } 21.79$  per cent.

Found . . . . . =  $\text{Cu } 21.54$  " "

The amorphous copper salt of l-proline, when dried at  $110^\circ$ , weighed 28.02 gm., equivalent to 22.10 gm. of l-proline.

For identification a portion was converted into the phenylhydantoin. The substance crystallized from water in the characteristic flat prisms, which melted at  $143^\circ$ .



Carbon and hydrogen, 0.2045 gm. subst., gave 0.4973 gm. CO<sub>2</sub>, and 0.1082 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.60; H 5.61 per cent.

Found . . . . = C 66.32; H 5.88 " "

Fraction.	Temp of bath up to	Pressure.	Weight.
IV { A	150°	0.84 mm.	107.03 gm.
{ B	146°	0.97 "	44.37 "
Total . . . . .			151.40 "

The fraction was treated with water, and the phenylalanine ester shaken out with ether, according to the procedure described by Fischer and Abderhalden.<sup>1</sup> The ester was saponified by evaporation with hydrochloric acid. The phenylalanine hydrochloride weighed 23.53 gm., equivalent to 19.27 gm. of phenylalanine.

For identification the substance was converted to free phenylalanine, and to the difficultly soluble copper salt.

Carbon and hydrogen, 0.1814 gm. subst., gave 0.4355 gm. CO<sub>2</sub> and 0.1107 gm. H<sub>2</sub>O.

Nitrogen, 0.2012 gm. subst., required 1.68 c.c. 5/7 N-HCl.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N = C 65.40; H 6.73; N 8.50 per cent.

Found . . . . = C 65.47; H 6.78; N 8.35 " "

The copper salt gave the following analysis:

Carbon and hydrogen, 0.2678 gm. subst., dried at 110°, gave 0.5421 gm. CO<sub>2</sub> and 0.1223 gm. H<sub>2</sub>O.

Calculated for C<sub>18</sub>H<sub>20</sub>O<sub>4</sub>N<sub>2</sub>Cu = C 55.12; H 5.15 per cent.

Found . . . . . = C 55.21; H 5.07 " "

The aqueous layer was saponified by warming with excess of baryta. It separated on standing, 32.38 gm. of aspartic acid as the barium salt.

Carbon and hydrogen, 0.3230 gm. subst., gave 0.4286 gm. CO<sub>2</sub> and 0.1599 gm. H<sub>2</sub>O.

Nitrogen, 0.3335 gm. subst., required 3.47 c.c. 5/7 N-HCl.

Calculated for C<sub>4</sub>H<sub>7</sub>O<sub>4</sub>N = C 36.06; H 5.31; N 10.55 per cent.

Found . . . . = C 36.19; H 5.50; N 10.40 " "

<sup>1</sup> FISCHER and ABDERHALDEN: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 274.

The filtrate from barium aspartate, freed from barium, yielded 8.63 gm. of hydrochloride of glutaminic acid, equivalent to 6.91 gm. of the free acid.

The free acid decomposed at about 202°–203° with effervescence.

*Carbon and hydrogen*, 0.3761 gm. subst., gave 0.5593 gm. CO<sub>2</sub> and 0.2063 gm. H<sub>2</sub>O.

*Nitrogen*, 0.4622 gm. subst., required 4.34 c.c. 5/7 N–HCl.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.82; H 6.12; N 9.52 per cent.

Found . . . . = C 40.56; H 6.09; N 9.39 “ “

After freeing the remainder of the fraction from chlorine with silver sulphate, there was further obtained 35.95 gm. of copper aspartate, equivalent to 17.36 gm. of aspartic acid. The substance crystallized from water in the characteristic tyrosine-like bundles of needles.

*Copper*, 0.1022 gm. air-dried subst., gave 0.0292 gm. CuO.

*Nitrogen*, 0.1871 gm. air-dried subst. required 0.96 c.c. 5/7 N–HCl.

Calculated for C<sub>4</sub>H<sub>5</sub>O<sub>4</sub>NCu · 4½ H<sub>2</sub>O = Cu 23.06; N 5.09 per cent.

Found . . . . . = Cu 22.80; N 5.13 “ “

From the filtrate from the copper aspartate no definite substance could be isolated.

Fraction.	Temp. of bath up to	Pressure.	Weight.
V { A	200°	0.85 mm.	37.34 gm.
{ B	200°	0.74 “	44.25 “

The phenylalanine was separated as in fraction IV, and isolated as the hydrochloride. There were obtained of the latter 15.42 gm., equivalent to 12.63 gm. of phenylalanine.

The aqueous layer after saponification with baryta separated, on prolonged standing, large clusters of the barium salt of glutaminic acid. The substance was as good as free from barium aspartate; for on decomposition with sulphuric acid it yielded 14.20 gm. of glutaminic acid, which, after once recrystallizing from water, was obtained in perfectly pure condition. It decomposed at about 202°–203° with effervescence.

*Carbon and hydrogen*, 0.3888 gm. subst., gave 0.5818 gm. of CO<sub>2</sub> and 0.2201 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.82; H 6.12 per cent

Found . . . . = C 40.81; H 6.29 “ “

The filtrate<sup>1</sup> from barium glutamate was freed from barium with sulphuric acid, and, after concentrating to small volume under reduced pressure, was saturated with gaseous hydrochloric acid. After long standing at 0°, 9.16 gm. of glutamic acid hydrochloride had separated. Recrystallized from strong hydrochloric acid, it melted at 198°, while the free acid decomposed at about 202°–203° with effervescence.

There was further isolated from fraction V, 1.66 gm. of aspartic acid as the copper salt. After removing the copper the remainder of the fraction was examined for serine, and of this substance 2.88 gm. obtained by fractional crystallization of the free amino-acids.

The serine decomposed at about 243° to a brownish mass with effervescence. The aqueous solution tasted sweet.

*Carbon and hydrogen*, 0.2624 gm. subst., gave 0.3290 gm. CO<sub>2</sub> and 0.1591 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>3</sub>N = C 34.29; H 6.67 per cent.

Found . . . . = C 34.19; H 6.73 " "

#### DISTILLATION RESIDUE.

From the undistilled residue of A and B there was further isolated, after saponification with excess of baryta, 87.92 gm. of the hydrochloride of glutamic acid, which makes the total weight of free glutamic acid, isolated by the ester method, 98.91 gm., or 10.1 per cent of the protein, which is about 70 per cent of the yield obtained by the direct method. As glutamic acid separates from the hydrolysis solutions of phaseolin with difficulty, we made an examination of the filtrate remaining from the former determination of Osborne and Gilbert<sup>1</sup> and succeeded in obtaining 2.21 per cent more glutamic acid, making the total 14.54 per cent.

#### RESIDUE AFTER ESTERIFICATION.

The residue which remained after removing the esters with ether from the original solution of the products of hydrolysis was examined for oxy-proline,<sup>2</sup> with a negative result. In its place there was obtained, as in gliadin and glutenin,<sup>3</sup> 0.87 gm. of pure serine, which decomposed at about 243°.

<sup>1</sup> OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

<sup>2</sup> FISCHER, E.: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 2660.

<sup>3</sup> OSBORNE and CLAPP: This journal, 1906, xvii, pp. 242, 255.

Carbon and hydrogen, 0.2743 gm. subst., gave 0.3444 gm. CO<sub>2</sub> and 0.1717 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>3</sub>N = C 34.29; H 6.67 per cent.

Found . . . . = C 34.24; H 6.95 " "

#### CYSTINE.

No attempt was made to isolate cystine, as phaseolin contains less than 0.4 per cent of sulphur.

#### TYROSINE.

Forty grams of phaseolin, equal to 37.2 gm. dry and ash-free, when decomposed by boiling with three parts of sulphuric acid and six parts of water, yielded 0.8108 gm. of tyrosine, or 2.18 per cent.

Nitrogen, 0.2533 gm. subst., required 2.02 c.c. 5/7 N-HCl.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>3</sub>N = N 7.75 per cent.

Found . . . . = N 7.97 " "

#### ARGININE.

By the method of Kossel and Patten 44.76 gm. of dry and ash-free phaseolin gave a solution containing arginine, in which the nitrogen was equal to 2.187 gm. of arginine, or 4.89 per cent.

Nitrogen, 50 c.c. solution required 6.82 c.c. 5/7 N-HCl = 0.0682 gm. N = 0.682 gm. N in 500 c.c. = 2.115 gm. arginine, adding 0.072 gm. for solubility of the silver arginine = 2.187 gm. = 4.89 per cent.

The arginine was converted into the picrolonate in the usual way.

Nitrogen, 0.1161 gm. subst., gave 26.8 c.c. moist N<sub>2</sub> at 758 mm. and 25°.

Calculated for C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>N<sub>4</sub> · C<sub>10</sub>H<sub>5</sub>O<sub>5</sub>N<sub>4</sub> = N 25.62 per cent.

Found . . . . . = N 25.71 " "

The picrolonate decomposed at 226°-227°.

#### HISTIDINE.

The solution of the histidine from 44.76 gm. phaseolin was made up to 500 c.c., and found to contain nitrogen equal to 1.97 per cent of histidine.

Nitrogen, 100 c.c. solution required 4.8 c.c. 5/7 N-HCl = 0.0480 gm. N = 0.2400 gm. N in 500 c.c. = 0.8843 gm. histidine = 1.97 per cent.

A solution containing the histidine from 37.2 gm. of another preparation of phaseolin contained nitrogen equal to 1.74 per cent of histidine.

*Nitrogen*, 50 c.c. required 1.79  $\frac{5}{7}$  N-HCl = 0.0179 gm. N = 0.179 gm. in 500 c.c. = 0.66 gm. histidine = 1.74 per cent.

The histidine contained in the phosphotungstic acid precipitate obtained from the residue of the esters was converted into the dichloride which crystallized in the characteristic rhombohedral crystals, and gave, on warming, the biuret reaction. It melted at about 230° with effervescence.

*Chlorine*, 0.2115 gm. subst., dried at 120°, gave 0.2642 gm. AgCl.

Calculated for  $C_6H_{11}O_2N_3Cl_2 = Cl$  31.08 per cent.

Found . . . . . = Cl 30.88 “ “

#### LYSINE.

The lysine picrate yielded by 44.76 gm. of phaseolin weighed 4.11 gm. = 1.6000 gm. lysine, or 3.57 per cent.

The lysine picrate yielded by 37.2 gm. phaseolin weighed 3.7485 gm. = 1.4593 gm. lysine, or 3.92 per cent.

*Nitrogen*, 0.1626 gm. subst., gave 27 c.c. moist  $N_2$  at 758.3 mm. and 22°.

Calculated for  $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3 = N$  18.70 per cent.

Found . . . . . = N 18.76 “ “

The following table gives the results of this hydrolysis of phaseolin calculated to a water and ash-free basis:

	Per cent.		Per cent.
Glycocoll . . . . .	0.55	Serine . . . . .	0.38
Alanine . . . . .	1.80	Tyrosine . . . . .	2.18
Valine . . . . .	1.04	Oxyproline . . . . .	undetermined
Leucine . . . . .	9.65	Arginine . . . . .	4.89
Proline . . . . .	2.77	Histidine . . . . .	1.97
Phenylalanine . . . . .	3.25	Lysine . . . . .	3.92
Aspartic acid . . . . .	5.24	Ammonia . . . . .	2.06
Glutamic acid . . . . .	14.54	Typtophane . . . . .	present
• Total . . . . .			54.27

After our analysis of phaseolin was nearly completed Abderhalden and Babkin<sup>1</sup> published an analysis of the monoamino acids of legumin

<sup>1</sup> ABDERHALDEN and BABKIN: Zeitschrift für physiologische Chemie, 1906, xlvii, p. 354.

prepared by Ritthausen's method from the white bean. Although the statements of Abderhalden and Babkin leave us in some doubt as to the identity of the seed used by them with that used by us, and also as to which of the three methods employed by Ritthausen was used by them in making their legumin, it seems probable that the same protein substance served for their analysis as for ours. On this assumption a comparison of their results with ours shows that a satisfactory agreement can be obtained with Fischer's method by different operators working wholly independently.

The results obtained by Abderhalden and Babkin were: glycocoll, 1.0; alanine, 2.8; valine, 1.0; leucine, 8.2; proline, 2.3; phenylalanine, 2.0; aspartic acid, 4.0; glutaminic acid, 16.3; tyrosine, 2.8; total, 40.4 per cent.

In conclusion we wish to acknowledge our indebtedness to Mr. I. F. Harris for the service which he rendered in the preparation of the large quantity of phaseolin used in this analysis.

We also wish to express our thanks to Prof. W. P. Bradley, of Wesleyan University, for the abundant supply of liquid air which he has generously placed at our disposal for all the analyses of the proteins thus far hydrolyzed in this laboratory.

EFFECT OF PARTIAL STARVATION FOLLOWED BY A  
RETURN TO NORMAL DIET, ON THE GROWTH OF  
THE BODY AND CENTRAL NERVOUS SYSTEM OF  
ALBINO RATS.

By SHINKISHI HATAI.

[From the Wistar Institute of Anatomy and Biology, Philadelphia.]

IN my previous research (:04) on the effect of partial starvation on the brain of the albino rat, it was shown that in the experimented rats, fed with starch, beef fat, and water for twenty-one days (younger group, rats thirty to forty days old; older group, rats one hundred and fifty to two hundred days old), not only the growth of the brain was stopped, but brain substance was lost, the actual loss from the weight before starvation being, on the average, 4.67 per cent. In such experimented animals, also, the percentage of water in the brain was diminished (79.08 per cent control, and 78.84 per cent experimented) and of the ether-alcohol extracts increased (46.69 per cent control, and 47.61 per cent experimented — Hatai, :04).

As the absolute weight of the brain in the starved group was diminished, and as the relative amount of the extracts was increased, the writer inferred that the protein substances had been most affected. The total loss in the body weight in the experimented rats at the end of twenty-one days was 29.7 per cent.

Having established the fact that the brain is definitely modified as the result of partial starvation for twenty-one days, the next question was:

Can the nervous system thus affected recover when the animal is returned to a normal diet? The present research was undertaken in order to answer this question.

Altogether thirty-two rats, representing seven litters, were used. One half of each litter was subjected to partial starvation, and the other half used for control. The rats were so grouped that at the beginning of the observation the average body weight in the control group balanced that in the experimented. As soon as the young

albino rats reached thirty days of age, the experimented groups were fed with starch (Oswego cornstarch) and water alone,<sup>1</sup> while the control groups were fed with the usual diet, — corn, cabbage, milk, bread, meat, etc. The supply of food for both groups was abundant. After twenty-one days of starvation the experimented rats were at once put on the full normal diet, and then fed for the succeeding one hundred and forty-nine days with the same diet as was given to the control group. When the rats became two hundred<sup>2</sup> days old, they were killed, and the weight of the central nervous system, percentage of water, and percentage of the ether-alcohol extracts were determined. The dissection of the rats, and the procedure for the determination of weight, etc., were carried out with all the precautions used in the previous experiments.

**Body weight.** — As is shown in Table I, the initial weight of the experimented male rats was slightly greater (6.5 per cent) than that of the male controls, while that of the experimented females was slightly less (5.5 per cent) than that of the female controls. As the result of the partial starvation, the loss in the body weight of the experimented group was 24 per cent in the male, and 21 per cent in the female, while the increase in the control group for the same period amounted to 44.2 per cent in the male, and 46.4 per cent in the female. Thus, after twenty-one days (*e. g.*, at the age of  $30 + 21 = 51$  days), the difference in the weight between the control and experimented groups became very large, amounting to 55 per cent in the male, and 60 per cent in the female, the weight of the control group being taken as the standard.

In my previous research the loss in the weight (younger series, thirty to forty days old) after twenty-one days of starvation was even greater; the average for both sexes being 32 per cent, as contrasted with 23 per cent in the present research. It must be remembered, however, that in the earlier experiments the rats were slightly older. Thus the greater loss in weight in the previous case was, perhaps, in some measure due to the reduction of fat.

After twenty-one days of starvation the experimented rats showed marked changes, the vertebral spines were evident, the trunk slightly curved, the pink color had entirely disappeared from the soles of the feet as well as from the external ear, the eyelids were partly closed.

<sup>1</sup> The beef fat was omitted in the present experiment.

<sup>2</sup> It was found in our laboratory that the albino rats of two hundred days old are fully matured. The sexual maturity takes place at about seventy days of age.



and movements were rather unsteady. The rats in this state were removed to the other cages and at once given a full normal diet. The rapidity of recuperation was surprising, as it took only three or four days for them to return to their initial weight.

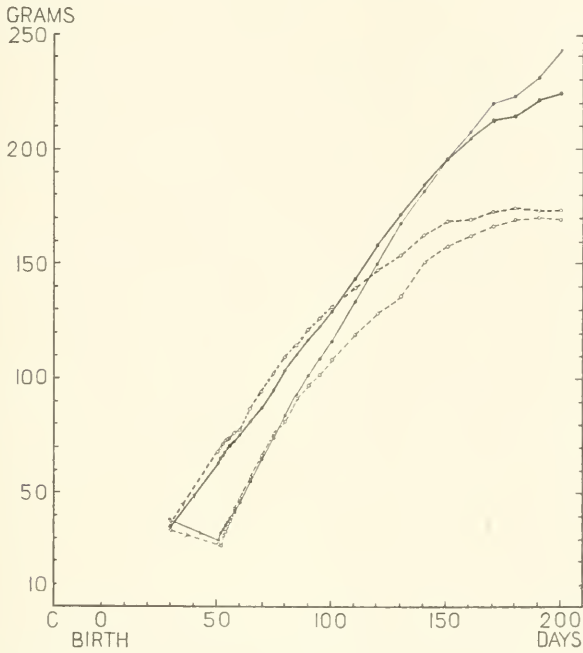


FIGURE 1.—Curves showing the body weights of albino rats at different ages. C, conception, and O, the date of birth twenty-one days after conception. •—• Males Starved. ○—○ Female Starved. ●—● Male Control. ○—○ Female Control.

It was naturally asked whether or not the mere distention of the stomach was responsible for the rapid recovery in the body weight. A careful examination, however, showed that this was not the case. Within three to four days almost all the evidences of disturbed nutrition, enumerated above, entirely disappeared. The pink color reappeared in the soles of the feet and the external ear; the eyes were held widely opened, the trunk became straight. The movements, however, were not so steady or so active as in the normal rats. An interpretation of this rapid recovery is postponed until the composition of the urine during such a period, as well as some other points, have been determined.

The daily increase or decrease in weight is shown in Fig. I. At

TABLE I.

CONTROL RATS (MALES).						
Body weight.			Weight of		Percentage, water.	
Initial.	After 21 days.	Final.	Brain.	Sp. cord.	Brain.	Sp. cord.
					per cent.	per cent.
23.6	60.3	211.5	1.7810	0.5520	77.36	69.61
25.6	48.6	233.8	1.8030	0.5348	77.79	70.26
27.2	56.0	205.0	1.7988	0.5614	77.79	69.50
28.2	54.0	188.4	1.7214	0.5330	77.13	69.60
31.4	55.6	228.8	1.9264	0.5894	77.28	69.90
36.2	59.8	199.2	1.7641	0.5168	77.60	70.54
42.7	76.4	223.4	1.8847	0.5814	77.27	69.38
43.2	74.2	211.2	1.8634	0.5415	77.80	69.56
58.6	83.0	318.4	2.1862	0.6548	77.51	69.06
....	....	....	....	....	....	....
AVERAGES.						
35.2	63.1	224.4	1.8587	0.5628	77.50	69.71
CONTROL RATS (FEMALES).						
24.2	72.4	161.2	1.6644	0.4770	77.60	70.10
26.5	59.0	141.3	1.6864	0.4866	77.44	69.21
26.8	54.8	154.2	1.7018	0.4864	77.65	69.10
42.5	75.8	188.8	1.8664	0.5582	77.34	69.13
46.2	74.6	201.6	1.9063	0.5834	77.39	69.22
51.6	70.2	188.2	1.9272	0.5587	77.58	69.62
....	....	....	....	....	....	....
AVERAGES.						
36.3	67.8	172.6	1.7905	0.5250	77.50	69.40

TABLE I (Continued).

EXPERIMENTED RATS (MALES).						
Percentage, water.		Weight of		Body weight.		
Sp. cord.	Brain.	Sp. cord.	Brain.	Final.	After 21 days.	Initial.
per cent.	per cent.					
71.06	78.05	0.5108	1.7930	199.7	20.8	27.2
69.97	77.84	0.5482	1.7507	196.3	22.8	27.5
70.95	77.90	0.4978	1.6138	194.0	22.2	28.8
69.79	77.74	0.5714	1.9514	228.7	23.8	31.1
70.20	77.82	0.5940	2.0681	262.0	25.0	32.0
70.47	77.68	0.5460	1.7696	215.8	26.4	32.0
69.97	77.91	0.5902	1.8779	256.0	30.4	40.8
69.66	77.39	0.5988	1.9493	281.0	32.2	43.9
69.05	77.52	0.5926	1.9774	259.0	38.2	52.2
69.46	77.61	0.7162	2.1154	327.0	42.4	60.6
AVERAGES.						
70.05	77.75	0.5766	1.8866	242.0	28.4	37.6
EXPERIMENTED RATS (FEMALES).						
71.11	77.90	0.4120	1.5614	130.0	20.2	24.0
70.81	78.01	0.4520	1.5696	138.2	21.4	24.6
69.56	77.64	0.5152	1.6800	176.0	28.9	36.1
69.46	77.91	0.5804	1.8472	198.8	29.7	36.9
70.85	77.82	0.4858	1.6372	152.0	26.3	37.8
69.92	77.60	0.5593	1.8811	181.0	32.4	40.0
69.21	77.41	0.5574	1.8608	199.0	30.0	40.9
AVERAGES.						
70.10	77.75	0.5089	1.7196	167.8	27.0	34.3

the end of two hundred days the final weight in the experimented and in the control was 242 and 224 gm. respectively, in the case of the males, and 173 gm. and 168 gm. in the case of the females; that is, the experimented male rats were slightly heavier (7.4 per cent) than in the controls, while in the case of the females the control rats were the heavier (2.5 per cent).

The comparison between the initial and final weight is shown in Table II.

TABLE II.

	Initial.	Body weight.		Total gain.	Ratio between initial and final.
		After 21 days.	Final.		
Male, controls . . .	35.2	63.1	224.4	189.2	1 : 6.37
Male, experimented . .	37.6	28.4	242.0	204.4	: 6.43
Female, controls . . .	36.3	67.8	172.6 <sup>1</sup>	136.3	: 4.75
Female, experimented .	34.3	27.0	167.8 <sup>1</sup>	133.5	: 4.89

From the above it is seen that the absolute gain in weight is slightly greater in the experimented males, and slightly smaller in the experimented females, as compared with their controls. The ratios, however, show that the relative gain is approximately the same in both experimented and control groups, although slightly greater in the case of the experimented rats in both sexes.

I therefore conclude that so far as the body weight is concerned, the experimented rats have completely recovered from the effect of the twenty-one days of partial starvation.

It has been frequently observed by different investigators — Coudeureau ('69), Pagliani ('79), and others — that the growth of the body in recuperation is very rapid in the children whose growth has been temporarily disturbed by illness or other unfavorable conditions. This observation is well supported by the present experiments. As is shown in Table I, as well as in Fig. I, the recovery in the weight is most astonishing, especially during the first three or four days, within which time the starved rats regain the weight lost during the twenty-one days of starvation. Later the increase in weight is very steady, though not as rapid as during the first few days, until the rat has reached the age of one hundred and fifty days, and after this age increase in weight is relatively slow.

What will happen to such rats during the later portions of the

<sup>1</sup> The body weight in both control and experimented is small for the age.

span of life has yet to be determined in order to answer the question whether this partial starvation in early life has any influence either on longevity or the onset of old age.

**Weight of brain and spinal cord.**—After the body weight had been taken, the brain and spinal cord were removed separately, and their weights were carefully determined. The spinal cord was

TABLE III.

	Body weight.	Encephalon.	Sp. cord.
Male, controls . . .	224	1.8587	0.5682
Male, experimented . .	242	1.8866	0.5766
Female, controls . . .	173	1.7905	0.5250
Female, experimented . .	168	1.7196	0.5089

severed from the encephalon at the tip of the calamus scriptorius. The spinal roots were cut off as close to the cord as possible. The results obtained are seen in Table III.

As it stands, the weight of the central nervous system in the experimented male is heavier, and in the experimented female lighter, when compared with the corresponding controls. Since the brain weight is closely correlated with the body weight, we should expect a heavier brain weight in the heavier individuals, and therefore it is desirable to determine whether or not the brain weights found in the experimented rats correspond with the given body weights. Dubois ('98) found that in man, at maturity, the brain weights were related as the fourth roots of the body weights.

Dhéré and Lapique ('98) have determined a like relation for dogs of different sizes where they found a good accordance between the observed and calculated results. An application to the present data of this law given by Dubois for the human brain weights, shows that in the case of the males 1.8866 gm. of brain in the experimented males should correspond to the body weight of 238.3 gm., instead of 242 gm., when the relations in the control group are used as a standard. The difference (3.7 gm.) is too small to be considered significant. It is therefore quite safe to conclude that so far as the relation between brain weight and body weight in the male is concerned, the starvation effect has been completely removed. In the case of the female we have some difficulty in applying Dubois' law, since the brain weight in the control group is too high<sup>1</sup> for the given body weight. Accord-

<sup>1</sup> This fact was determined by examining some ten records preserved in our archives, for female rats having a body weight of about 173 gm.

ing to Dubois' law, the figures for the control group being taken as the standard, a brain of 1.7196 gm. found in the experimented group should correspond to body weight of 147.3 gm., instead of 168 gm., as observed. It was found that according to our standard curve 1.7196 gm. of brain weight there corresponds to the body weight of 162 gm., or 6 gm., less than that observed. This difference is negligible in view of the variability in this relation, and therefore it seems

TABLE IV.

	Percentage of water.	
	Encephalon. per cent.	Sp. cord. per cent.
Male, controls . . . . .	77.50	69.71
Male, experimented . . . . .	77.75	70.05
Female, controls . . . . .	77.50	69.40
Female, experimented . . . . .	77.75	70.10

safe to conclude that in the experimented female group partial starvation has not permanently modified the relation of brain weight to body weight.

In regard to the weight of the spinal cord, it is clearly shown that the cord also follows body weight in the same manner as does the brain, but the details have not been worked out.<sup>1</sup>

**Percentage of water in the central nervous system.** — From Table IV it is clearly seen that percentage of water in both encephalon and spinal cord is higher in the experimented rats than in the control rats.

On the average, the difference between the experimented and control is 0.25 per cent in the encephalon of both sexes, and 0.34 per cent in the male spinal cord, and 0.70 per cent in the female, always in favor of the experimented groups. Although the difference shown is small, nevertheless it is significant, since the difference appears in all but one instance when the representatives of the same litter are compared.

From unpublished observations in this laboratory, it has been concluded that during normal growth the percentage of water in the

<sup>1</sup> An application of Dubois' law to the weight of the spinal cord shows that, when the relations in the control group are used as a standard, 0.5766 gm. of spinal cord in the experimented males correspond to the body weight of 247.4 gm. (difference 5.4 gm.), and 0.5089 gm. of the experimented female spinal cord to 152.8 gm. of the body weight (difference, 15.2 gm.). These calculated values of the body weight agree with those calculated from the brain weight, indicating that the relation of the spinal cord to the body is similar to that of the brain.

TABLE V.

LITTER 1.					
CONTROL RATS.			EXPERIMENTED RATS.		
Body weight.	Percentage, water.		Percentage, water.		Body weight.
	Brain.	Sp. cord.	Sp. cord.	Brain.	
	per cent.	per cent.	per cent.	per cent.	
223.4 M.	77.27	69.38	69.66	77.39	M. 281.0
188.8 F.	77.34	69.13	69.35	77.41	F. 199.0
LITTER 2.					
318.4 M.	77.51	69.06	69.46	77.61	M. 327.2
201.6 F.	77.39	69.22	69.46	77.91	F. 198.8
188.2 F.	77.58	69.22	69.90	77.60	F. 181.0
			69.56	77.64	F. 176.0
LITTER 3.					
211.2 M. <sup>1</sup>	77.80	69.56	69.05	77.52	M. 259.0
LITTER 4.					
199.2 M.	77.60	70.54	69.97	77.91	M. 256.2
			70.47	77.68	M. 215.8
			70.85	77.82	F. 152.0
LITTER 5.					
233.8 M.	77.79	70.26	70.20	77.82	M. 262.0
228.8 M.	77.28	69.90	71.06	78.05	M. 199.7
205.0 M.	77.79	69.50	70.95	77.90	M. 194.0
188.4 M.	77.13	69.60	71.11	77.90	F. 130.0
154.2 F.	77.65	70.10			

<sup>1</sup> Litter 3 alone is an exceptional case where the percentage of water in the control is higher than in the experimented rat.

TABLE V (continued).

LITTER 6.					
CONTROL RATS.			EXPERIMENTED RATS.		
Body weight.	Percentage, water		Percentage, water.		Body weight.
	Brain.	Sp. cord.	Sp. cord.	Brain.	
	per cent.	per cent.	per cent.	per cent.	
211.5 M.	77.36	69.61	69.79	77.74	M. 228.7
141.3 F.	77.44	69.21	69.97	77.84	M. 196.3
LITTER 7.					
161.2 F.	77.60	69.10	70.81	78.01	F. 138.2

nervous system of the rat is mainly a function of its age, and is but slightly modified by the weight of the central nervous system or of the size of the body (Donaldson). In these experiments, however, the relation of age to the nervous system is considerably modified in the experimented rats, since not only the growth of the nervous system has been completely stopped for twenty-one days, but the percentage of water was diminished (79.08 per cent control, and 78.84 per cent experimented — Hatai, :04) by this treatment. Therefore the higher percentage of water found in the central nervous system of the experimented rats after recovery may mean one of two things: (1) As the result of partial starvation, the destructive process which has been traced (see previous paper, Hatai, :04,) might produce a diminution of nerve tissue, and the relatively higher content of water might be due merely to an accumulation of fluid occupying the spaces which had been so developed; (2) It may indicate a much more active metabolic process, and be an indication of recuperative activity.

The second assumption seems the more probable, since the weight of the central nervous system was normal in relation to the body weight, indicating that there was not a mere accumulation of the fluid occupying newly formed spaces, for this would have tended to reduce the brain weight.



Recently Watson (:05) made observations on the effect of the bearing of young upon the body weight and the weight of the central nervous system of female albino rats, where he has shown that the brain and spinal cord of the mated individuals contained a slightly higher percentage of water.

He gives the following percentage values :

	Percentage of water.	
	Brain. per cent.	Sp. cord. per cent.
Mated (average — 8 rats) . . .	77.47	68.51
Unmated (average — 10 rats) . . .	77.37	68.29

Watson's experiments may be considered analogous to mine, as he is also dealing with animals that have passed through a period of partial starvation, since during lactation the mother loses body weight to quite an extent (Minot, '91; Watson, :05). Although it is impossible at the present moment to make any definite statement, our observations on partial starvation suggest that the higher percentage of water found in the rats bearing young might have been produced as the effect of temporary retardation of the growth of the nervous

TABLE VI.

	Percentage of extracts.	
	Encephalon. per cent.	Sp. cord. per cent.
Male, controls . . . . .	50.61	68.83
Male, experimented . . . . .	50.36	68.54
Female, controls . . . . .	49.56	68.87
Female, experimented . . . . .	49.16	68.40

system followed by recovery. This can be determined only by a study of the nervous system during the period of greatest retardation in rats bearing young.

It remains, moreover, to be determined whether or not our experimented rats will in this respect recover entirely from this condition which is produced by the twenty-one days of partial starvation.

In the normally grown rats the percentage of extracts in the nervous system is also a function of age, and is inversely related to the percentage of water (Donaldson). Thus the determination of the percentage of extracts would furnish us further evidence as to the normal condition of the growth of the nervous system with respect to age and to the percentage of water.

It is clear from the preceding Table VI that the percentage of ex-

tracts is less in the experimented group in both encephalon and spinal cord than in the control. In the case of the encephalon 0.25 per cent in the male and 0.40 per cent in the female, and in the case of the spinal cord, 0.29 per cent in the male and 0.47 per cent in the female are in favor of the control rats. These are the results that one would expect. It is therefore concluded that the amount of extract, as compared with the residue, found in the nervous system of the experimented rats is normal with respect to the age and percentage of water in that organ.

#### CONCLUSIONS.

From what has been presented, the following conclusions are drawn:

(1) So far as the weight of the body and central nervous system are concerned, the effect of a twenty-one day period of partial starvation on albino rats thirty days old is eventually completely compensated.

(2) The chemical composition of the brain and spinal cord is, however, not entirely free from the effect, as is indicated by the higher percentage of water, and lower percentage of ether-alcohol extracts, in the experimented rats, as compared with the controls.

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CHEMICAL STUDIES ON THE CELL AND ITS MEDIUM.  
— PART II. SOME CHEMICO-BIOLOGICAL RELATIONS  
IN LIQUID CULTURE MEDIA.

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I. CHEMICAL DATA.

A STUDY of the conditions under which organisms live must concern itself largely with the analysis of a given complex set of conditions into its component factors. The attempt to study the factors singly is an afterthought resulting from some degree of previous analysis sufficient to show the relevancy of the supposedly simpler study. Our ability to analyze the media in which nearly all cells live — *e. g.*, sea water, fresh water, pond water, blood, lymph, fermenting solutions, etc. — into factors of determined biological value is a measure, not only of progress in this field, but also of the fruitfulness of all the studies made upon the separate conditions involved. Experience shows that progress as measured by this standard, which is based upon ability to interpret, is not very rapid. In view of the above considerations the present study, which was made upon a hay infusion regarded as a fair type of liquid medium, requires no apology, even though the results presented come far short of making an adequate picture of what occurs. The methods (except biological) by which the following data were obtained have been described, in the main, in Part I of this paper. During the past three years numerous cultures of the kind here described have been studied in this laboratory both by myself and by students. The matter was found to be well adapted to serve as an introduction to the general principles of physiological zoölogy, or better to the important subject of chemical

biology, which is neither pure chemistry nor simply descriptive biology. I shall first present, in tabular form, the chemical and physical data in regard to a number of cultures, and subsequently the biological data which ran parallel with the preceding and which were definitely determined for certain of these cultures. The significance of the numerical values is the same as in Part I. They express millionths of a gram molecule, or of a gram, of either the reagent used or of the substance measured as described in Part I.

The data for Table I were taken from cultures promiscuously selected, and they have no known relation to each other except that all were old when the data were obtained. This table serves merely to give an indication of the general pitch of the values which may prevail in cultures of this nature.

TABLE I.

Culture No. . .	5-2-21	5-2-27/1	5-2-27/2	5-2-28,1	5-2-28,2	5-3-2
Age, days . .	31	25	24	23	22	19
Phth. Ac. . .	0.34	0.58	1.11	1.0	1.1	2.0
Mthor. Alk. . .	9.6	8.8	8.6	8.4	9.0	9.6
Elect. Cond. . .	793.0	781.0	793.0	747.0	828.0	793.0
O consumed . .	44.0	44.0	68.0	32.0	52.0	52.0
Total Org. N. .	18.0	9.0	12.0	14.0	16.0	8.0
Ammon N. . .	1.8	4.4	1.8	1.8	2.4	2.0

The remaining tables of cultures give the continuous history of each but they differ in the variety of data.

TABLE II.

CULTURE NO. 5-3-27.

Age, days . .	0	1	2	3	4	5	6	7
Phth. Ac. . .	0.58	0.34	1.84	2.0	1.4	1.0	0.9	0.9
Mthor. Alk. . .	7.8	8.0	7.0	7.0	7.4	8.2	8.0	8.0
Total Org. N. .	10.0	18.0	16.0	14.0	8.0	..	25.0	10.0
Ammon. N. . .	4.4	5.0	1.2	1.4	1.4	..	1.4	2.2

TABLE III.  
CULTURE No 5-4-10.

Age, days . . . .	0	1	2	3	4	5
Phth. Ac. . . . .	0.26	0.58	1.94	1.26	1.0	0.9
Lacmoid Alk. . . .	6.8	7.4	5.8	7.8	7.4	7.6
Ca. . . . .	....	....	....	1.8	3.6	5.2
Elect. Cond. . . .	660.0	675.0	761.0	761.0	761.0	701.0
O consumed . . . .	63.0	60.0	66.0	51.0	42.0	48.0
Total Org. N. . . .	22.0	22.0	25.0	24.0	....	....
Ammon. N. . . . .	5.2	1.8	1.6	5.2	....	....

TABLE IV.  
CULTURE No. 5-4-19.1.

Age, days	0	1	2	3	4	5	6	7	8	9
Phth. Ac. . . . .	0.5	1.84	2.2	1.4	1.7	1.7	1.3	1.0	1.2	0.9
Lacmoid Alk. . . .	7.0	7.0	5.6	..	7.2	7.0	6.2	7.0	6.8	6.8
Ca. . . . .	3.8	5.6	6.2	..	5.6	4.2	6.0	4.4	3.0	3.8
Elect. Cond. . . .	637.0	660.0	597.0	..	628.0	628.0	675.0	731.0	716.0	..
O consumed . . . .	42.0	36.0	42.0	..	27.0	24.0	27.0	24.0	27.0	..
Total Org. N. . . .	....	4.0	5.0	..	18.0	8.0	12.0	20.0	....	..
Ammon N. . . . .	....	0.8	1.0	..	3.2	0.8	1.2	0.8	....	..

TABLE V.  
CULTURE No. 5-6-0.1.

Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.	Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.
0	0.38	8.0	637	8	1.5	8.4	703
1	2.5	7.6	647	11	1.0	8.4	703
2	2.0	8.0	452	12	0.75	8.4	609
4	2.5	9.0	688	13	0.63	8.6	661
5	2.5	8.4	731	14	0.63	8.6	647
6	2.0	8.6	702	15	0.5	8.6	661
7	1.75	8.4	703	18	0.5	8.8	661

TABLE VI.  
CULTURE No. 5-6-0.2.

Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.	Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.
0	0.25	8.0	541	8	0.25	6.4	563
1	0.25	7.6	563	11	....	6.0	461
2	1.25	7.5	563	12	....	5.8	397
4	2.0	7.2	574	13	....	6.2	442
5	0.25	7.0	563	14	....	6.2	425
6	0.25	6.8	530	15	....	6.0	442
7	0.25	6.6	563	18	....	5.8	442

TABLE VII.  
CULTURE No. 5-6-0.3.

Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.	Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.
0	0.38	7.8	610	8	0.25	8.6	746
1	1.5	7.2	647	11	0.25	8.6	675
2	1.0	7.8	661	12	0.13	8.6	635
4	1.0	8.2	702	13	....	8.7	622
5	0.75	8.2	688	14	....	8.7	622
6	0.5	8.4	689	15	....	8.7	622
7	0.5	8.4	765	18	....	8.6	661

TABLE VIII.  
CULTURE No. 5-10-31/1.

Age, Days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.	Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.
....	0.4	7.0	582	10	0.8	7.6	733
1	1.4	7.2	676	11	0.74	....	....
2	2.2	6.8	683	13	0.74	7.6	733
3	1.8	7.4	682	14	0.76	7.7	682
6	0.7	7.0	682	17	0.8	8.0	704
7	0.8	7.2	682	20	0.76	8.2	665
8	0.9	7.3	649	23	0.8	8.3	704
9	0.9	7.4	662	29	0.76	8.5	718

TABLE IX.  
CULTURE No. 5-10-31/2.

Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.	Age, days.	Phth. Ac.	Mthor. Alk.	Elect. Cond.
0	0.35	7.3	636	10	0.6	7.5	733
1	1.44	6.8	582	13	0.64	7.5	704
2	2.0	7.0	718	14	0.68	7.6	778
3	1.6	7.2	682	17	0.76	8.0	682
6	1.0	7.3	704	20	0.8	8.1	649
7	0.9	7.4	748	23	0.86	8.4	663
8	0.7	7.3	662	29	0.8	8.6	718
9	0.68	7.5	718				

TABLE X.

Simultaneous data for six cultures which were set differently as follows:

Culture No.

6-10-31/1. Solid hay + hay infusion + tap water + seed.

6-10-31/2. Solid hay + hay infusion + tap water + seed + yeast.

6-10-31/3. 0 + hay infusion + tap water + seed.

6-10-31/4. 0 + 0 + tap water + seed.

6-10-31/5. Solid hay + hay infusion + tap water + 0.

6-10-31/6. Old No. 6-7-24 + refeeding + reseeded.

Ph = phenolphthalein acidity; M = methyl-orange alkalinity; O = oxygen consumed.

Age.	6-10-31/1			6-10-31/2			6-10-31/3			6-10-31/4			6-10-31/5			6-10-31/6		
	Ph.	M.	O.	Ph.	M.	O.	Ph.	M.	O.	Ph.	M.	O.	Ph.	M.	O.	Ph.	M.	O.
0	0.8	8.3	28	1.2	8.0	33	0.8	7.6	16	0.8	8.2	1	0.9	8.4	16	0.74	9.1	36
1	1.4	7.8	28	2.4	7.0	22	1.2	7.5	4	1.0	8.2	4	1.0	7.3	20	1.2	9.0	24
2	1.4	7.0	24	2.5	6.7	30	1.2	7.8	12	1.0	8.0	3	1.0	7.6	16	1.2	9.0	27
3	1.2	8.3	18	2.4	6.5	26	0.6	8.0	9	0.4	9.0	5	1.0	7.8	10	1.2	9.2	24
4	..	..	..	..	..	..	..	..	..	Alk.	..	..	..	..	..	..	..	..
5	1.4	8.2	24	3.6	6.8	28	0.4	8.2	8	Tr.	8.2	3	0.72	8.1	20	1.4	9.4	25
6	1.0	8.6	20	2.6	6.6	18	0.4	8.2	2	Tr.	8.0	2	0.32	8.0	9	1.2	9.3	23
7	0.6	8.4	23	2.8	6.6	26	0.2	8.1	5	0.4	8.9	2	0.32	8.8	16	0.8	9.4	20
8	0.8	8.7	24	2.4	6.6	36	0.2	8.3	9	0.5	8.1	0	0.8	8.6	17	0.6	9.6	16
9	0.8	8.2	11	2.9	6.4	33	0.12	8.3	4	0.6	8.0	3	0.6	8.2	16	0.4	9.8	21
10	0.76	8.4	..	1.3	6.4	..	0.04	8.2	..	0.7	7.8	..	0.6	8.1	..	0.4	9.6	..
11	..	..	..	..	..	..	Alk.	..	..	..	..	..	..	..	..	..	..	..
12	0.8	8.0	4	1.0	6.4	35	Tr.	8.0	3	0.6	8.3	5	0.4	8.6	20	0.36	9.6	24
13	0.6	8.4	23	0.8	7.2	33	Tr.	8.4	4	0.8	7.4	2	0.6	8.4	16	0.2	9.6	22
14	0.4	8.6	22	1.0	7.6	32	0.2	8.4	5	0.8	7.4	2	0.6	8.6	18	0.2	9.6	20
15	0.4	8.4	23	1.2	8.2	31	0.2	8.4	7	0.9	7.4	1	0.2	8.6	20	0.4	9.5	22
16	0.4	8.2	24	1.6	9.2	24	0.2	8.4	7	0.9	6.9	3	0.32	8.6	19	0.4	9.6	16
17	..	..	25	..	..	26	..	..	21	..	..	7	..	..	13	..	..	14
19	0.4	8.6	..	0.6	8.8	..	0.5	8.2	..	0.6	6.6	..	0.4	8.6	..	0.2	9.6	..
23	0.2	8.8	26	0.6	9.4	35	0.3	2.3?	7	0.2	6.2	6	0.2	8.6	18	0.2	9.6	22



## II. BIOLOGICAL METHODS.

All the cultures of which chemical data have been given in the preceding tables, and many others, were kept under observation for their biological phenomena also. It is not the intention of the writer to make a catalogue of all the numerous organisms, plant and animal, which flourish in these cultures. My attention was directed to a certain selection from this number, which seemed favorable to my purpose of correlating their presence, number, and order of succession, with the changing conditions of their environment. The ordinary methods of microscopical examination were used. The material was in all cases examined in its living condition and in many cases also after killing and fixing. Qualitative examinations by various simple methods were frequently made, and even these were sufficient, when made regularly, to convince the observer that the different kinds of organisms showed their maximal numbers at different periods in the history of the same culture. It was desirable to express this fact in a quantitative way, and the following method was developed. For my purpose, as above stated, it was necessary to express only the relative frequency of the organisms, not their absolute numbers. The sample of culture liquid to be examined was placed in a shallow flat-bottomed watch-glass in a layer of such small depth (about a millimetre) as to suit the optical apparatus to be used for direct observation. The observer, having first identified the forms present, at least those which are most numerous, then fixes his attention upon their *relative numbers*, selecting first that form which is most numerous, and he places this at the head of his recorded list for that day as No. 1. By comparison with No. 1 the organism showing the next lower frequency is determined and given its serial position in the day's list as No. 2, and so on. At different times in the history of the same culture, or in cultures of different nature, a given organism may of course occupy a different serial position. These serial lists, when taken on the same culture from its beginning and at intervals of a day, will soon show that a given organism passes through certain serial positions, which may then be plotted as ordinates, the days being laid off on the abscissa. There is thus obtained a curve representing the relative frequency of this organism. A little practice is necessary to gain accuracy in the discrimination of the relative numbers of objects, whose size may range all the way from a bacterium to a Stentor easily visible with the naked eye.

Some matters subsidiary to this method call for remark. It was usually applied to living organisms. By forcing them into and out of a medicine dropper they may be kept uniformly distributed in the watch-glass. But if it be desirable to examine them in stationary condition, they should first be evenly distributed as just described, and then there may be added a few drops of Worcester's formol-sublimate. After thus killing the organisms they may be redistributed, if necessary or desirable, so that the observations for their serial positions, *i. e.*, for their relative numbers, may be made more accurately. The observation of these watch-glass preparations and the estimation of relative numbers in them were much facilitated by the use of a Zeiss binocular microscope in which a field of large area and great depth was easily examined at one observation. The results obtainable with this instrument for all forms except the bacteria enable the observer to feel certain regarding their accuracy.

Some observations which I have made upon the use of killing and fixing agents for the examination of the micro-organisms of these cultures, particularly the protozoa, may be permissible here. It seems to me that *Paramæcium*, owing to its sufficient size, extensive differentiation, and its delicate protoplasm, serves as an excellent test of the efficiency of killing and fixing agents for all micro-organisms of its class. The experimenter who does not content himself with the mere use of formulæ according to authority, but who is interested in the rationale of their action, can find in *Paramæcium* a good object to illustrate some principles which have been found applicable. Discussions and investigations of this nature can be found in Mann's *Physiological Histology*. Tests which I made upon *Paramæcium* with nearly all of the killing and fixing agents in common use showed that very few of them preserved the natural form of the animals. In some cases it was evident that the agent used failed to accord with the osmotic conditions which were necessary for a good preservation of form. Evidence for this view was the fact that modification of the osmotic concentration from that recommended by the author of the formula gave preserved forms more nearly resembling those of living animals. The practical application and the selection and composition of these agents should be guided, far more than has been customary, by an intelligent view of the principles of osmosis and dissociation. For *Paramæcium*, and in general for protozoa that have a distinct cell wall I have found Worcester's formol-sublimate to give a most excellent preservation. This agent consists of a ten per cent solution

of formaldehyde in water, which solution is then saturated with corrosive sublimate. The proportions may be varied, thus altering the osmotic conditions. However, for the objects tested, *Paramæcia*, and under the special conditions of adjustment to their various media in which I found them, the strong concentrations above given seemed to be well adapted. In the composition of this liquid, as in most good killing and fixing mixtures, the swelling action of one constituent, formaldehyde, is balanced by the shrinking action of another, in this case corrosive sublimate. It should further be noted that both these constituents are non-electrolytes. To use this agent, the organisms should be brought into a pipette in as small a volume of their culture liquid as possible, and they should then be ejected suddenly into a watch-glass containing the formol-sublimate. Of other fixing mixtures I obtained good results also with strong solution of Flemming and with acetic alcohol. The latter is especially advantageous when it is desirable to avoid water entirely in subsequent processes. Under some conditions osmic acid and also iodine gave good results. Formol-sublimate, and for special purposes acetic alcohol, were found sufficient.

Bacteria in living condition were examined by means of a well-known arrangement consisting of a hanging drop on the under side of a cover-glass which formed the top of a ring cell, the bottom of this cell consisting of the glass slide. The rings may be made of glass, one-half to one centimetre in height, their end surfaces should be parallel, and they may be fastened water-tight to the slide by means of a thin coating of vaseline. In the bottom of the cell is placed at least enough water to cover the surface. The rings may be made of cardboard, and in that case they should be impregnated with melted vaseline. Whether they are made of glass or of paper, their end surfaces must be of sufficient extent to make good contact with slide and cover-glass. If the latter with its hanging drop be sealed to the ring with vaseline, the whole preparation may be kept for days. It is a convenient and instructive form of moist chamber, and serves well for the study of both the bacteria and the protozoa in the living condition. For the estimation of relative numbers of bacteria and of protozoa very low rings cut out of paper were used, and the drop was made to occupy the central area of the cell and to make contact with both cover-glass and slide. For making fixed and permanent preparations of the bacteria the usual bacteriological technique for that purpose was pursued, using Ziehl's carbol-fuchsin as a stain. An

instructive series to show the progressive numerical development and decline of bacteria in these cultures can be obtained by making a permanent preparation daily.

The selection of a fair sample of the micro-organisms of the culture requires considerable care. They seem to be located in selected places, and it is not desirable to mix uniformly the contents of the culture, because that would interfere with its normal progress and with the proper taking of some of the chemical data, *e.g.*, the acidity. The plan I have used in order to avoid the disturbance of the whole culture was to take numerous samples from various places in the jar by means of a medicine dropper, to mix these samples in a watch-glass by forcing them into and out of the pipette. If for any reason it became necessary to concentrate the micro-organisms (except bacteria) I have made use of a centrifuge. This method is especially useful if one desires to make permanent preparations. The ordinary processes necessary for this purpose can be performed in glass tubes about 5 mm. in diameter and about 60 mm. in length, which have been sealed at one end in the form of a cone. This tube can be made easily from the glass part of an ordinary medicine dropper. By means of the centrifuge and of sufficiently long capillary pipettes, all changes of liquids can be made at will. The above-described tube may be fastened on any centrifuge by means of a perforated cork of the proper size.

### III. THE BIOLOGICAL CORRELATIONS OF CHEMICAL DATA.

An examination of the numerical values for the phenolphthalein acidity of the fourteen cultures of Tables II to X shows that these data have such uniformities as to produce a characteristic curve. Additional observations upon other cultures have shown that this curve is one of general validity for all the cultures I have raised under the conditions described. In each case the values rise suddenly and fall more slowly. Each curve has its maximum during the first few days of the culture history. In other words, the day of maximum acidity divides the curve into two arms, the first of which is short and steep, the second long and gradual. The following table shows the time and the value of the maximum acidities of the cultures reported in Tables II to X.

TABLE XI.

Day . . .	3	2	2	3	4	3	2	2	3	5	1.5	1.5	2	5
Acidity .	2.0	1.94	2.2	2.5	2.0	1.0	2.2	2.0	1.4	3.6	1.2	1.0	1.0	1.4

The sum of the days, = 39, divided by the number of cultures tabulated, = 14, gives an average age of 2.8 days as the time at which the maximum acidity occurs. Under some conditions, as shown in Table X under cultures 6-10-31<sup>3</sup> and 6-10-31<sup>4</sup>, a culture may become alkaline. In Curves 1 and 2 are plotted the acidities for cultures 5-10-31<sup>1</sup> and 5-10-31<sup>2</sup> according to the data given in Tables VIII and IX. Not all the curves obtained have such sharply defined maximal points as those here shown, but they all conform to the general type above described, of which these two are fair representatives.

What organisms stand in relation to this acidity? Primarily the bacteria, and secondarily the protozoa. Chemical examination has shown that the acid is volatile and consists very largely of carbonic acid. Biological examination shows the presence of relatively few bacteria when the culture is started, of enormous numbers during the next few days, and of greatly diminished numbers subsequently. The bacteria of the early period are of the rod form; later occur the filamentous forms. Several layers of zooglœa may succeed each other and fall in turn to the bottom of the jar. The cultivation on the standard media and the determination of the kinds of bacteria were not attempted. There probably was much variety here, because the gross characters exhibited by cultures from seed materials of different origin varied noticeably. In all cases gas formation was prominent during the early period, *i. e.*, when the rod bacteria were abundant. It is evident that we are here dealing with bacterial fermentations, mostly of carbohydrates and more particularly with such fermentations as occur in an acid medium. I have not dealt thoroughly with the bacterial aspect of the cultures, but the data obtained show that these organisms are the first cause of decomposition and the foundation of the food supply for all other organisms that at the same time or subsequently inhabit the medium. Owing to the small size of the bacteria, the methods I have described for determining the relative numbers of organisms of various kinds must give much less reliable results from the quantitative point of view when applied to bacteria than when protozoa only are compared numerically. However, the examinations made left no doubt that, qualitatively, the curve for bacteria runs approximately parallel to the curve for acidity.

except that its descending arm runs to a minimum value much faster than that of the corresponding arm of the acidity curve. The maximum of the bacterial curve might also vary a little from the period of maximum acidity. From the observations that have been made it is probable that the bacteria, like the protozoa, succeed each other in some order depending upon their relative adjustment to the changing conditions of their environment. Hence one curve to represent all the bacteria has but little significance for that group itself, but this general curve is of some use for comparison with the curves for protozoa. The decline of the bacteria as a whole may be ascribed first to the exhaustion of their food supply. The determination of oxygen consumed, *c. g.*, in Table X shows the presence of an abundance of organic matter at all times in these culture liquids, but the kind and condition of this material are not indicated by this estimation. That which is in available form for a given species is soon seized upon and its supply thus exhausted. The second important factor in the decline of the bacteria is the accumulation of their own waste products, especially the acid resulting from their metabolic activity. A low degree of acidity or of alkalinity may act as a stimulant, but it is well known that increased concentrations of acid inhibit the fermentations producing them.

In all the curves it is noticeable that the second arm representing the fall of acidity shows regularly a period of initial rapid fall succeeded by a much longer period of low and of more or less uniform acidity. The level maintained in this region of the curve is always higher than was the acidity of the culture on the day when it was set. Owing to the method by which the culture liquid was prepared (described in Part I of this paper), the original culture liquid contained a normal concentration of carbonic acid, for the boiling of the entire quantity of culture liquid was purposely avoided. The increase of acidity must of course be attributed to the activity of the contained organisms, and in the beginning mostly to the bacteria. The more or less level region of the acid curve I attribute to organisms also, but in the main to a different class, the protozoa. Evidence bearing upon the question of their presence, kind, and number will be given subsequently. The fact that the level region of the curve maintains itself above the initial acidity of the culture is best explained by the continual respiratory activity, during this period, of at times enormous numbers of protozoa. During the period indicated these supply the carbonic acid at a somewhat greater rate than

that of the escape of carbon dioxide, which latter is influenced by the partial atmospheric pressure of carbon dioxide and by the undisturbed condition of the culture liquid. Owing to the slowness of diffusion, each upper layer of the liquid protects the adjacent lower layer from rapid interchange with the air and enables the deeper layer to maintain a higher acidity. Direct titration of the acidity of samples taken carefully from different depths during the early period of the culture when the production of acidity was great, showed increasing acidity for each increase of 5 to 10 cm. in depth. If the culture were agitated either continuously or at intervals, the curve of acidity would of course present an entirely different form, and correlated with this would be a considerably different set of environmental conditions, which again would carry with it a corresponding modification of biological content of both qualitative and quantitative character. The existence of these relations is easily demonstrated by running, for comparison, cultures agitated by a continual current of air. For the bacteria we are familiar with the fact that slight alteration of the conditions of the nutrient medium may result in the growth or the destruction of a given species. Experiments in cultivation will soon show that other unicellular organisms also have a delicate adjustment to their environment. In the discussion of these cultures and especially of the acidity curve it must be remembered that we are less concerned with the total amount of acid produced than with that portion which does not immediately escape and with which the organisms must live in contact. This is the quantity of biological significance which the curve of acidity, obtained under the described conditions, shows. The earlier and later periods of the curve represent times when the bacteria and the protozoa respectively were predominately numerous. Biological observation and chemical estimation made these extremes easily recognizable. The intervening interval was of course characterized by the abundance of both groups of organisms. We have thus far identified successive regions of the curve characterized by rapid rise, then one of more or less rapid fall, then one of continued level or of very slow fall. After a sufficient number of weeks or months, depending upon the quantity and character of organic matter originally present, the curve falls to its lowest acidity, comparable to that with which it began, or even to neutrality or alkalinity. In this last period few or no bacteria and protozoa are present, but sometimes a number of crustacea, *c. g.*, cyclops, can maintain their existence. It is at this time that certain

green algæ, which locate themselves upon the side of the jar in a dense layer, become the predominating organic form, provided the conditions of illumination are favorable. The mineralization of organic matter resulting from the preceding biological activities has provided a salt solution which fulfils one of the conditions of algal growth. The series, bacteria — protozoa — algæ, is a fair representation of the biological succession which takes place in almost any culture under ordinary conditions if the culture be long enough continued. The changes within the protozoan group I shall describe in more detail presently. Of the chemical conditions which are correlated with this series that of acidity occupies the most prominent place in the early history of the culture. Further evidence bearing upon the relation between acid content and organic content will be given in connection with the discussion of the protozoa.

A study of the numerical data for the methyl-orange alkalinity, as given in Tables II to X, shows that these values pursue a more or less zigzag course in the history of any given culture, but with one or two notable exceptions the general tendency of this curve is upward. In the following table (XII) are shown the initial and the final values for the methyl-orange alkalinity of all the cultures in Tables II to X, in the order in which they are recorded, and in Curves 3 and 4 is plotted the alkalinity for cultures 5-10-31/1 and 5-10-31/2 respectively.

TABLE XII.

Initial	. 7.8	6.8	7.0	8.0	8.0	7.8	7.0	7.3	8.3	8.0	7.6	8.2	8.4	9.1
Final	. 8.0	7.6	6.8	8.8	5.8	8.6	8.5	8.6	8.8	9.4	2.3?	6.2	8.6	9.9

As explained in the previous part of this paper, the above numbers represent the amount of alkali and alkali earth bicarbonate. The increase of this quantity, as shown in the majority of the data above given, is an expression of the process of mineralization of organic matter which takes place continually in all the cultures. It is probable that not all the mineral matter enters into such chemical combinations as to make it determinable as methyl-orange alkalinity, yet the data in Tables II to X and in Table XII show that, upon the whole, this estimation gives an index to progressive mineralization. The difficulty of making an accurate estimation of this process has been referred to in Part I of this paper. As there described, one other and important use of the determination of methyl-orange alkalinity is its indication of approximately the osmotic conditions. A



curve would serve to show the general pitch of the numerical value of the possible osmotic pressure, and under certain assumptions a calculation could be made, whose results, however, could be regarded

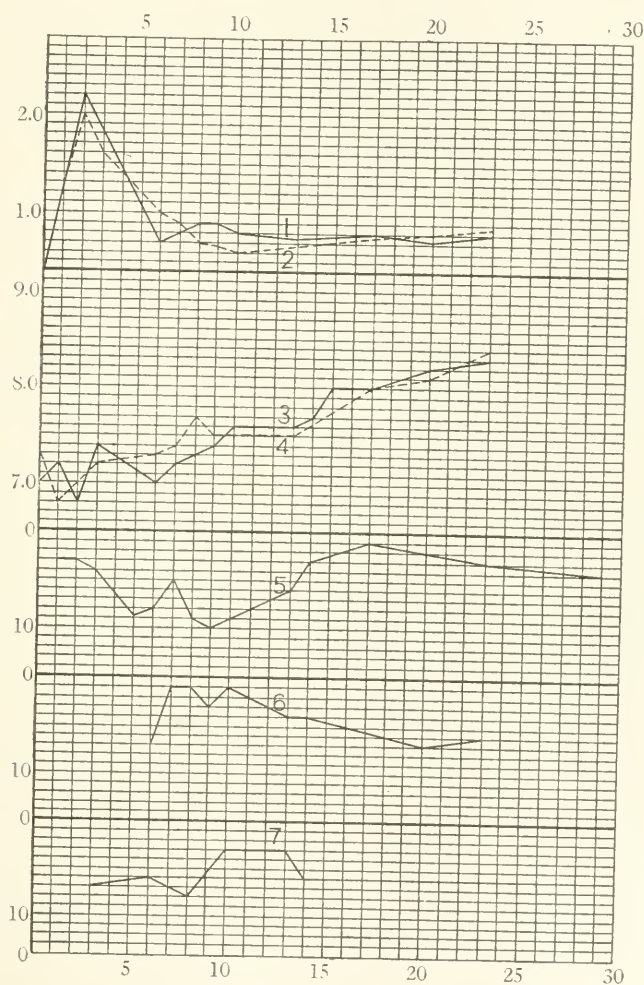


FIGURE 1.— The accompanying curves are all plotted to the same scale of days, showing the ages of the cultures on the ordinates from 0 to 30. Curves 1 to 4 are plotted in the first quadrant, numbers 5 to 13 in the fourth, so that uniformly a rising curve indicates an increasing quantity as in curves 1 to 4, or an increasing numerical rank as in curves 5 to 13. The sudden beginning or cessation of a curve indicates that the animals are at that time entirely absent or perhaps too few to attract attention in making the counts.

as only a rough approximation. In any case the methyl-orange determination is capable of frequently showing pronounced differences in the physiological environment prevailing in apparently similar cultures.

The numerical values for the electrical conductivities of the culture liquids, as given in Tables III to IX, show the same general upward tendency which we have noted in the alkalinity curves. The initial and final values of the electrical conductivities in the order in which they occur are shown in the following table:

TABLE XIII.

Initial . . . . .	660	637	637	541	610	582	636
Final . . . . .	701	716	661	442	661	718	718

The conditions which these curves represent much resemble those expressed by the alkalinity curve. The upward tendency of the curve is due to increase of electrolytes, and this has been brought about by disorganization of organic matter. But in the present curve we find expressed not only the salt content, capable of being measured as alkalinity, but also the electrical conductivity due to the free acidity. The influence of this factor can be traced in the numerical data, which in some cultures show a series of higher values corresponding with the falling arm of the acidity curve. That the conductivity curve should follow so irregular a course is comprehensible when one considers the variety of decomposition or of metabolic products which must occur in the progressive degradation of the organic matter of these cultures. This matter includes not only the metabolic products of living organisms, but also the products from the dead forms of all the numerous groups of organisms which have succeeded each other in the course of the culture history.

From the nitrogen determinations no regularities of any sort were discoverable. Almost the same report must be made regarding the data for oxygen consumed. Both these determinations are still useful as an index of conditions which exist at the time when the estimations are made. To forecast the probable course of either of these curves would require a much more intimate knowledge of the bacterial and protozoan content of these cultures than we now possess. A comparison of the analysis of events occurring in these culture liquids, as here made, and an analysis of the stages of decomposition of sewages, as made by Rideal,<sup>1</sup> leads to the conclusion that the culture

<sup>1</sup> RIDEAL: *Sewage and the Bacterial Purification of Sewage*, 1901, pp. 50-111.

liquids here discussed are to be regarded as mild sewages, and that, practically, the same factors are operative in both cases. Although much more investigation has been made of sewage, yet no such understanding has been even approached of the complex biological and chemical factors as would be necessary to explain the varying course of the two curves above described. Adopting the principles which Rideal developed for sewages, we can say that there are here both ærobic and anærobic bacteria, some of which convert protein nitrogen into ammonia, others which transform this into nitrites, and still others which transform nitrites into nitrates. Not only does the process operate in this direction, but there are also denitrifying bacteria which may partially reverse this process, and some nitrogen may be freed as gas. When we contemplate the interaction and the succession of these factors, it becomes clear that a lack of precise biological knowledge is a serious deficiency to be removed by no small amount of research. As previously stated, nitrates added to cultures in active decomposition quickly disappeared, showing that the period of predominant nitrification had not yet been reached.

#### IV. THE PROTOZOAN SERIES AND ITS IRREVERSIBILITY.

In Tables VIII and IX are reported the chemical data for cultures 5-10-31/1 and 5-10-31/2 respectively. For these two cultures I have more complete data for their protozoan content and history than for any others. Having examined many other cultures with essentially the same results, I shall describe these two as types for cultures raised and managed as previously described. The relative numerical rank of a number of species of protozoa was daily determined, as above described under "Biological Methods." The curves obtained by plotting these daily records will be given. The age of the culture in days is laid off on the abscissa. The relative numerical rank of the organism under discussion is plotted on ordinates running downward from the abscissa, so that the lower in rank a given protozoan stands at a certain time the lower will its curve extend below the abscissa. In other words, the curves are plotted in the fourth quadrant instead of the first. In the original record all the curves are plotted in the same figure as the successive plotting of each day's record would produce them, but I shall now have to reproduce them singly. This series of observations is not complete for all the kinds of protozoa which occurred in these cultures, but the

record is complete for each organism reported for the length of time the culture was systematically examined, which in this case was twenty-nine days. Some of the observed forms (usually of very small size) were not certainly identified, and hence were known by some arbitrary designation, *e. g.*, Heterotricha A, Holotricha A, etc. The forms thus incompletely classified were familiar because of their frequent and regular appearance in different cultures, and they usually attracted the attention by their great numbers at some stage in the history of the culture, and by their complete disappearance subsequently.

In Curve 5 are shown the data for *Stentor cœruleus*. The ninth day divides it into two regions, the first of which is characterized by the numerical decline of these animals as compared to others present during the same period. During the second period *Stentor* flourished more than any other organism and attained at one point the first rank in relative numbers. The curve has not been long enough continued to show the duration of their period of prosperity. Different cultures have shown that this period may continue from one to three weeks. During their period of predominance these animals may become exceedingly numerous, forming a dense blue-green layer always located on that side of the jar which is turned away from the source of light for the room. It is characteristic of the successful *Stentor* period to occur in the later history of the culture, when the most active bacterial fermentations have ceased. The liquid which constitutes their favorable medium is clear, well aerated, and of low acidity. Another condition of much significance is the salt content of the medium. In a previous paper<sup>1</sup> I have discussed the relation of *Stentor* to salt solutions. The cultures described in the present part of this paper were set according to methods detailed in Part I,<sup>2</sup> using a tap water an analysis of the salt content of which is there given. To these salts are added those originating from the hay. It is not probable that the total resulting salts differed enough, either qualitatively or quantitatively, in the periods of decline and of predominance of *Stentor* to account for the differentiation of these two periods. I think the same may be justly said regarding other animals for which I shall present curves. It is of course possible for changes of the appropriate kind and amount to produce periods of decline and of pre-

<sup>1</sup> PETERS: Metabolism and division in protozoa, Proceedings of the American Academy of Arts and Sciences, 1904, xxix, pp. 441-516.

<sup>2</sup> PETERS: This journal, 1907, xvii, pp. 443-477.

dominance such as here occur, owing to the normal adjustments of the animals to different conditions of salt content. To demonstrate that such precise adjustment exists requires only a few experiments directed to this end, and I have reported numerous tests of this kind in my paper under date of 1904, to which I have above made reference. Another factor which might be considered in accounting for the two parts of the Stentor curve is the food supply. I do not believe that this factor here plays an effective part. By compressing Stentors under a cover-glass by the withdrawal of the water which supports it much of the food of the animals can be examined. The transparency of the uninjured animal also permits direct observation of the ingested matter. By this means it is easily determined that Stentor *cœruleus* is omnivorous. I have seen ingested Paramœcia, making active movements and finally disappearing by digestion, thus showing that Paramœcia *can* serve as food. I have also observed Stentors to become pale and die in a culture containing many Paramœcia, and repeated observation showed that the latter animals were not being utilized as food. Arcellæ, various flagellates, and algæ seemed to be more favorite foods. Owing to the omnivorous habits of the animal, it is safe to say that plenty of available food was at all times present previous to their period of abundance, and their failure to flourish during the early period must be attributed to some other cause. The same reasoning, based upon similar but less numerous observations, leads me to the opinion that the curves which I shall give for other protozoa cannot be accounted for by the conditions of the food supply. While aeration, salt content, and food supply must be favorable to produce a period of abundance of a given species, it is also true that these very conditions may be and frequently are present when this same species maintains only a low numerical rank in comparison with others. This peculiarity which is so pronounced in the cases for which I shall present curves is best accounted for, it seems to me, by a consideration of those environmental conditions whose parallel changes can best be correlated with the physiological variations of the animals. That one of these conditions which is chemically most prominent in its variation and which is known to produce strong physiological effects upon living protoplasm is in the present case the condition of acidity. Other important factors of a physical or chemical nature may also have been present, but, if so, they were not detected. The hypothesis that the concentration of acid is probably the most efficient factor in determining the biological succession in

these cultures is strongly supported by the results obtained in experiments made in this laboratory, on certain commonly occurring protozoa, to test their relation to an acid environment. *Colpidia* and *Paramæcia* were raised in great abundance in the same culture whose medium was a hay infusion made as previously described. It was observed, however, that the period of maximum abundance of *Colpidium* always occurred earlier in the history of the culture than that of *Paramæcium*, and that the *Colpidium* period always coincided with that of high acidity of the medium, the *Paramæcia* reaching their period of numerical predominance at some time beyond that of maximum acidity. Of course both animals existed in the culture at the same time, but the above description fairly describes the numerical relations. After a time *Colpidia* disappeared, but *Paramæcia* remained throughout a long period of the culture history. Owing to the above conditions, these two animals offered good material upon which to test the question of their relative adjustment to acid in their medium. An index to this adjustment was obtained by determining the minimum concentration of hydrochloric acid which would kill the animals instantly when the concentration of acid was suddenly raised by the addition of hydrochloric acid to their natural culture medium. It is important to observe that the animals were not first brought into similar and supposedly normal physiological condition by changing their medium to distilled water, as has been done in some investigations, but these tests were made with the natural culture medium which was the same for both animals. Of course some of the acid was neutralized by the abundance of bicarbonates in the hay infusion, but this amount was the same for both animals, and we desired for our purpose of comparison to determine the relative, not the absolute killing points. A detailed description of these experiments has not been published, and I shall use here only the results obtained. By the method pursued the experimenter became so practised as to be able to detect with reliability killing points which differed from each other by the concentration of a 0.0001 normal solution of hydrochloric acid. As the result of numerous experiments it was found that *Colpidia* and *Paramæcia* originating from the same naturally acid culture medium required minimum concentrations of hydrochloric acid to kill them instantly, which differed by a several times greater amount than the limit of error of the experimenter. Furthermore *Colpidium* always required a much higher concentration of acid to kill than *Paramæcium*.

From these results we may fairly conclude that *Colpidium* is

much better adjusted to normally occurring high acidities than *Paramæcium*, and that this is one of the chief factors in determining the numerical superiority of *Colpidia* over *Paramæcia* during the early days of a culture history, and the reversal of this relation in the subsequent period even leading to complete disappearance of *Colpidium*. These experiments do not lead to any necessary conclusion as to how the acidity acts in determining the proportionate numbers of the two species. In particular the action of the acid should not be interpreted as exerting an inhibitive influence only, as if it were simply a poison in all concentrations. It is possible, and probable from some experiments which I have made, that in low concentrations, such as might normally occur in these cultures, the acid acts as a stimulant upon multiplication, and moreover influences one species more strongly than another. The experiments with acid were not extended to other animals of the series occurring in cultures, but I have never found *Stentors* numerous and in healthy condition in any medium whose acidity was high, and, as before stated, the media in which they occurred abundantly have shown very low acidity. In its relation to neutral salts contained in its medium *Stentor* has shown itself to be very sensitive, and in view of all the facts thus far developed it may be considered highly probable that the condition of acidity of the natural cultures described is a determining moment in causing the decline of the *Stentor* curve in its initial period. I have merely taken the curve for *Stentor* as a type of all those I shall present, and I regard the principles illustrated in its explanation as applicable to all the curves. Therefore I shall illustrate from *Stentor* another proposition of general validity for all the species discussed.

It might be supposed that if the different species of protozoa were placed in the culture liquid in sufficient numbers at the beginning they would not show the phenomenon of occurrence in series. In other words, a preponderance in numbers in the seed material used gave to some species an advantage over others. To test this possibility the *Stentors* were seeded into the cultures, for which the *Stentor* curves are given in very large numbers. The curve shows that they were third in rank on the first and second days. Since they naturally occur in predominant numbers only in the later period of a culture history this abundant initial seeding would show whether mere force of numbers could account for this fact. If so, they ought to flourish well in the early period under the condition of abundant

seeding. The curve actually obtained shows that they suffered a period of decline extending through the first nine days of the record. Observation of the Stentors during this period showed that they became pale instead of keeping their healthy blue-green color and that many of them disintegrated. This period of decline is to be attributed to an unfavorable condition of the medium, and in all probability to the acidity, which was of the most pronounced character during this interval. Sometimes Stentors bear the fermentation period much better than in the above typical case and begin to flourish much earlier. It is impossible to change their period of predominance by merely introducing them in sufficient numbers at the time when the change in numerical rank is desired. In other words, the serial position of these animals in a list showing the order of numerical predominance of the different species of protozoa is not reversible. Similar experiments made to test this proposition for several other species besides Stentor leads to the conclusion of the general validity of the principle that the serial order of the biological groups in cultures managed as previously described is irreversible. This statement does not exclude the possibility that some groups may have a range of adjustment so wide as to flourish at all periods of a culture history, though I have not found any group that fell strictly within this class. Paramæcia occur abundantly through a wider interval of time than almost any other form, but even they are adversely affected by the early conditions. The effort was made to seed the two cultures from which the curves were obtained in as great abundance as taking the seed from other old cultures would permit. Hence it is easy to understand why, as in the case of Stentor, many of the animals have a high position in the beginning which they fail to maintain. These curves may be regarded as containing an abnormal period.

Amœba is almost always found in these cultures in the zooglœa which in successive layers cover their surface. Their numbers may be small, but in many cases they are enormous. I have not estimated their numbers in such way as to represent them by a curve. Their presence always coincides with the downward arm of the acidity curve. I have never observed them to be numerous before this time nor to continue long after the principal fall of the acidity curve. They are never large in size, but one can note an increase in this respect from day to day as samples of the material are taken for examination. Finally, when their number becomes smaller, it



is observed that frequent cases occur of the fusion of many individuals into one. These features in conjunction with some other observations lead to the suspicion that the forms seen are amœboid stages in the life history of some unknown organism. To what extent this may be true of all amœbæ we of course do not know.

Curve 6 shows the data for a form designated as *Holotricha A*. It was present in the culture from the sixth to the twenty-third day. It appeared suddenly in great numbers, had a well-defined maximal period, and then completely disappeared.

Curve 7 represents *Hypotricha A*, a form having a still more limited time range, extending from the eighth to the fourteenth day, but during its short maximal period it made itself noticeable by a large relative as well as absolute abundance.

Curve 8 represents *Heterotricha A*, whose curve much resembles that for *Holotricha A* in Curve 6, but its numbers are pitched upon a lower scale.

Curve 9 represents *Heterotricha B*, a form which was always characterized by reaching its maximum during the later stages of the culture history.

Curves 10 and 11 represent two different species of rotifers. Like *Stentor*, the rotifers always belonged to the later culture history, but with this difference that they are much more resistant to adverse conditions than *Stentor*. When seeded in large numbers into a culture at its beginning, the curve shows much the same regions as that for *Stentor*, and I would attribute its course largely to the same influences, among which the early acidity of the culture plays a prominent part. The eggs of rotifers were easily obtained from these cultures, and their development was observed in separate preparations, using methods previously described.

Curve 12 represents *Arcella vulgaris*, whose number was always large in the early and middle periods of the culture history.

Curve 13 represents *Paramœcium*. *Paramœcia* were seeded into the medium in very large number, and hence the curve does not fairly represent the position of their optimum conditions, which occurs beyond the period of greatest acidity. Owing to their adjustment to a wide range of conditions, their number did not diminish as pronouncedly during the period of high acidity as was the case with *Stentor*, an organism of much narrower range of adjustment. Experiments which will not be detailed here have shown that a certain low concentration of acidity acts as a stimulus upon the rate of

division of *Paramæcium*, but at a higher concentration becomes inhibitory. The large range of adjustment of *Paramæcium* also explains why they are a numerically prominent organism throughout the greater part of a culture history.

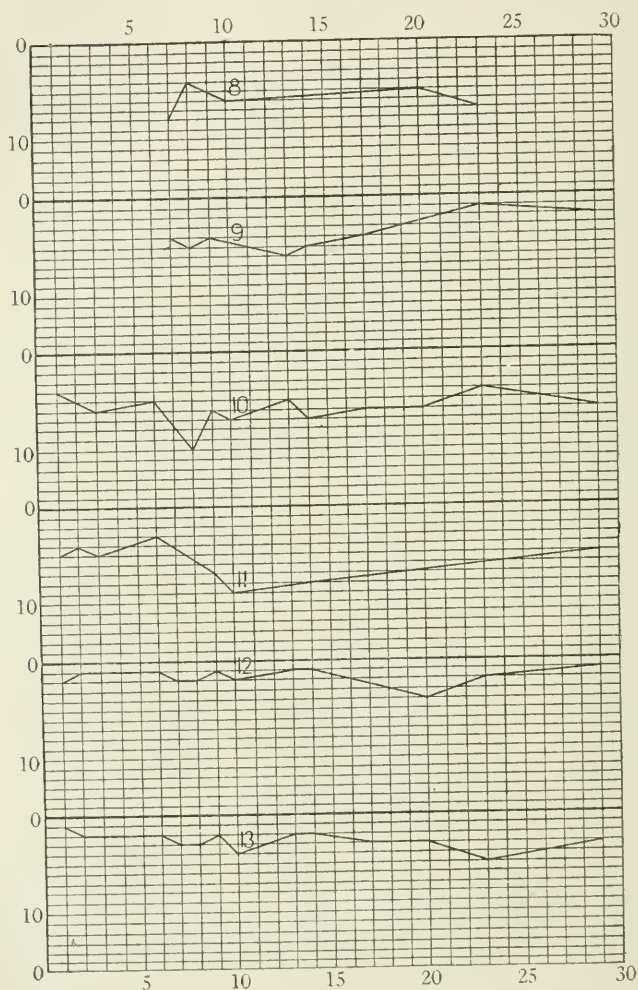


FIGURE 2. — The accompanying curves are all plotted to the same scale of days, showing the ages of the cultures on the ordinates from 0 to 30. Curves 1 to 4 are plotted in the first quadrant, numbers 5 to 13 in the fourth, so that uniformly a rising curve indicates an increasing quantity, as in Curves 1 to 4, or an increasing numerical rank as in Curves 5 to 13. The sudden beginning or cessation of a curve indicates that the animals are at that time entirely absent, or perhaps too few to attract attention in making the counts.

The discussion of the preceding curves shows that in order to determine from them the period of an animal's optimum conditions due allowance must be made for the effect produced upon the curve at its beginning by seeding the organism in question into the medium in very large numbers.

A study of all the data shows that the entire history of a culture managed as described falls into a number of easily recognizable periods. I would describe these in broad, general terms as follows: The first is the period of high acidity characterized biologically by an abundance of bacteria. The next succeeding period, that of falling acidity, is characterized by some well-adjusted forms which appear in sudden abundance but whose time range is limited. To this group belong, roughly, *Amœba*, *Holotricha A*, *Hypotricha A*, *Heterotricha A*. The third period I have usually referred to as the later culture history. It is characterized by *Stentor* and rotifers, and by the development of a rich growth of green algæ which continues into the next period. *Paramœcium*, as above explained, extends through both the second and third periods. The fourth period is marked by the practical exhaustion of available organic food for animals, and it may be well called the period of faunal sterility. Small crustacea, *e. g.* cyclops, frequently live for a considerable time during this period. The period is characterized by the conspicuous absence of numbers and variety of organisms and the medium is frequently destitute of living things except for the presence of spores and cysts or of eggs. It is worthy of note that a culture can be revived by refeeding it, by the same method as originally used, when it has arrived at the end of the third or the beginning of the fourth period. If thus refeed *without reseeding*, practically the same phenomena will recur as the culture has already once exhibited.

#### V. SUMMARY.

1. A liquid medium consisting of water, salts, gases, and organic matter from the extraction of hay has been studied as a type representing the essential conditions of the solutions in which the vast majority of cells pass their existence. After seeding this medium with a considerable variety of plant and animal organisms its progressive chemical changes from day to day were quantitatively determined. These estimations included mainly the (1) phenolphthalein acidity, (2) methyl-orange alkalinity, (3) electrical conductivity, (4) oxy-

gen consumed, (5) organic nitrogen. A determination of the parallel biological changes was also made by methods which showed the relative abundance of the different organisms present at a given time. The ranks attained from day to day by different organisms have been plotted in curves which show variations in relative abundance throughout the history of a culture.

2. The data obtained indicate that of the chemical conditions the concentration of acid, which is due primarily to bacterial metabolism, is one of the chief factors determining the biological content and history of a culture managed as previously described. The biological data show, besides the facts regarding the relative numbers of each individual organism studied, that the history of a culture shows four recognizable periods. The first is characterized by maximum acidity and by the very abundant development of certain bacteria. The second period shows a falling degree of acidity, and in it develop certain forms which appear suddenly in large numbers but which are of only temporary supremacy. The third period is of longer duration, shows low acidity, and has its characteristic forms, which may have been present previously, but whose maximum occurs here and is more or less prolonged. The fourth period proceeds with an exhausted condition of organic matter and a more or less favorable salt solution for the nutrition of algæ, which solution owes its origin partly to the previous mineralization of the organic matter by biological processes. Few faunal organisms live in this period, but green algæ may develop abundantly under favorable conditions of illumination and temperature.

3. When an organism has a pronounced optimum position at either extreme of the culture history it is not possible to reverse the position of its optimum by seeding it into the culture at a different time than that of its optimum. The organism so seeded speedily undergoes diminution in numbers in consequence of the unfavorable conditions of the medium. The normal sequence of narrowly adjusted organisms is irreversible.

## GASTRIC PERISTALSIS IN RABBITS UNDER NORMAL AND SOME EXPERIMENTAL CONDITIONS.

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### INTRODUCTION.

UNTIL quite recently knowledge of the behavior of normal gastric peristalsis was derived from studies by various methods, all of which were carried out under more or less distinctly pathological conditions. These methods were as follows: (1) Observation of the excised stomach placed in a moist, warm chamber (Hofmeister and Schütz<sup>1</sup>). The abnormality of this procedure is evident and requires no comment. (2) Observation of the stomach in the living animal with opened abdomen without anæsthesia (Wepfer,<sup>2</sup> Schwartz<sup>3</sup>) or under the influence of morphine or curare (Rossbach<sup>4</sup>). We know now that pain and rage as well as morphine and curare modify the behavior of normal peristalsis. The profound effect which opening of the abdomen exerts upon peristalsis will be discussed later. (3) Study of the peristalsis through a gastric fistula in man and animals by introducing into the stomach a thermometer (Beaumont<sup>5</sup>), a manometer (Uffelman<sup>6</sup>) or a balloon connected with a graphic apparatus (Ducceschi<sup>7</sup>). The adhesions of the stomach to the abnormal wall and the presence of foreign bodies in the stomach surely influence normal peristalsis. (4) Peroral introduction of a stomach

<sup>1</sup> HOFMEISTER and SCHÜTZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1886, xx, p. 7.

<sup>2</sup> WEPFER: *Historia cicutæ aquaticæ*, Basel, 1679, p. 152.

<sup>3</sup> SCHWARTZ: HALLER'S *Dissertationes anatomicæ*, Göttingen, 1746, i, p. 337.

<sup>4</sup> ROSSBACH: *Deutsches Archiv für klinische Medizin*, 1890, xlv, p. 296.

<sup>5</sup> BEAUMONT: *Physiology of digestion*, Burlington, 1847.

<sup>6</sup> UFFELMAN: *Deutsches Archiv für klinische Medizin*, 1877, xx, p. 546.

<sup>7</sup> DUCCESCHI: LUCIANI'S *Physiologie des Menschen*. Translated by BAGLIONI and WINTERSEIN, 1906, ii, pp. 163 *et seq.*

tube, the tip of which is provided with a balloon (Morat<sup>1</sup>). Besides the fact that we have here again a foreign body in the stomach, the presence of the tube in pharynx and œsophagus and the reflexes it produces are probably not without modifying influences upon the normal course of gastric peristalsis. Moreover, tracings so obtained without any other control are open to a variety of interpretations.

The only method which can claim to investigate normal peristalsis under normal conditions is the fluoroscopic inspection of the stomach after giving the animal food mixed with bismuth. As is well known, this method has been extensively employed by W. B. Cannon<sup>2</sup> for the last ten years, and the numerous results which it brought to light need not be pointed out to readers of this journal. In these studies the observations were mostly made on the stomachs of cats. A disadvantage of this method is the fact that it demands complex apparatus, and furthermore the method requires also an investigator who is an expert in this mode of observation.

Considering the numerous researches which deal with gastric peristalsis, it is somewhat surprising that a method was overlooked by means of which stomach motility could be studied in a common laboratory animal without any operation, and under conditions that are as physiological as any method can give that demands fixation of the animal. In the rabbit mere inspection reveals gastric peristalsis. In the literature no statement was found indicating this fact. On the contrary, many writers point out especially the tardiness or lack of peristalsis of the rabbit's stomach. But these writers studied the stomach in the opened abdomen, where its motility is indeed strikingly retarded, if not entirely absent.

#### NORMAL PERISTALSIS OF THE RABBIT'S STOMACH.

**Methods of observation.** — The animals were observed under normal conditions; the only abnormal state was their extension and fixation on a holder. They were placed on cotton and well covered to prevent loss of heat as much as possible. Disturbing and exciting influences were avoided. No narcotics were administered except in experiments devoted to the study of their effects.

The observations were carried out, in the first place, by simple inspection. With a little practice any one may recognize the stomach

<sup>1</sup> MORAT: Archives de physiologie, 1893, v, p. 142.

<sup>2</sup> CANNON, W. B.: Science, June 11, 1897, p. 902; This journal, 1898, i, p. 359.

waves and follow out their course. It is a great advantage that a number of persons may observe the peristaltic movements at the same time, and individual subjective observations can thus be satisfactorily controlled. Besides simple inspection, tracings of the gastric movements were obtained. These tracings have the advantage that their interpretation was controlled by the inspection method.

*Inspection.*—If a rabbit, well fed shortly before the experiment, is stretched on its back and the hair of the upper abdominal region cut short, a large part of the stomach can easily be outlined by inspection and by palpation. The position occupied is usually either diagonally from the right chondro-xiphoid region downward and to the left, or the organ lies transversely. Full-grown rabbits are preferable, for their stomachs are larger and a greater area is available for observation, due to a perhaps normal gastroptosis.

Inspection of the stomach area for the first few minutes reveals no motion (Fig. 3, *a*), but after three to ten minutes a shallow depression may be seen apparently arising at the junction of the fundic and middle thirds of the stomach. This depression courses slowly over the viscus from left to right, increasing moderately in depth as the pyloric third is approached. The initial depression on the abdominal wall of the rabbit over the stomach apparently does not reach the greater curvature. Whether this wave involves the lesser curvature cannot be stated, for this portion of the stomach is hidden from sight by a lobe of the liver. Preceding the depression is a bulging, which increases and reaches its maximum at the beginning of the pyloric third. On reaching this point the depression often seems to pause for an appreciable period of time, and then a contraction of the bulging pyloric third sets in. This latter contraction does not seem peristaltic, but apparently occurs more or less as a whole; the bulging sinks away without showing any peristaltic wave. During this sinking the contraction causing the bulging relaxes, and the contents of the pyloric third may often be seen forced partially into the middle third of the stomach again, thus simulating a short anti-peristaltic wave. At other times, especially in young rabbits, and when the stomach was moderately dislocated downward in order to render the pyloric third more completely visible, the contraction of this portion of the stomach seemed to be caused by a peristaltic wave which traversed the bulging.

When peristalsis is fully established, the prominence of the pyloric third at the end of a gastric wave is very marked, and its peculiar

contraction may then be best noted. The contraction may reduce the mass to the same level occupied by that stomach region before diastole (Fig. 1, *a*), or the systole may be so powerful that the pyloric third disappears entirely from view (Fig. 1, *b*). During a contraction of the latter type a bubbling sound is often audible.

The waves increase slowly in vigor and frequency, and are most marked about one to two hours after feeding (Fig. 1, *a, b*). Then the constriction at the beginning of the pyloric third and the bulging of that portion become extremely marked. In many instances the constriction was so marked that separation of this part from the rest of the stomach cavity seemed to be produced, the organ showing an hourglass shape. This constriction was a few millimetres in width at its base, and either maintained its position and strength during the ensuing contraction of the pyloric third or relaxed during it.

*Pyloric sounds.* — If the powerful constriction described above was more or less maintained, a musical gurgling sound was frequently heard during the contraction of the pyloric third. This sound seemed to be produced in the right hypochondriac region, and on lightly pressing a finger beneath the pyloric portion of the stomach over the duodenum, a fine bubbling thrill of varying length and intensity could be easily felt. This thrill occurred at irregular intervals, and seemed undoubtedly to be produced by the expulsion of liquid material from the stomach into the gas-containing duodenum. Palpation frequently showed a thrill in the duodenal region, when the unaided ear heard no sound, and when the constriction above noted was not extreme. It must be mentioned that a loop of the cæcum passes just beneath the pyloric third of the stomach, and in this loop cæcal sounds may be heard and felt. Their dull, popping character and coarse thrill is quite different from the rather musical, high-pitched sound and fine thrill produced by the expulsion of material through the pyloric sphincter.

*Graphic registration.* — The frequency of the waves varied from one to five or more per minute; two waves crossing the stomach at the same time could always be seen when peristalsis was active. In order to count them for long periods of time graphic registration was used.

This was easily accomplished by placing a Marey receiving tambour, about four centimetres in diameter, over the pyloric third of the stomach, and then connecting with a Marey writing tambour which traced the volume curve of the stomach area covered on smoked paper. The receiving



tambour was held in place by four pieces of tape radiating from the tambour stem, the tape ends passing through holes in the rabbit board where they were fixed by plugs. The position of the tambour itself was marked on the abdominal wall with pencil, so as to permit readjustment when dislodged by movements of the animal. In this connection it may be permitted to state that considerable patience is often required with some rabbits, for active movements usually require rearrangement of the receiving tambour. After preparing the animal in this fashion it was covered with cloths and cotton wool, so as to prevent loss of heat as much as possible.

In the tracings the *upward* direction of the curve means an increase in volume of the stomach area covered by the tambour, a diastole; the *downward* movement means a diminution in volume of that area, a systole.

By this method not only the stomach waves are written, but the respirations are also shown as oscillations superimposed on the stomach waves. With delicate adjustment of the writing lever the

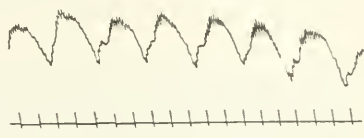


FIGURE 1 a.— All the tracings are taken from the pyloric third of the rabbit's stomach by means of tambours. The large waves are stomach waves, the rising limb representing an increase in volume, diastole; the descending limb representing a diminution of volume, systole, of the pyloric third. The smaller curves superimposed on the larger are respiratory oscillations. Time marks represent six seconds. Tracing taken twenty minutes after feeding.

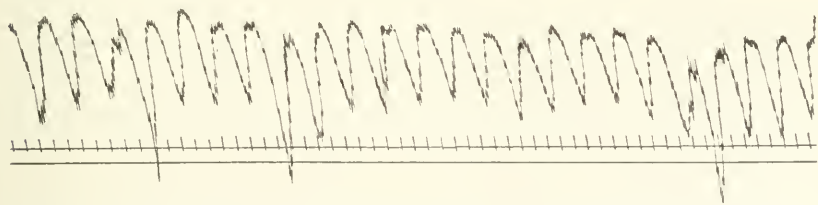


FIGURE 1 b.— Same animal eighty minutes after feeding. The three strong contractions are to be noted; pyloric sounds as a rule are associated with them.

heart beats are shown as small notches on the respiratory oscillations. The pyloric third was usually chosen for registration because the waves are there most marked. The tracings bring out well the points already described, and emphasize the almost machine-like regularity of the waves, which occur one or two hours after feeding. Then there is usually only a slight pause, or none at all, between the rhythmic play of systole and diastole (Figs. 1, a, 2, b, 3, c). If a considerable

length of time has elapsed since the last feeding and when peristalsis is slow, the pause between systole and diastole may be marked (Figs. 1, *c*, 3, *a*) and the waves do not occur with such marked regularity, both as to time and strength of contraction.

The length of time necessary for a wave to traverse the stomach cannot be determined by this method with accuracy, for the real

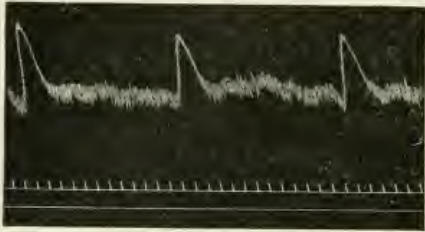


FIGURE 1*c*. — Five hours after feeding. Taken from another rabbit which showed rapid respiration.

starting time cannot be noted, because the depression caused by a beginning gastric wave is probably so slight that the abdominal wall shows no sign of it. Bearing this in mind, it may be stated that the waves take approximately about twenty seconds to cross the stomach. The contraction of the pyloric third, from the beginning of diastole

to the end of systole, consumes about fifteen seconds. This time interval is fairly constant, as an examination of the tracings will show.

As already stated, the site of origin of a gastric wave cannot be determined with accuracy. Inspection shows the first sign of a depression on the anterior surface of the stomach, about at the junction of the fundic and middle thirds. This corresponds about to the region below the entrance of the œsophagus. When the receiving tambour is placed over the fundus, changes in volume are registered; but this can by no means be interpreted as due to a wave starting at that place; it might well represent only the bulging caused by the transmission of pressure produced by a wave beginning nearer the pylorus. The method described can give no definite answer to the question, whether or not gastric waves begin in the fundus.

When the abdomen of a rabbit is opened so as to identify the different portions of the stomach seen through the intact abdominal wall, it is found that the pyloric third is composed of two well-defined divisions, an antrum and a preantrum. The marked bulging which the abdominal wall shows towards the end of a gastric wave, as described in the preceding pages, is caused by the filling of the preantral portion. This preantral division is well marked off from the rest of the stomach by a pouching which occurs both on the lesser and greater curvatures, the one on the greater curvature being the longer and larger of the two; the preantrum thus forms a knee

connecting the stomach with the antrum. But these outpocketings are not the only features that serve to differentiate this region; its musculature is thick, practically as thick as that of the antrum itself, and the border of the preantral bag is quite sharply marked off from the thin musculature of the fundic part of the stomach. The musculature of the preantrum shows strong circular fibres, some of which apparently insert in tendinous patches, of which each surface, anterior and posterior, shows one. The preantrum is flattened anteroposteriorly, and the level of the two surfaces is about that of the stomach proper.

The antrum itself is very muscular, and forms a cone-shaped cap, closing the preantral cavity. Between antrum and preantrum is a definite constriction marking the *antral* sphincter; between preantrum and the rest of the stomach is another perfectly definite constriction, and at this region the preantral musculature abruptly loses its thickness, its border forming the *preantral* sphincter.

Inspection of a rabbit's abdomen, as a rule, shows only the preantral portion of the pyloric third of the stomach; the antrum is too deeply located to affect during its activity the surface conformation of the abdomen under ordinary conditions.

**Discussion.** — From the description given above, it will be seen that the preantral region of the rabbit's stomach is definitely differentiated anatomically, and possibly has also a different function. The literature does not contain definite statements on the preantrum. Hofmeister and Schütz,<sup>1</sup> it is true, speak of a "preantral constriction," which marks the end of the first phase of their "Peristole," but it is evident that they only mean to localize the site of the constriction by this term; there is no evidence that they meant to differentiate a preantrum from the antrum. Cannon,<sup>2</sup> in a diagram of the cat's stomach, distinguishes a preantrum from the antrum, but no special statement is made regarding its structure and function. Ellenberger and Baum<sup>3</sup> make no mention of a preantrum in the dog. The textbooks on human anatomy describe no preantral region. For the rabbit itself this anatomical differentiation of the pyloric third of the stomach into an antrum and preantrum seems to be unknown; Krause<sup>4</sup> states nothing bearing on this question. Nor do

<sup>1</sup> HOFMEISTER and SCHÜTZ: *Loc. cit.*

<sup>2</sup> CANNON: This journal, 1898, i, p. 364.

<sup>3</sup> ELLENBERGER and BAUM: *Anatomie des Hundes*, 1891, p. 290.

<sup>4</sup> KRAUSE: *Die Anatomie des Kaninchens*, 1884.

Oppel<sup>1</sup> and Wiedersheim<sup>2</sup> mention or give a drawing of the preantrum in rabbits.

It is interesting to note, however, that the behavior of the rabbit's *preantrum* during a gastric wave tallies quite well with the description Hofmeister and Schütz<sup>3</sup> give of the rôle played by the *antrum* in the dog. Those authors state that a gastric wave, as seen in the excised stomach of a dog, consists of two phases. During the first phase a peristaltic wave, increasing in intensity as it progresses, ends in a deep preantral constriction. During the second phase the sphincter antri contracts powerfully, the preantral constriction relaxing meanwhile, so that the stomach assumes an hourglass shape, the antral cavity being separated from the rest of the stomach; then the antrum contracts, not peristaltically, but more or less as a whole. It is unfortunate that the data at hand do not justify any definite statement regarding the behavior of the rabbit's antrum during a gastric cycle.

Moreover it must be mentioned that Hofmeister and Schütz's description of gastric peristalsis, while corroborating Beaumont's<sup>4</sup> account, has not in its turn been corroborated by more recent investigators. Rossbach,<sup>5</sup> Cannon,<sup>6</sup> Roux and Balthazard,<sup>7</sup> all state that the gastric wave sweeps peristaltically over the antrum in cats, dogs, and man.

Whether in the rabbit the antrum behaves like its preantrum, and whether there is a difference in the peristaltic contraction of the stomach between herbivorous and carnivorous animals, are problems whose solution must be left to future studies.

#### GASTRIC PERISTALSIS UNDER SOME EXPERIMENTAL CONDITIONS.

This method lends itself readily to the study of gastric motility under various experimental conditions, and some of the facts obtained will now be described.

<sup>1</sup> OPPEL: *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere*, 1896, i, p. 386.

<sup>2</sup> WIEDERSHEIM: *Vergleichende Anatomie der Wirbelthiere*, 1902, p. 376.

<sup>3</sup> HOFMEISTER and SCHÜTZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1886, xx, p. 8.

<sup>4</sup> BEAUMONT: *Loc. cit.*

<sup>5</sup> ROSSBACH: *Deutsches Archiv für klinische Medicin*, 1890, xlvi, p. 296.

<sup>6</sup> CANNON: *This journal*, 1898, i, p. 367.

<sup>7</sup> ROUX et BALTHAZARD: *Comptes rendus de la société de biologie*, 1897, iv, p. 705; *Archives de physiologie*, 1898, xxx, p. 88.

**Fasting.** — If a well-fed animal, whose stomach motility has been noted, is starved from twelve to twenty-four hours, the peristaltic waves are greatly reduced in strength and may be absent entirely. Full-grown animals must be used for this study, because a twenty-four-hour fast reduces the volume of the stomach, so that in young rabbits the pyloric third is largely hidden by the right costal arch. In older rabbits the viscus is larger, lies lower in the abdominal cavity, and fasting for a moderate time does not markedly decrease the stomach surface available for study. This reduction, or cessation of stomach movements during fasting, has been noted by many observers in different animals. The cessation is particularly interesting in rabbits, because this animal's stomach is never empty; after a fast of ten days the stomach is considerably reduced in size, but it still contains a fair amount of material.



FIGURE 2*a*. — Stomach was moderately distended with air before tracing was taken. No movements visible before distention, as animal was starved.

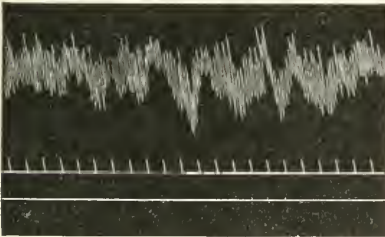


FIGURE 2*b*. — Air was removed from the stomach per tube: diminution of gastric waves.

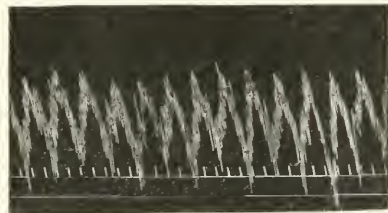


FIGURE 2*c*. — Same animal. Stomach re-distended with air: increase in strength of waves.

**Distention.** — From the above it would seem as if a certain distention were a factor in the production of the peristalsis. This supposition was established by an experimental test. When the stomachs of starving rabbits, which show only slight or no gastric motility, were distended with air or water, peristaltic waves arose, or were increased, if they had been present before. Figure 2, *a*, shows strong and regular gastric pulsations obtained from the pyloric third, after moderate distention of the viscus with air introduced per stomach tube; before the distention no waves were visible. Some time after distention, the air was removed as much as possible through a catheter passed into the stomach; the stomach waves were again greatly

reduced (Fig. 2, *b*). On redistention with air (catheter), vigorous peristalsis was re-established (Fig. 2, *c*). The distention must be moderate; the amount varies with each animal. Too much pressure not only fails to cause increased activity, but reduces whatever motility existed before distention; and if too little air or water is introduced the increase may be only slight or absent entirely. After distention with air pyloric sounds frequently occur, and by this method they may be produced at will.

This distention reflex has been described by Schütz,<sup>1</sup> who used the dog's excised stomach, and by Ducceschi.<sup>2</sup> The latter gives tracings illustrating the primary increase on distention and the decrease in strength of the contractions by overdistention of the dog's stomach.

Distention, however, is probably not the only factor in producing strong peristalsis. It was stated above that peristalsis of the rabbit's stomach was most vigorous one to two hours after feeding, and that the increase in vigor was gradual (Fig. 1 *a, b*). If the distention were the sole cause of the peristalsis, it should set in immediately after feeding, since it is then that the chief distention takes place; unless it is assumed that the stomach volume is considerably augmented by the formation of gases in some fashion or other within the stomach during digestion. However, the view expressed by some writers, that the products of digestion assist in stimulating the stomach movements possibly explains the gradual increase in a more plausible fashion.

#### CONDITIONS INHIBITING PERISTALSIS.

The stomach movements are easily inhibited by a variety of influences.

**Psychic influence.** — The necessary handling to which the rabbit is subjected when being tied on the board stops gastric peristalsis for a variable length of time, as has already been mentioned (Fig. 3, *a*). Within ten minutes, as a rule, movements again appear. But if the animal be startled in any way, or if it struggles, motion is again abolished for some time. Occasionally the stomach stops moving for no cause appreciable to the observer. The influence of emotions upon gastric peristalsis is not new; Cannon<sup>3</sup> has found that rage,

<sup>1</sup> SCHÜTZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1886, xxi, p. 343.

<sup>2</sup> See LUCIANI'S *Fisiologie del' Uomo*, 1901, i, p. 680, for tracings.

<sup>3</sup> CANNON: *Loc. cit.*, p. 380.

fright, anxiety, abolish movements of the stomach in the cat; and Rossbach<sup>1</sup> has noted stoppage of intestinal peristalsis after the same causes in the human subject.

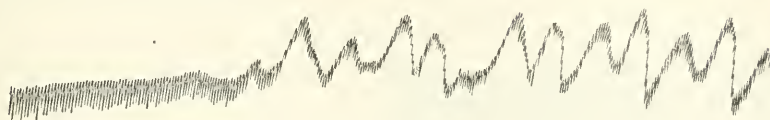


FIGURE 3 *a*. — Tracing taken shortly after animal was stretched out on board. Note the initial inhibition (only partly shown in tracing).

**Ether.** — When ether is given continuously to a rabbit, advancing the cone very slowly toward the animal and avoiding respiratory stoppage, the first effect, as a rule, is cessation of peristalsis. This inhibition of gastric peristalsis lasts from thirty seconds to several

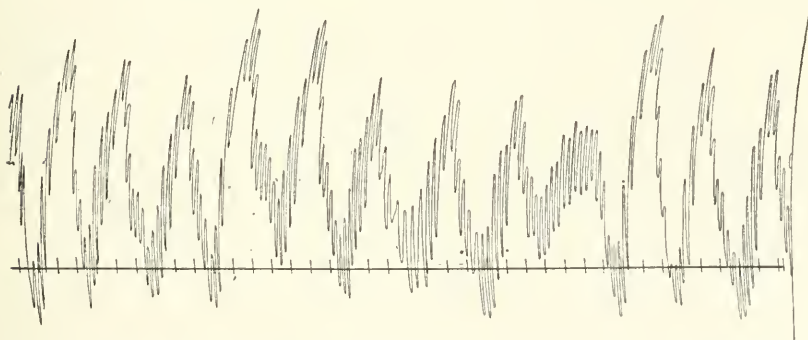


FIGURE 3 *b*. — Same animal now fully anesthetized by ether. The peristalsis is now more regular and powerful than before anesthesia.

minutes, respiration continuing uninterruptedly with careful administration of the ether (Fig. 3, *a*). This naso-gastric inhibitory reflex is thus seen to be more sensitive than the well-known naso-respiratory inhibitory reflex described by Kratschmer<sup>2</sup> and by Holmgren. Not all animals, however, show this period of inhibition at the beginning of ether anesthesia. (Some of these refractory animals suffered from a moderate nasal discharge, and it is possible that in those cases in-

<sup>1</sup> ROSSBACH: *Deutsches Archiv für klinische Medizin*, 1890, xlv, p. 326.

<sup>2</sup> KRATSCHEMER: *Sitzungsberichte der Wiener Akademie, math.-phys. Kl.* 2 Abt., 1870 lxii, p. 147.

flammation of the nasal mucous membranes was the cause of failure.) When ether was administered through a tracheal cannula, no inhibition was caused.

After the more or less long period of inhibition, gastric peristalsis is re-established; at first it is somewhat irregular, but later it is at

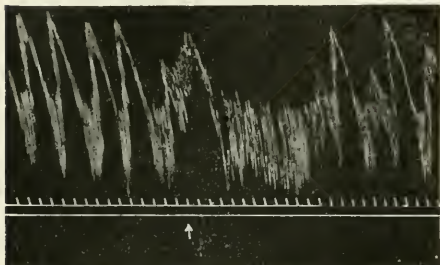


FIGURE 3 *c*. — Shows inhibition of gastric peristalsis when ether was started. Note slight effect on respiration.

least as regular as before the anæsthesia. This regularity is maintained even when the corneal reflex is absent (Fig. 3, *b*). During complete ether anæsthesia, therefore, gastric peristalsis is not abolished. In numerous instances, while the animal was completely under ether, the peristalsis was even more regular than before the anæsthetic was given (Fig. 3, *b*).

The course just described above is the rule; but there are exceptions. Ether anæsthesia sometimes retards the peristalsis and renders it irregular; periods of gastric quiet are occasionally interrupted by a series of waves of practically normal strength and duration. This course, however, forms an exception, and its character closely approaches that usually seen during chloroform anæsthesia.

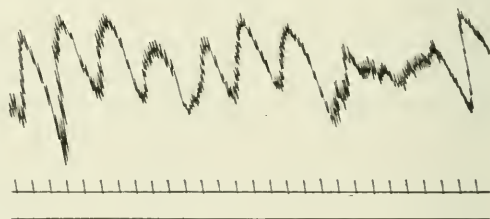


FIGURE 4 *a*. — Tracing taken under normal conditions. Note short inhibition of gastric waves.

**Chloroform.** — When a rabbit is allowed to inhale chloroform, at first in a very dilute form so as to avoid struggles of the animal, the initial effect is inhibition of gastric movements, respiration not being necessarily affected. The inhibition lasts a variable length of time, in general much longer than with ether. Then a few apparently normal waves appear, to be succeeded again by a period of inhibition. This play is repeated, inhibition alternating with the appearance of a few gastric waves (Figs. 4, *b*, *c*). These gastric waves are not weak, but are practically normal in strength. This course may be considered the type for chloroform. Here, again, there are exceptions to the rule. After induction of anæsthesia some rabbits may



fail to show a single gastric wave perceptible to the tambour; in others chloroform produces irregular waves throughout the anæsthesia. In general, however, the difference between ether and chloroform in their effect upon gastric peristalsis is well marked.

The administration of chloroform requires more care than that of ether, and the stage of full anæsthesia is more difficult to judge.

With chloroform the lid reflex in rabbits is only abolished shortly before onset of rapid extensive respirations, associated with a high-pitched crying of the animal, which marks the beginning of respiratory failure.<sup>1</sup>

**The inhibitory effects of laparotomy.** — It has been shown that gastric peristalsis, as a rule, is active when a rabbit is anæsthetized with ether. If the abdomen of such an animal is now opened in the middle line and the stomach inspected, not a sign of motion will be detected. The organ which a few moments before showed active powerful waves lies there inert,

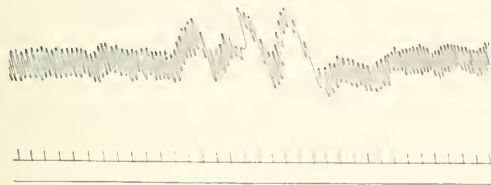


FIGURE 4c. — Same animal. Under chloroform fifty minutes.

no motion can be seen. Well might Johannes Müller<sup>2</sup> say, “Die peristaltischen Bewegungen des Magens habe ich nie deutlich gesehen. . . .” If the viscus is allowed to become dry, slight waves may occasionally appear. Opening the peritoneal cavity under 0.9 per cent saline solution kept at a temperature of about 39° does not alter the picture; the stomach shows no movements worth mentioning. Stomach motility is lost as long as the peritoneal cavity remains open. If the abdominal wound be closed and the stomach area inspected after a few hours, gastric peristalsis may be observed

<sup>1</sup> It is worth noting that under ether as well as under chloroform the lid and corneal reflexes do not disappear at the same time; the corneal reflex seems the more sensitive and fails, while the lid reflex may still be active.

<sup>2</sup> MÜLLER: *Handbuch der Physiologie des Menschen*, dritte Auflage, 1838, i, p. 499.



FIGURE 4b. — Same animal. Chloroform has been administered twenty minutes.

again. *Mere opening of the abdomen, therefore, exerts a profound inhibitory effect of long duration upon the stomach.*

It is obvious from the foregoing that any method which demands opening of the peritoneal cavity for the study of stomach motility is incapable, at least in the rabbit, of throwing light upon the normal physiological behavior of the stomach.

Most investigators do not seem aware of the profound inhibition which exposure of the abdominal viscera produces. As far as I know Pal<sup>1</sup> is the only author who has called attention to the inhibition which abdominal section produces. But while he clearly and sharply notes the strong inhibitory effect of this operation upon the small and large intestines, only scanty attention is paid to the stomach.

It must be pointed out that Pal's method of experimentation did not entirely justify his conclusions, at least as far as the stomach is concerned. He experimented upon curarized animals, and observed the motility of the gastro-intestinal canal during cessation of artificial respiration. The cessation of artificial respiration is not an indifferent factor, as can be seen from the following observations made in the present investigation. In a rabbit fully curarized by intravenous injections of curarine, and kept alive by artificial respiration, the stomach shows excellent and regular peristalsis. But if artificial respiration be stopped, stomach peristalsis ceases at once, or within a few seconds. If artificial respiration be resumed in one to two minutes, gastric peristalsis will not set in until about two to four minutes have elapsed. This shows that interruption of artificial respiration in curarized rabbits stops gastric peristalsis. The stoppage of the peristalsis in Pal's experiments, at least as far as the stomach is concerned, could therefore have been due as much to the cessation of the artificial respiration as to the opening of the abdomen.

#### SUMMARY.

1. Gastric peristalsis may be observed in a well-fed rabbit without any operative interference whatsoever, by mere inspection of the epigastrium.
2. These movements are easily registered by placing a tambour

<sup>1</sup> PAL, J.: STRICKER'S Arbeiten aus dem Institut für experimentelle Pathologie der Wiener Universität, 1890, p. 31.

over the stomach area to be studied and connecting it with a writing tambour.

3. There is a striking difference between the effects of ether and chloroform upon gastric peristalsis; ether does not, as a rule, abolish gastric peristalsis, chloroform considerably reduces the stomach motility.

4. Opening the peritoneal cavity inhibits stomach peristalsis completely. This remarkable inhibition remains in force as long as the abdominal cavity is open; closure of the cavity is followed in a few hours by a return of gastric peristalsis.

5. Fasting reduces or completely abolishes stomach peristalsis.

6. Distention of the fasting stomach with air or water causes active peristalsis.

7. Curarization does not affect gastric peristalsis as long as artificial respiration is maintained. Stoppage of lung ventilation causes almost immediate cessation of stomach movements.

8. Temporary inhibition of gastric motility is produced easily by a variety of causes: handling of the animal, fright, struggles, irritating odors. Ether and chloroform vapors inhibit the stomach movements without causing respiratory stoppage.

9. Pyloric sounds in the rabbit are apparently produced by the expulsion of liquid material into the gas-containing duodenum; these sounds occur at irregular intervals, and when peristalsis is well established.

10. The rabbit's stomach shows a well-defined preantrum and preantral sphincter.

It is with great pleasure that I acknowledge the invaluable help and stimulating counsel of Dr. S. J. Meltzer during this investigation.

## THE ANALYSIS OF URINE IN A STARVING WOMAN.<sup>1</sup>

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RECENT contributions to the physiology of inanition, especially on professional fasters, have included the more exact study of the transformations of matter as measured by the urinary constituents. While the earlier literature furnishes a number of instances of observations on starving women, the number of recorded observations made with modern methods is small, and this paper presents the record of the urinary examinations of a woman patient in the Connecticut Hospital for the Insane at Middletown. This patient, who was otherwise organically sound, voluntarily refused food for six days. During this period feces were passed, and the report of their examination is likewise here included.

The earliest record of the examination of the urine of a fasting woman with which we are familiar is that of Schultzen,<sup>2</sup> who reports a case of œsophagus stenosis in a nineteen-year-old girl, as a result of poisoning with sulphuric acid. In 330 c.c. of urine excreted two days before death, there were 6 gm. of urea corresponding to 2.794 gm. of nitrogen.

Since the patient was in a delirium and voided the urine irregularly, an exact study could not be made.

The earliest accurate observations on a starving woman are those made by Seegen,<sup>3</sup> who reported a case of partial inanition in a twenty-four-year-old girl with a constriction of the œsophagus, which was diagnosed as carcinoma ventriculi. From the 10th of July until the 21st the patient took only 35 gm. of fresh cow's milk per day. This cor-

<sup>1</sup> The expense of this investigation was borne by the Carnegie Institution of Washington.

<sup>2</sup> SCHULTZEN: *Archiv für Anatomie und Physiologie*, 1863, p. 31.

<sup>3</sup> SEEGEN J.: *Sitzungsberichte mathematisch-naturwissenschaftlichen Classe*, 1871, lxiii, Abth. 11, pp. 429-438.

responds to the ingestion of about .29 gm. of nitrogen per day. Under these conditions the patient passed 2230 c.c. of urine in the twelve days, or 185 c.c. per day. The daily quantities ranged from 125 to 240 c.c. The amount of urea ranged from 6.1 to 11.9 gm., with an average of 8.9 gm., corresponding to 4.51 gm. of nitrogen. The patient remained most of the time in bed, and the pulse varied from 72 to 80.

Tuczek<sup>1</sup> describes two cases of fasting in insane women. One of the patients, who was previously very well nourished, and weighed 65 kgm., eliminated on the last eight days of a twenty-two-day fast an average of 4.26 gm. of nitrogen per day. The second patient, who weighed 55 kgm., excreted, on almost complete inanition, an average of 4.28 gm. of nitrogen per day for sixteen days. The nitrogen was determined as urea by precipitation with mercury salts, making due allowance for the chlorides present.

Tuczek discusses in considerable detail the influence of mental disorders on metabolism, but his conclusions are not supported by the more recent opinions of Folin, who was unable to find any positive alteration in general metabolism that could be ascribed to mental disturbance.

Senator<sup>2</sup> described a case of fasting in an insane patient who weighed 50 kgm. During four fasting days there was an average excretion of 12.14 gm. of urea, equal to 5.65 gm. of nitrogen per day. On a later fast day the same patient excreted 3.78 gm. of nitrogen.

Mueller<sup>3</sup> reported a case of oesophagus stenosis, caused by drinking lye, in a nineteen-year-old girl. The amounts of urine passed on four successive days were 150, 170, 140, and 130 c.c. respectively. The corresponding specific gravities were 1.026, 1.030, 1.025, and 1.029. The amounts of nitrogen were 4.92, 4.15, 3.25, and 3.01 gm., respectively. The feces for the four days of fasting, which were separated only with great difficulty, weighed in a dry form 17.4 gm. The four days during which observations were made corresponded to the fifth to the eighth days of complete inanition. The body weight was unusually low, 34.5-33.0 kilos.

Schaefer<sup>4</sup> reports seven cases of fasting insane women, in which

<sup>1</sup> TUCZEK: *Archiv für Psychiatrie und Nervenkrankheiten*, 1884, xv, pp. 784-799.

<sup>2</sup> SENATOR: *Charité Annalen*, 1887, xii, p. 325.

<sup>3</sup> MUELLER: *Zeitschrift für klinische Medizin*, 1889, xvi, pp. 496-549.

<sup>4</sup> SCHAEFER: *Allgemeine Zeitschrift für Psychiatrie*, 1897, liii, pp. 525-537.

four took no water. The nitrogen was determined in the twenty-four-hour urine by the Kjeldahl method. Of the seven cases water was taken in small amounts by cases 1, 2, 5. No water was taken by the other four cases. The results are given in Table I.

TABLE I.  
SUMMARY OF RESULTS OBTAINED BY SCHAEFER ON SEVEN FASTING INSANE WOMEN.

Case.	Day of fast.	Volume.	Nitrogen.	Body weight.	Nitrogen per kilo. of body weight.	
		c.c.	gm.	kilos.	gm.	
1	{	4	410.0	5.7441	63.6	0.0903
		5	450.0	6.2351	63.2	0.0986
2	{	5	220.0	3.4151	49.3	0.0692
		6	470.0	5.9194	48.8	0.1213
3	{	7	380.0	4.5146	48.0	0.0940
		5	550.0	8.5222	38.7	0.2202
4	{	6	405.0	6.9110	37.85	0.1825
		4	265.0	6.0590	38.2	0.1586
5	{	6	260.0	6.2814	36.2	0.1735
		4	465.0	7.8123	36.1	0.2164
6	5-6	262.5	6.0239	46.3	0.1301	
7	2-5	162.5	4.0747	33.0	0.1234	

The observations of Van Hoogenhuyze and Verploegh<sup>1</sup> on the professional fasting woman, Flora Tosca, included the determinations not only of the total nitrogen (Kjeldahl), but also of the creatinine. The creatinine determinations were made by the Folin<sup>2</sup> method, and hence are of unusual interest. On the day before the fast began the nitrogen excretion was 13.99 gm. The nitrogen excretion on the succeeding days of the fast were 8.76, 8.38, 10.73, 9.40, 7.87, 7.73, 6.11, 6.70, 7.35, 6.80, 6.14, 6.97, 5.62, 4.08, and 4.38 gm., respectively. The creatinine excretion on the last day with food was 1.087 gm. On the fasting days it was 0.904, 0.577, 0.581, 0.634, 0.663, 0.590, 0.469,

<sup>1</sup> VAN HOOGENHUYZE and VERPLOEGH: *Zeitschrift für physiologische Chemie*, 1905, xlvi, pp. 468-471.

<sup>2</sup> FOLIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223.

0.689, 0.715, 0.602, 0.453, 0.566, 0.548, 0.426, and 0.715 gm., respectively. On the first day after food was taken it again rose to 1.028 gm.

Mohr<sup>1</sup> and his collaborators have recently reported a fifteen-day fast of a professional fasting woman (Schenk). The fluctuations in body weight were accurately recorded, and the total nitrogen in the urine. No observations on the creatinine elimination during this fast have thus far been reported.

The body weight fell from 54.4 kilos to 48.2 kilos. The nitrogenous output in the urine, as determined by the Kjeldahl method, was 8.408; 6.592; 7.789; 7.856; 7.815; 7.128; 6.195; (5.400); 4.384; 5.166; (5.784); (8.124); 5.958; 5.040, and 4.060 gm. on the succeeding days of the fast. The values in parentheses represent days on which amino-acids were ingested.

#### EXPERIMENT WITH S. H.

In connection with a lengthy series of experiments on fasting men which were made in this laboratory, the apparently anomalous observations noted in several cases lead to the belief that data should be accumulated regarding the influence of inanition on the metabolism of women. Accordingly, when the following case presented itself in the clinic of one of us (A. R. D.), arrangements were made to conduct the experiment in such a manner as to secure positive evidence as to the authenticity of the fast, and the collection of the total amounts of urine and feces. A nurse was detailed to watch the patient carefully. Unfortunately a study of the respiratory exchange of this subject was impossible.

**Subject.** — In accord with fixed religious delusions, the subject, S. H., thirty-six years of age, fasted three weeks, taking only cold water. From this time she abstained absolutely from eating meat in any form, including soups. Her diet consisted of one slice of bread, a cup of tea, one potato, or an equal quantity of some other vegetable, and one-half glass of milk three times daily. She remained on this diet for twenty-four months. Her weight, which was 76.2 kilos at the beginning of this period, varied considerably, at one time reaching as high as 96.6 kilos. At the end of the period it was 88.5 kilos. At this time, March, 1905, the patient, in accord with similar delusions, began to restrict her diet to one and one-half pints of milk per day, and did

<sup>1</sup> MOHR: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, pp. 638-645, 675-687, and 687-691.

not drink water. This diet continued for eight and one-half months, and her weight dropped from 88.5 kilos to 49.9 kilos.<sup>1</sup> The weights were taken on the first of each month, and were as follows:

March 1, 88.5 kilos.	September 1, 55.4 kilos.
April 1, 76.2 "	October 1, 52.6 "
June 1, 66.2 "	November 1, 51.3 "
July 1, 63.5 "	November 20, 49.9 "
August 1, 58.5 "	

During all of this period, as was her custom, the patient was of sedentary habits, sewing continuously for about ten hours daily.

On November 22, at 5 P. M., the patient began a fast, and during the succeeding three days took no water or food of any kind. Thus the fast was complete. The body weights for the succeeding days were

November 24, 48.2 kilos.	November 28, 47.2 kilos.
November 27, 47.4 "	December 1, 45.9 "

The weight taken thirty-nine hours after the fast was begun shows that she had lost 1.7 kilos since the last time of weighing (November 20).

This fast without water or food lasted one hundred and ten hours. During the last seventy-one hours of this one hundred and ten hours fast without food and water, she lost 0.9 kilo. During the next twenty-four hours the patient took water freely (fourteen glasses, equivalent to about 3900 c.c.). Although no food was taken, the subject drank on the two subsequent days three glasses (840 c.c.) of water per day. From the one hundred and tenth to the one hundred and thirty-fourth hour of the fast she lost in weight 0.11 kilo. The fast ended at 10 A. M. November 29, after a duration of one hundred and sixty-one hours. The next weight was not taken, because the patient was confined in bed until December 1, forty-eight hours after end of fast, when it was found that she had lost 1.25 kilos. At the termination of the fast, the patient sipped ten ounces of milk, and thirty minutes later another ten ounces of milk, fifteen minutes later she became profoundly nauseated. The nausea continued throughout the day and

<sup>1</sup> At the time this experiment was made no measurements were recorded of this subject's height and her various girths. In a subsequent experiment (see the following paper, p. 383) with a body weight of 61.2 kilos the subject measured around the shoulders 1.06 metres, around the waist 0.81 metres, and around the hips 1.07 metres. Her height is 1.65 metres.



evening. At 11.30 A. M. there was a movement of the bowels, at which time she nearly fainted. At 1 P. M. she sipped another ten ounces of milk, and again at 4 P. M. another ten ounces. Meanwhile the patient was on her feet and walking most of the time. At 6 P. M. she sipped twenty ounces of milk; thirty minutes later she vomited fifteen ounces of curdled milk. At 8.30 P. M. she vomited a huge quantity of milk and fluid, aggregating two quarts. At 3 A. M. the next morning she arose from bed to urinate, and then fainted. This specimen was lost (probably about 150 c.c.). During this day, November 30, the patient took no food, but drank twenty ounces of water. The following day the patient began again to drink three pints of milk daily.

**Methods of analysis.**—The total nitrogen was determined by the Kjeldahl method. The creatinine was determined by the Folin<sup>1</sup> method.

The heat of combustion was determined by drying 15 c.c. of fresh urine, to which 0.05 gm. of pure salicylic acid had been added, in a vacuum desiccator. The dried mass was then transferred to a nickel capsule, and dried a second time, until in condition to burn. It was then burned in the calorimetric bomb<sup>2</sup> with compressed oxygen. In the computation of the results allowance was made for the salicylic acid used.

#### URINE.

For five days before the fast began, the urine was collected, the bladder being emptied each morning at 7 A. M. This patient was unusually intelligent, entered into the spirit of the investigation thoroughly, and we have every reason to believe that the separation at 7 A. M. in the morning was as accurate as could be made with a normal individual. After the collection of the twenty-four-hour urine a preservative was added, usually chloroform, and then the sample submitted to an analysis. The samples were all given precisely the same treatment. This procedure was emphasized, since, if there was a change of creatinine to creatine by reason of the somewhat delayed analyses, it would affect all days alike. The

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

<sup>2</sup> The Berthelot-Atwater bomb calorimeter was used in all of these determinations. For description of this instrument, see ATWATER and SNELL: *Journal of the American Chemical Society*, 1903, xxv, p. 693.

determinations were usually made within forty-eight hours of [the collection of the urine.

The results for the analyses of urine are given in detail in the table. As the table shows, the results are given for five days before the fast began and ten days after the fast ended. Unfortunately the nitrogen of the income was not determined during these days accurately, neither were the feces collected; hence no nitrogen balance can be obtained.

**Volume.**—Aside from the volume of urine during the fasting periods, the quantities voided by this patient presented no unusual feature. On the other hand, for the three days of fasting when water was not taken, November 23, 24, and 25, the volume of urine was exceedingly low. The lowest amount, 237 c.c., is remarkable when the body weight of this subject is taken into consideration. It is furthermore to be noted that this low volume occurred on the first rather than the later days of the fast.

The marked increase in the volume of urine accompanying the ingestion of large amounts of water on the fourth day of the fast is of unusual interest. On this day the subject drank approximately 3900 c.c. of water, and the urine volume increased from 404 to 2100 c.c.

**Specific gravity.**—The specific gravities of the urine obtained during the first three days of fasting, *i. e.*, when no water was consumed, are remarkably high, 1.035, 1.035, and 1.030, respectively. The specific gravity of 1.035 is higher than any thus far reported for fasting women.

The high specific gravity observed on the first two days of complete fast are to be expected from the low volume of urine passed during these days.

**Total nitrogen.**—In the earlier literature the main index to protein katabolism was the determination of urea, which, as is well known, was almost invariably made by faulty methods, and therefore represents neither the true proportion of urea nor the measure of the true protein katabolism. The Kjeldahl method of determination gives results, indicating the total nitrogen in the urine, and consequently it furnishes a much more accurate index of protein katabolism, but even this has recently been improved upon by Folin, who has elaborated methods for the partition of the nitrogen, and has thus given more definite clues to the nature of the different forms of protein katabolized. In this experiment it was only possible to determine the total nitrogen and one of the nitrogenous urinary

TABLE II.  
COMPOSITION OF THE URINE FOR STARVING WOMAN.

Date.	Volume. (a)	Specific gravity. (b)	Total nitrogen.		Heat of combustion per c.c. (c)	Total energy of urine. $\left(\frac{a \times c}{1000}\right)$ (f)	Ratio calories $\frac{f}{N}$ ( $f \div d$ ) (g)	Total creatinine. gm. [0.34]	Preformed creatinine. gm. [0.23]	Preformed creatine. <sup>1</sup> gm. [0.11]
			per cent. (c)	$a \times c$ (d) gm.						
Nov. 18	c.c. 965		0.68	6.56	small calories. 62.14	kilo. calories. 59.97	9.14	0.50	0.50	....
" 19	1100		0.59	6.49	57.90	63.69	9.81	0.66	0.61	0.05
" 20	950		0.70	6.65	64.35	61.13	9.19	0.63	0.60	0.03
" 21	920		0.76	6.99	66.05	60.77	8.69	0.67	0.61	0.06
" 22	1050	1.013	0.71	7.45	64.39	67.61	9.07	0.68	0.65	0.03
" 23	237	1.035	1.77	4.19	179.70	42.59	10.16	0.65	0.61	0.04
" 24	360	1.035	1.68	6.05	217.70	78.37	12.96	0.66	0.57	0.09
" 25	404	1.030	1.58	6.38	222.20	89.72	14.06	0.61	0.54	0.07
" 26	2100	1.009	0.33	6.93	43.87	92.13	13.29	0.50	0.44	0.06
" 27	780	1.015	0.79	6.16	128.50	100.23	16.27	0.65	0.49	0.16
" 28	630	1.017	0.70	4.41	129.90	81.84	18.56	0.49	0.34	0.15
" 29	362 <sup>2</sup>	1.019	0.84	3.04	165.90	60.05	19.75	0.67	0.56	0.11
" 30	502	1.017	0.97	4.87	130.60	65.56	13.46	0.67	0.56	0.11
Dec. 1	495	1.013	0.64	3.17	54.46	26.96	8.51	0.55	0.50	0.05
" 2	925	1.019	0.55	5.09	51.42	47.56	9.34	0.64	0.61	0.03
" 3	960	1.010	0.63	6.05	51.92	49.84	8.24	0.61	0.60	0.01
" 4	470	1.016	1.18	5.55	95.00	44.65	8.05	0.52	0.50	0.02
" 5	650	1.015	1.18	7.67	91.01	59.16	7.71	0.65	0.63	0.02
" 6	590	1.017	1.09	6.43	94.02	55.47	8.62	0.59	0.58	0.01
" 7	915	1.013	0.75	6.86	....	....	....	0.59	0.56	0.03
" 8	1070	1.014	0.62	6.63	51.45	55.05	8.30	0.55	0.54	0.01

<sup>1</sup> Expressed as creatinine.

<sup>2</sup> Some urine lost.

constituents, *i. e.*, creatinine. The per cent of nitrogen and the total excreted per day are recorded in the table. Of especial significance is the total quantity excreted. Fortunately during the six days of the fast no known accidents interfered with the collection and analysis of the complete day's urine. On the first day after the fast, there was a loss of urine, as has been stated previously. The nitrogenous output during the first four days of fasting steadily increased from 4.19 to 6.93 gm. There is, then, a subsequent marked decrease on the last two days, and on the last day of fasting the total nitrogenous output was but 4.14 gm. This increase in the nitrogenous output for the first three or four days of fasting has been very commonly observed. Thus, with Flora Tosca,<sup>1</sup> on the first three days without food, there were 8.76, 8.38, and 10.73 gm. of nitrogen excreted, respectively. Contrary to the experience observed with fasting men and with the fasting woman, Tosca, in the case of (Schenk) the subject of Brugsch and Hirsch,<sup>2</sup> the nitrogenous output for the second, third, and fourth day of fasting were 8.41, 6.59, and 7.78 gm. respectively. In other words, the largest excretion occurs on the second day of fasting. As has frequently been pointed out, the nitrogenous intake the day before the fast began may affect noticeably the nitrogenous output of the first day, but it is hardly probable that the nitrogenous output for the second day should be affected by the food prior to the fast, and this experiment obviously presents a somewhat different picture from the others.

The increase in the nitrogenous output in the first three days of fasting with the subject of the experiments here reported is, however, wholly in accord with the studies on the fasting men made in this laboratory and published elsewhere.<sup>3</sup> In those experiments it was possible to determine the glycogen katabolized, and by these determinations the hypothesis of Prausnitz<sup>4</sup> that the glycogen protects the nitrogenous material from disintegration was wholly substantiated. It would appear that also with the fasting woman, S. H., in all probability there is a larger katabolism of glycogen on the first two or three days of the fast which temporarily checks the disintegration of nitrogenous material.

<sup>1</sup> Reported by VAN HOOGENHUYZE and VERPLOEGH, *loc. cit.*

<sup>2</sup> BRUGSCH and HIRSCH: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 639.

<sup>3</sup> Carnegie Institution of Washington, Publication No. 77, 1907.

<sup>4</sup> PRAUSNITZ: *Zeitschrift für Biologie*, 1892, xliii, p. 638.

At this period the body weight of the subject was about 47.5 kilos, and the nitrogenous excretion corresponds to the average proteid disintegration on the five days of 34 gm., or, on the basis of per kilo of body weight, there was katabolized during this fast on an average about 0.72 gm. of protein. Of especial interest in this discussion is the fact that when there is a marked diuresis caused by the ingestion of a large quantity of water, the volume increased from 404 c.c. on the third day of the fast to 2100 c.c. on the fourth. This was accompanied by a slight increase in the nitrogenous excretion amounting to 0.55 gm. The discussions regarding the effect of large quantities of water on the elimination of nitrogen have resulted in the assumption that there may be either a washing out of preformed nitrogenous end products of protein katabolism, or there may actually be an increase in metabolic activity by virtue of the large quantity of water passing through the glands. From the slight increase in the output of nitrogen, but little evidence is here secured to substantiate either hypothesis. On the 29th of November the nitrogenous output was but 3.04 gm., but, as has been before stated, there was a loss of urine on this day of probably not less than 150 c.c. The remarkably low nitrogenous output on the first of December, *i. e.*, 3.17 gm., is, however, the total output of twenty-four hours since no urine was lost. On the day preceding no food had been taken, and on this day, December 1, the subject consumed a large amount of milk, a portion of which was vomited. It is entirely conceivable, however, that a not inconsiderable portion of the milk was digested and absorbed, and hence this low nitrogenous output would indicate a marked storage of nitrogen on this day. This, again, is wholly in accord with the studies on fasting subjects, in which a study of the effect of the subsequent ingestion of food on the nitrogen output was made. It is interesting to note that five days after the fast ends the subject excretes approximately the same amount of nitrogen as before the fast began, namely, 6.8 gm.

**Creatine and creatinine.** — Evidence regarding the creatinine excretion of women is extremely meagre, and hence determinations of the creatinine in the urine of this patient were made. We are indebted to Miss Charlotte R. Manning for making these determinations. By means of the Folin method it was possible to determine the preformed creatinine and the creatine. The preformed creatinine was determined on the fresh urine by heating with hydrochloric acid before producing the Jaffé reaction, and a subsequent determination

was made in which a sample was used without heating with acid. The difference between the total and the preformed creatinine gave the preformed creatine expressed in terms of creatinine. The total creatinine excretion observed in this patient is as constant as is usually found. The amount excreted was relatively low, averaging 0.60 gm. of total creatinine and 0.55 preformed creatinine. In averaging the results for November 29 were omitted, since urine was unavoidably lost on this day. The body weight during the whole experimental period, November 19–December 9, was not far from 50 kilos, the excretion of creatinine per kilo of body weight, or so-called creatinine coefficient, was not far from 11.

The most noticeable feature of the creatine observations was the marked increase in the preformed creatine excreted during the fast. On one day, November 27, there was as much as 0.16 gm. of preformed creatine (expressed as creatinine) in the urine. On the resumption of the food, namely, on the 1st of December, the preformed creatine practically disappeared. It is, however, to be noted that on November 29, when the subject took a large quantity of milk, and although vomiting much, must have retained some, the preformed creatine excretion was still relatively high, 0.15. This appearance of creatine in the urine is wholly in accord with observations made on fasting men in this laboratory. They have also been verified by observations made on a fasting man in the laboratory of Dr. Folin at Waverley, Massachusetts.

As pointed out in a discussion of the creatine determinations on fasting men,<sup>1</sup> the creatine excretion during fasting appears to be the result of the disintegration of flesh, and the hypothesis was there advanced that as the flesh was katabolized or broken down, the creatine which existed in the flesh as such was excreted by the body as such and not converted to creatinine. According to this hypothesis, therefore, the creatine and creatinine of the urine have two entirely distinct origins.

**Energy.**—In all fasting experiments previously made in this laboratory, a part of the routine has been to determine the heat of combustion of urine, and this determination was likewise made on the urines in this experiment. Unfortunately it was not possible to determine the carbon owing to pressure of other work. The heat of combustion of the urine, expressed in terms of small calories, is recorded in column *e* of Table II. The total potential energy excreted in the

<sup>1</sup> Carnegie Institution of Washington, Publication No. 77, 1907.

urine for each day, expressed in large calories, is recorded in column *f* of the same table. The quantity of energy thus excreted for this fasting woman is very much less, on the whole, than that excreted by fasting men. As an average of a large number of experiments, the total energy excreted in the urine of fasting men was 88, 115, 120, 121, 120, 146, and 139 calories on the first to the seventh day of fasting, respectively. Only on the third, fourth, and fifth fasting days of the experiment with S. H. does the energy reach the lowest average observed with fasting man. The small amount of energy thus excreted corresponds with the much smaller general output of urinary constituents, based in large part, probably, upon the fact that this woman was of much smaller body weight than the subjects of the fasting experiments with men reported previously.

**Ratio of energy to nitrogen.** — While unfortunately the carbon could not be determined, the ratio of energy to nitrogen is readily computed. With ordinary diet it has been found that as a result of a large number of experiments for every gram of nitrogen in the urine there are not far from 8 to 9 calories of energy.<sup>1</sup> In the fasting experiments above referred to, the ratio of energy to nitrogen on the first days of fasting showed that for every gram of nitrogen there was not far from 8 to 9 calories of energy. In the experiments with this subject, it is seen that the ratio of energy to nitrogen expressed in column *g* is in no wise abnormal on the days before the fast. There is, however, a striking increase in this ratio which reaches a maximum on the first day after the fast, namely, 19.75. On December 1 the ratio again returns to its usual value, and remains not materially different during the remainder of the study. This marked increase in the ratio of energy to nitrogen has been the subject of considerable discussion in the report of the study with fasting men. In that report the carbon of the urine was likewise determined, and it was possible to compare the ratio of energy to nitrogen and also that of nitrogen to carbon and carbon to energy. As a result of these ratios, it seemed highly probable that there was in the urine some constituent of non-nitrogenous or low nitrogenous nature which yielded energy. Sugar and albumen were proved absent. Although not strictly demonstrated, the explanation of the high ratio may well be based upon the assumption of an acidosis. The acidosis as measured by the calorie-nitrogen ratio disappears on the conclusion of the fast. In

<sup>1</sup> ATWATER and BENEDICT: Bulletin 136, U. S. Dept. of Agriculture, Office of Experiment Stations, 1903, p. 114.

the fasting experiments with men the calorie-nitrogen ratio did not in all experiments increase. Even in several experiments in which the fast lasted three days and over, there was no material disturbance of this ratio, but in one experiment of seven days and one of four days the ratio actually increased, until it became 1 : 14.87, the highest ratio observed during the series of experiments on men. The ratios observed with this fasting woman are indeed very considerably higher, and we know of no ratios thus far reported which approximate these high ratios. Unfortunately no tests were made for  $\beta$ -oxybutyric acid and similar organic acids, although in the fasting experiment with Succi, reported by Brugsch,<sup>1</sup> a marked acidosis was observed, and in the more recent experiments on a fasting woman reported by Bönninger and Mohr,<sup>2</sup> a marked acidosis was recorded, the total amount of  $\beta$ -oxybutyric acid at times amounting to from 18 to 25 gm. per day.

When it is considered that the body during fasting is living upon essentially a protein-fat diet, and that, as has been experimentally demonstrated, an acidosis is frequently observed on such diets, the assumption that we are here dealing with a true acidosis is not improbable.

Loewy,<sup>3</sup> in making observations upon metabolism at high altitudes, determined, among other factors, the calorie-nitrogen ratio in the urine, but, finding this somewhat larger than is common, he was able to separate varying amounts of amino-acids. In discussing the question, Loewy points out the fact that lactic acid has also appeared in the urine.

It is much to be regretted that in the experiment with S. H. the direct determinations of the organic acids were not made, but the calorie-nitrogen ratio points strongly to the assumption that there was a marked acidosis on the later days of the fast. This is wholly in accord with the observations in at least two of the longer studies during inanition made in this laboratory with men.

**Feces.** — The great difficulty in properly separating the feces during fasts with men has been experienced by most investigators. It was impracticable in this experiment to administer any coloring material

<sup>1</sup> BRUGSCH: *Zeitschrift für experimentelle Pathologie und Therapie*, 1905, i, p. 419.

<sup>2</sup> MOHR: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 675.

<sup>3</sup> LOEWY: *Deutsche medicinische Wochenschrift*, 1905, p. 1919.



for separating the feces, and hence it was possible to examine only the feces which were passed during the actual fasting period. During this period, and including the movement one and one-half hours after taking food, the patient had four bowel movements. This is directly contrary to all experience with fasting subjects. The total mass in the fresh condition weighed 265.1 gm. A macroscopic examination showed several hard pillular lumps, mixed with a large amount of soft, watery material. A specimen was examined microscopically, and found to contain large amounts of yellowish brown, bile-stained crystalline material.

Most of the crystals disappeared upon the addition of glacial acetic acid. There was also present a large number of columnar epithelial cells. No starch granules or other food residues were identified. No fatty acid crystals were observed. In addition to the above there were some small colorless crystals which were undisturbed by glacial acetic acid. The air-dried material weighed 15.26 gm.

In the air-dry material there was 2.07 per cent of nitrogen; 37.04 per cent of ash; 18.05 per cent of crude ether extract (neutral fat + free fatty acids) and 38.0 per cent of total fatty acids obtained by extracting the feces after drying with alcohol and hydrochloric acid to break up the soaps. The heat of combustion was 4.736 calories per gm. of air-dry material. The per cent of nitrogen is scarcely one half that found in ordinary feces.

The per cent of ash was unusually large, nearly twice that commonly found in feces, and much larger than with any sample of feces which we have ever examined in this laboratory. Furthermore, nowhere in the literature have we been able to find a sample of feces with such a large proportion of ash. On the assumption that air-dried feces contains not far from 5 to 7 per cent of water, it will be seen that on the water-free material there would be not far from 40 per cent of ash.

The large amount of fatty acids when compared to the amount of crude ether extract shows that there must have been a very considerable excretion of soap. While unfortunately no determinations were made of calcium, it is highly probable that this element was combined and excreted with the fatty acids in the form of calcium soaps. On this assumption the high ash content may partly be accounted for by the fact that the ignition of the calcium soaps would result in a relatively large residue of calcium carbonate, which would not be converted to the oxide at the low temperature of incineration at which these ash determinations are commonly made.

While the feces cannot be strictly designated as fasting feces, they are of interest as having a composition closely analogous to one of those of the fasting men who took small quantities of food after fasting, and the feces were characterized by a very large quantity of ash, accompanied by a large excretion of fatty acids.

#### SUMMARY.

The results of this investigation may be summarized as follows:

1. The volume of urine of a fasting woman (without water) may be as low as 237 c.c. in twenty-four hours.

2. The specific gravity of two twenty-four-hour amounts of the urine during fasting (without water) was observed to be as high as 1.035.

3. The nitrogen output during fasting increased for the first three days and then decreased. On one day, at the conclusion of the fast, the subject excreted but 3.17 gm. of nitrogen.

4. The total potential energy of the urine was much less than that commonly observed in fasting men, and on but one day did the energy excreted reach 100 calories.

5. The ratio of energy to nitrogen during the fast increased rapidly as the fast progressed. On all fasting days it was unusually high (on one day 1:19.75), thus indicating an acidosis, although a verification of this hypothesis was not made by determining the organic acids.

6. The preformed creatinine excretion of this subject was low, the creatinine excretion per kilogram of body weight being but 11 mgm.

7. The preformed creatine in the urine increased markedly with fasting, and practically disappeared on the conclusion of the fast.

8. A chemical examination of the feces which were not sharply separated, and therefore not, strictly speaking, fasting feces, showed an abnormally high content of ash and fatty acids, thus indicating the excretion of a large amount of soap. These observations were in accord with the microscopical examination.

## THE ELIMINATION OF CREATININE IN WOMEN.<sup>1</sup>

BY FRANCIS GANO BENEDICT AND VICTOR CARYL MYERS.

[From the Chemical Laboratory of Wesleyan University.]

THE significance of the creatinine elimination as a factor in metabolism has received unusual attention in the past two or three years, due to the researches of Folin,<sup>2</sup> who not only has devised a simple colorimetric method for the exact determination of creatinine, but has viewed the creatinine output from the standpoint of a new theory of protein metabolism. Folin,<sup>2</sup> Van Hoogenhuyze and Verploegh,<sup>3</sup> Closson,<sup>4</sup> Klercker,<sup>5</sup> Koch,<sup>6</sup> and Shaffer<sup>7</sup> have reported results, giving the creatinine elimination of a large number of individuals.

Of the results thus far reported but few are on women. In reporting the results of some metabolism studies with special reference to mental disorders, Folin<sup>8</sup> with his collaborators, Shaffer and Hill,

<sup>1</sup> The expenses of this investigation were shared by the Carnegie Institution of Washington and the Connecticut Hospital for the Insane at Middletown, Connecticut. The study was made possible through the hearty co-operation of Drs. W. E. FISHER, A. B. COLBURN, and L. F. LAPIERRE. Throughout the investigation we enjoyed the counsel of Dr. A. R. DIFENDORF, in charge of the Laboratory of the Connecticut Hospital for the Insane, where the analytical work incidental to this investigation was completed.

<sup>2</sup> FOLIN: This journal, 1905, xiii, pp. 46-138; *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223; *American journal of insanity*, 1904, lx, p. 609, and lxi, p. 299.

<sup>3</sup> VAN HOOGENHUYZE and VERPLOEGH: *Zeitschrift für physiologische Chemie*, 1905, xlvi, p. 415.

<sup>4</sup> CLOSSON: This journal, 1906, xvi, p. 252.

<sup>5</sup> KLERCKER: HOFMEISTER'S *Beiträge zur chemischen Physiologie*, 1906, viii, p. 59.

<sup>6</sup> KOCH: This journal, 1905, xv, p. 15.

<sup>7</sup> SHAFFER: In a private communication.

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

gives the creatinine output of seven women patients in the McLean Hospital for the Insane at Waverley, Massachusetts.

Mrs. A. W. excreted 0.644, 0.653, 0.525, 0.600, 0.646, 0.598, 0.615, 0.568, 0.561, 0.674, 0.688, 0.540, 0.617, and 0.612 gm. of creatinine on consecutive days, an average for the fourteen days of 0.614 gm.

Mrs. R., with a body weight of 40.86 kilos, excreted on five consecutive days 0.66, 0.54, 0.54, 0.57, and 0.62 gm. of creatinine, an average of 0.586 gm. per day, or 14.3 mgm. of creatinine per kilo of body weight.

Mrs. G. W. P., with a body weight of 63.78 kilos, excreted on four consecutive days 0.94, 0.79, 0.90, and 0.73 gm. of creatinine, of an average of 0.84 gm. per day, or 13.3 mgm. of creatinine per kilo of body weight.

Mrs. W. S. H., with a body weight of 52.8 kilos, excreted on four consecutive days 0.912, 1.07, 0.921, and 0.874 gm. of creatinine, or an average of 0.940 gm. of creatinine per day, or 17.7 mgm. of creatinine per kilo of body weight.

Miss F., with a body weight of 50.83 kilos, in one experiment lost in five consecutive days 1.103, 1.06, 1.16, 1.07, 1.313 gm., or an average of 1.104 gm. of creatinine per day, or 21.7 mgm. per kilo of body weight.

Mrs. E. M., with a body weight of 80.3 kilos, excreted on four consecutive days 0.755, 0.755, 0.733, 0.77, and 0.74 gm. of creatinine, or an average of 0.75 gm. per day, or 9.4 mgm. of creatinine per kilo of body weight.

Miss E. B. excreted 0.853 and 0.863 gm. of creatinine on two days respectively.

Van Hoogenhuyze and Verploegh<sup>1</sup> reported the creatinine elimination of a fasting girl, Flora Tosca. The amounts excreted for the fourteen days of the fast were 0.904, 0.577, 0.581, 0.634, 0.603, 0.590, 0.469, 0.689, 0.715, 0.602, 0.453, 0.566, 0.548, 0.426 gm. respectively. Unfortunately the body weight is not given.

Shaffer,<sup>2</sup> in experiments on a woman with a biliary fistula, observed an average excretion of 0.72, 0.59, and 0.89 gm. of creatinine in three series of experiments. While the body weight remained essentially 55 kilos, there were marked changes in the creatinine excretion per kilo of body weight.

Aside from the above observations, no other records of the creatinine elimination by women have, as far as we are aware, been published. Folin has already pointed out that the creatinine elimination seems to depend in a large measure upon the body weight of the

<sup>1</sup> VAN HOOGENHUYZE and VERPLOEGH: *Loc. cit.*, p. 470.

<sup>2</sup> SHAFFER: This journal, 1906, xvii, p. 362.

subject, although individuals of the same body weight may show variations according to the amount of adipose tissue. Hence there appears to be a more or less direct relationship between the creatinine elimination and the active mass of protoplasmic tissue. Numerous observations show that moderately corpulent men eliminate per twenty-four hours about 20 mgm. per kilo of body weight, while lean men yield about 25 mgm. per kilo. Shaffer,<sup>1</sup> in a recent paper before the American Physiological Society, has studied more in detail the limits of the normal creatinine elimination per kilo of body weight, and designates this ratio as the "creatinine coefficient." These limits vary from 18 to 30 mgm. per kilo.

Shaffer has also emphasized the importance of the varying degrees of muscular development, and more especially muscular tonus as affecting the creatinine elimination. Inasmuch as it is believed that the difference in adipose tissue in different individuals influences to a considerable extent the creatinine coefficient, it is of value to note the creatinine elimination in women, since the proportion of adipose tissue to the active mass of protoplasmic tissue is much larger with women than with men. A study of the creatinine elimination in women seemed extremely desirable to us with a view to throwing additional light on these three points; namely, the effects of the degree of muscular development, muscular tonus, and proportion of adipose tissue on the creatinine elimination.

While the use of insane patients for making observations of this kind, which are supposed to contribute to our knowledge of physiology rather than pathology, is open to criticism, yet from the observations of Folin<sup>2</sup> and others, it seems highly probable that mental disorders *per se* do not necessarily involve such changes in metabolism as to modify the creatinine output.

#### METHODS.

**Diet.** — In order to eliminate the possibility of the ingestion of preformed creatine and creatinine, the precaution was taken to make sure that the subject partook of a creatine-free diet both during and for twenty-four hours before the experiment proper began. While Folin<sup>3</sup>

<sup>1</sup> SHAFER: The effect of muscular activity on kreatinin excretion, with preliminary observations on the excretion of kreatinin in health and disease, reported by title: This journal, 1907, xviii, p. xx.

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

<sup>3</sup> FOLIN: Hammarsten Festschrift, 1906.

has recently shown that a large part of the creatine or creatinine ingested may not appear in the urine, either as preformed creatine or preformed creatinine, yet in any study of the endogenous creatinine elimination the food should be creatine-free. The diet consisted for the most part of bread, milk, and eggs.

**Analytical methods.**—The urine was collected in periods of twenty-four hours. In some instances it was necessary to catheterize the patient, but in the majority of cases the urine was collected as voided. It is always difficult to secure an absolute twenty-four hour separation for each day, nevertheless, if the measurements extend over a period of three days, it is fair to assume that the average excretion represents with considerable accuracy the amount excreted for each day. Aside from the creatinine determinations, microscopic examinations of the urine were made, together with tests for albumen and sugar, which are reported if significant in interpreting the results.

The creatinine was determined in all instances by Folin's method. Preformed creatinine was first determined. Then the urine was heated with hydrochloric acid either for three hours on the water bath or for one and one-half hours in an autoclave to convert all the creatine to creatinine. It was thus possible to obtain measurements of the amounts of preformed creatine present in the urine. In most instances the amounts found were negligible or within the limit of error of the analytical method employed.

In all of the early cases investigated the technique of preserving the urine is open to criticism in that the chloroform, which was then used as the preservative, was not added to the urine until the specimen reached the laboratory, thus leaving a slight opportunity for bacterial action. A recent study of the transformation of creatinine to creatine made in connection with this work shows that in some samples of urine there is a strong tendency for the conversion of creatinine to creatine even if chloroform or chloroform and thymol are present. The change is most noted in urines that are alkaline. Consequently in several samples, where pressure of other work precluded an immediate determination of the preformed creatinine, the results are reported as total creatinine.

In several instances a remarkably low creatinine elimination was observed, but repeated tests showed the constancy of the amounts excreted.

This paper deals with the preformed creatinine output, and hence

the discussion of creatine is not included here. Recent observations<sup>1</sup> imply that the creatinine and creatine of urine arise from independent sources.

EXPERIMENTAL DATA.

**Case I., D. H.** *Psychosis; Senile Dementia.* — Age, eighty-five years. Body weight, 39 kilos. This woman has lived on a milk diet for the last five years, the quantity before the experiment averaging 1700 c.c. per day. Subject in bed, old, feeble, withered, and very inactive. Clinical examination of urine revealed nothing pathological. Creatinine determinations were made during two different periods.

	1906	Feb. 7.	Feb. 8.	Feb. 9.
Quantity . . . . .		1352 c.c.	872 c.c.	1243 c.c.
Specific gravity . . . .		1.012	1.014	1.013
Performed creatinine . .		0.324 gm.	0.313 gm.	0.215 gm.

Average performed creatinine elimination for three days, 0.284 gm.

	1906.	June 19.	June 20.	June 21.	June 22.	June 23.	June 24.
Quantity . . . . .		1400 c.c.	1010 c.c.	1100 c.c.	1090 <sup>2</sup> c.c.	995 c.c.	1475 c.c.
Specific gr. . . . .		1.015	1.013	1.012	1.013	1.010	1.014
Performed creatinine {		0.322 gm.	0.238 gm.	0.245 gm.	0.287 gm.	0.269 gm.	0.428 gm.

Average daily performed creatinine elimination for six days, 0.298 gm.

**Case II., H. L.** *Psychosis; Dementia Præcox, Paranoid Form, 2d Group.* — Age, sixty-six years. Body weight, 40 kilos. Subject withered, active, but in bed. Nothing pathological found in clinical examination of urine.

	1906.	Feb. 11.	Feb. 12.	Feb. 13.
Quantity . . . . .		545 c.c.	806 c.c.	732 c.c.
Specific gravity . . . .		1.019	1.014	1.015
Total <sup>3</sup> creatinine . . . .		0.442 gm.	0.578 gm.	0.494 gm.

Average total creatinine elimination for three days, 0.505 gm.

<sup>1</sup> KLERCKER: *Loc. cit.*; SHAFFER: *Loc. cit.*; BENEDICT, Publication No. 77, Carnegie Institution of Washington, 1907.

<sup>2</sup> Ninety c.c. of urine lost, mixed with feces. (The amount lost was added to that actually collected, and the total amount passed is given in the table. Thus 1000 c.c. were collected and 90 c.c. lost.)

<sup>3</sup> In this and several other samples, although the urine had been preserved with chloroform (without the addition of thymol, however), there was an unavoidable delay in the determinations, and hence the total creatinine, *i. e.*, performed creatinine plus any performed creatine, is recorded.

**Case III., M. H.** *Psychosis; Dementia Paralytica.* — Age, fifty-two years. Body weight, 65 kilos. Subject in bed, has little control over muscular movements. Speech unintelligible, has difficulty in swallowing, sleeps most of the time, well nourished. No pathological findings in urine by routine tests.

	1906.	Feb. 16.	Feb. 17.	Feb. 18.
Quantity . . .	1555 c.c.	1065 cc.	1112 <sup>1</sup> c.c.	
Specific gravity .	1.017	1.018	1.018	
Total creatinine .	0.737 gm.	0.616 gm.	0.741 gm.	

Average total creatinine elimination for three days, 0.697.

**Case IV., M. C.** *Psychosis; Dementia Præcox, Catatonic Form.* — Age, forty-five years. Body weight, 48 kilos. Patient inactive, sits up, walks about the ward, young for her years, irritable heart. Clinical examination of urine revealed nothing pathological. Patient was subjected to experiment at three different periods. During the second period the urine was persistently alkaline, except on the last day, and though the determinations of the preformed creatinine were made as soon as the specimens reached the laboratory (three or four hours after last passage of urine for specimen), still the alkaline bacterial fermentation seemed to have destroyed considerable of the preformed creatinine.

	1906.	Feb. 20.	Feb. 21.
Quantity . . . . .	1120 c.c.	1065 c.c.	
Specific gravity . . .	1.015	1.017	
Preformed creatinine .	0.703 gm.	0.805 gm.	

Average preformed creatinine elimination for two days, 0.754 gm.

	1906.	June 26.	June 27.	June 28.	June 29.	June 30.
Quantity . . . . .	985 c.c.	795 c.c.	820 c.c.	660 c.c.	660 c.c.	
Specific gravity	1.020	1.025	1.021	1.023	1.025	
Reaction . . . . .	alkaline	alkaline	alkaline	alkaline	acid	
Preformed creatinine .	{ 0.282(?) gm.	0.502(?) gm.	0.517 gm. (?)	0.474(?) gm.	0.841 gm.	

The average preformed creatinine elimination for these five days was 0.525 gm.

	1906.	Oct. 12.	Oct. 13.	Oct. 14.	Oct. 15.
Quantity . . . . .	1340 c.c.	890 c.c.	1380 c.c.	847 c.c.	
Specific gravity . . .	1.018	1.022	1.017	1.019	
Preformed creatinine .	0.905 <sup>2</sup> gm.	0.580 <sup>2</sup> gm.	0.799 gm.	0.806 gm.	

The average preformed creatinine elimination for the four days was 0.772 gm.

<sup>1</sup> Five hundred c.c. lost, mixed with feces, quantity measured.

<sup>2</sup> The first day contains a portion of urine belonging to the second day as a result of imperfect collection.



This value is essentially the same as that found in the February experiment, and but slightly lower than that found on the one day of the June experiment on which the urine was acid. This therefore indicates that the alkaline urines of June 26-29 had undergone such changes as to decrease materially the amount of preformed creatinine normally excreted by this subject. The average for the subsequent table was taken from the first and third periods.

**Case V., S. L.** *Psychosis; Dementia Præcox, Catatonic Form.* — Age, thirty-two years. Body weight, 53 kilos. Muscular activity of subject practically normal, plump, active, good worker, younger than her years, irritable heart. Several weeks after experiment patient was sent home as recovered. From clinical examination urine was apparently normal in every respect.

	1906.	Feb. 24.	Feb. 25.	Feb. 26.
Quantity . . . . .	1070 c.c.	745 c.c.	785 c.c.	
Specific gravity . . . .	1.023	1.026	1.026	
Preformed creatinine . .	0.738 gm.	0.727 gm.	0.738 gm.	

Average preformed creatinine elimination for three days, 0.732 gm.

**Case VI., S. H.** *Psychosis; Dementia Præcox, Paranoid Form, 2d Group.* — Age, thirty-nine years. Body weight, 61 kilos at time of first experiment here recorded. Physically a normal individual, except that muscular activity is considerably under normal.

	1906.	March 15.	March 16.	March 17.
Quantity . . . . .	1780 c.c.	1480 c.c.	2040 c.c.	
Specific gravity . . . .	1.018	1.018	1.017	
Preformed creatinine . .	0.879 gm.	0.848 gm.	0.863 gm.	

Average preformed creatinine elimination for three days, 0.863 gm.

Body weight at time of second experiment, 78 kilos. During the last two days of the experiment patient was menstruating, and the urine was contaminated with a considerable amount of blood, making the urine albuminous and also rendering it alkaline. Urine collected in a bottle containing 10 c.c. of chloroform. Determinations made immediately after day's urine was collected.

	1906.	July 20.	July 21.	July 22.	July 23.
Quantity . . . . .	2080 c.c.	2230 c.c.	2345 c.c.	2480 c.c.	
Specific gravity . . . .	1.011	1.013	1.014	1.011	
Reaction . . . . .	acid	acid	alkaline	alkaline	
Albumen . . . . .	none	none	about 2 p. c.	about 2 p. c.	
Preformed creatinine . .	0.991 gm.	1.088 gm.	0.825 gm.	* 0.801 gm.	

Average preformed creatinine elimination for four days, 0.926 gm.

During the third period of experiment patient was on a very light diet as the result of some delusions she entertained, and her body weight fell from 80 kilos on September 1 to 74 kilos on October 1. Creatinine determinations were made in patient's urine during ten days of this period. The usual clinical examination revealed nothing pathological. On account of some irregularities the urine of the fourth day was discarded.

	1906.	Sept. 17.	Sept. 18.	Sept. 19.	Sept. 21.	Sept. 22.
Quantity . . . . .		1220 c.c.	680 c.c.	740 c.c.	535 c.c.	610 c.c.
Specific gravity . . . . .		1.010	1.011	1.008	1.008	1.011
Preformed creatinine . . . . .		0.898 gm.	0.798 gm.	0.895 gm.	0.778 gm.	0.833 gm.
	1906.	Sept. 23.	Sept. 24.	Sept. 25.	Sept. 26.	Sept. 27.
Quantity . . . . .		683 c.c.	900 c.c.	875 c.c.	685 c.c.	1030 cc.
Specific gravity . . . . .		1.011	1.009	1.008	1.013	1.018
Preformed creatinine . . . . .		0.768 gm.	0.858 gm.	0.779 gm.	0.867 gm.	0.959 gm.

Average preformed creatinine elimination for the ten days, 0.843 gm.

**Case VII., A. B.** *Psychosis; Dementia Præcox, Catatonic Form.* — Age, thirty years. Body weight, 42 kilos at time of experiment. Patient quiet in bed, apparently in fairly good physical condition; fed with tube. The clinical examination of the urine revealed that the patient had chronic nephritis, about 1 per cent of albumen continuously being present during the three days, together with a large number of hyaline casts and many pus cells.

	1906.	March 15.	March 16.	March 17.
Quantity . . . . .		1515 c.c.	445 <sup>1</sup> c.c.	1740 c.c.
Specific gravity . . . . .		1.013	1.020	1.013
Total creatinine . . . . .		0.662 gm.	0.250 gm.	0.783 gm.

The average total creatinine elimination for the three days was 0.565 gm.

**Case VIII., L. B.** *Psychosis; Dementia Præcox, Hebephrenic Form.* — Age, fifty-seven years. Body weight, 49 kilos during both experiments. Fair physical condition and moderately active during both observations, though possibly in slightly better physical condition during the second than during the first period. Clinical observations revealed nothing abnormal in the urine.

	1906.	July 7.	July 8.	July 9.	July 10.
Quantity . . . . .		1510 c.c.	1395 c.c.	1328 c.c.	1385 c.c.
Specific gravity . . . . .		1.014	1.014	1.014	1.014
Preformed creatinine . . . . .		0.588 gm.	0.582 gm.	0.664 gm.	0.676 gm.

Average preformed creatinine elimination for four days, 0.627 gm.

<sup>1</sup> Patient had only one passage of urine on the morning of the second day, and had to be catheterized the third day, so that the third day contained a portion of urine belonging to the second period.

1906.	Dec. 7.	Dec. 8.	Dec. 9.
Quantity . . . . .	1875 c.c.	1815 c.c.	1765 c.c.
Specific gravity . . . .	1.016	1.014	1.016
Preformed creatinine . .	0.799 gm.	0.707 gm.	0.821 gm.

Average preformed creatinine elimination for three days, 0.776 gm.

**Case IX., M. R.** *Psychosis; Paranoia.* — Age, forty-five years. Body weight, 43 kilos during both experiments. Subject anæmic, active, withered; no outdoor exercise. Clinical examination of urine revealed nothing pathological.

1906.	March 17.	March 18.	March 19.
Quantity . . . . .	495 c.c.	227 c.c.	345 c.c.
Specific gravity . . . . .	1.031	1.027	1.027
Total creatinine . . . .	0.702 gm.	0.314 gm.	0.444 gm.

Average total creatinine elimination for three days, 0.487 gm. On account of the lack of uniformity in the above results a second experiment was made.

1906.	Aug. 12.	Aug. 13.	Aug. 14.
Quantity . . . . .	220 c.c.	260 c.c.	280 c.c.
Specific gravity . . . . .	1.018	1.030	1.027
Preformed creatinine . . .	0.414 gm.	0.452 gm.	0.476 gm.

Average preformed creatinine elimination for three days, 0.447 gm.

**Case X., A. L.** *Psychosis; Manic Depressive. Maniacal Form.* — Age, sixty-three years. Body weight, 64 kilos. Subject well nourished, indolent, though taking a fair amount of exercise, but during the experiment the patient was undergoing a period of mental excitement. Clinical examination of the urine revealed that patient had chronic nephritis, about 1 per cent of albumen being found on each day, with many hyaline and granular casts and a considerable number of leucocytes.

1906.	March 17.	March 18.	March 19.
Quantity . . . . .	1046 <sup>1</sup> c.c.	695 c.c.	1160 c.c.
Specific gravity . . . . .	1.018	1.020	1.016
Preformed creatinine . . .	0.698 gm.	0.771 gm.	0.774 gm.

Average preformed creatinine elimination for three days, 0.748 gm.

**Case XI., E. K.** *Psychosis; Dementia Præcox, Paranoid Form, 2d Group.* — Age, thirty-one years. Body weight, 51 kilos. Subject has a rather poor circulation, but no organic trouble; moderate muscular activity. The collection of the urine was probably just following the menses. Clinical examination of the urine revealed nothing pathological.

<sup>1</sup> Two hundred and thirty-six c.c. lost.

1906.	March 17.	March 18.	March 19.
Quantity . . .	620 c.c.	515 c.c.	555 c.c.
Specific gravity .	1.020	1.026	1.029
Total creatinine .	0.739 gm.	0.749 gm.	0.807 gm.

Average total creatinine elimination for three days, 0.765 gm.

**Case XII., A. M.** *Psychosis; Melancholia.* — Age, forty-seven years. Body weight, 44 kilos. Subject in very poor physical condition, emaciated, muscular activity much less than normal. Clinical examination of the urine revealed nothing pathological.

1906.	March 23.	March 24.	March 25.
Quantity . . .	1460 c.c.	1349 c.c.	1209 c.c.
Specific gravity .	1.015	1.024	1.022
Total creatinine .	0.680 gm.	0.944 gm.	0.846 gm.
Urine lost . . .	none	294 c.c.	599 <sup>1</sup> c.c.

Average total creatinine elimination for three days, 0.823 gm.

**Case XIII., E. S.** *Psychosis; Manic Depressive, Depressed Form.* — Age, fifty years. Body weight, 47 kilos. During experiment patient was much excited, refused to keep on any clothes, and was consequently locked in her room. She was in fair physical condition and extremely active. Clinical examination of the urine revealed an occasional hyaline cast.

1906.	March 25.	March 28.	March 29.	March 30.
Quantity . . .	1040 c.c.	1375 c.c.	1096 c.c.	1926 c.c.
Specific gravity .	1.027	1.020	1.025	1.017
Total creatinine .	0.991 gm.	0.974 gm.	1.183 gm.	0.743 gm.
Urine lost . . .	none	none	237 c.c.	295 c.c.

Average total creatinine elimination for four days, 0.973 gm.

**Case XIV., E. B.** *Psychosis; Manic Depressive, Mixed Form.* — Age, seventy years. Body weight, 69 kilos. Subject well nourished, rather indolent, with only a moderate amount of activity. Clinical examination of the urine revealed nothing pathological.

1906.	March 24.	March 25.	March 26.
Quantity . . . . .	2490 c.c.	2782 c.c.	2525 c.c.
Specific gravity . . .	1.012	1.011	1.009
Preformed creatinine .	1.020 gm.	0.896 gm.	0.974 gm.

Average preformed creatinine elimination for three days, 0.963 gm.

<sup>1</sup> It might at first sight seem erroneous to assume that the creatinine content of the urine lost was exactly that of the urine saved, but numerous observations show that the hourly output is relatively constant, and unless a marked diuresis is assumed, it is highly probable that the reported results represent approximately the true creatinine output for twenty-four hours.

**Case XV., A. D.** *Psychosis ; Dementia Præcox, Paranoid Form, 1st Group.* — Age, fifty-one years. Body weight, 59 kilos. Subject leads practically a normal life. Clinical examination of the urine revealed the presence of a few hyaline casts in each of the three specimens examined.

1906	March 24.	March 25.	March 26.
Quantity . . .	752 c.c.	795 c.c.	1095 c.c.
Specific gravity .	1.027	1.021	1.020
Total creatinine .	0.812 gm.	0.683 gm.	0.768 gm.

Average total creatinine elimination for three days, 0.754 gm.

**Case XVI., N. M.** *Psychosis ; Senile Dementia.* — Age, ninety-two years. Body weight, 63 kilos in both experiments. Subject rather decrepit, partly paralyzed, fat and flabby. Spends most of time in bed. A few hyaline casts were found in the clinical examination of the urine during the first observation. On account of the low creatinine elimination and the advanced age of patient, she was subjected to two observations.

1906.	March 24.	March 25.	March 26.
Quantity . . . . .	401 c.c.	305 c.c.	385 c.c.
Specific gravity . . .	1.029	1.030	1.028
Preformed creatinine .	0.548 gm.	0.460 gm.	0.445 gm.
Urine lost . . . . .	59 c.c.	30 c.c.	30 c.c.

Average preformed creatinine elimination for three days, 0.484 gm.

1906.	June 21	June 22.	June 23.	June 24.	June 25.
Quantity . . . . .	520 c.c.	495 c.c.	255 c.c.	445 c.c.	313 c.c.
Specific gravity . . .	1.021	1.025	1.030	1.026	1.027
Preformed creatinine .	0.415 gm.	0.748 gm.	0.376 gm.	0.410 gm.	0.313 gm.
Urine lost . . . . .	none	250 c.c.	none	none	none

Average preformed creatinine elimination for five days, 0.452 gm.

**Case XVII., M. D.** *Psychosis ; Manic Depressive, Depressed Form.* — Age, twenty-five years. Body weight, 85 kilos. Subject in fair physical condition. Great mental pressure of activity in consequence of which patient was continuously active. Menstruated the three days previous to beginning the experiment. Clinical examination of urine revealed nothing pathological.

1906.	March 24.	March 25.	March 26.
Quantity . . . . .	1875 c. c.	2175 c. c.	2450 c. c.
Specific gravity . . .	1.017	1.023	1.013
Preformed creatinine .	0.999 gm.	1.024 gm.	1.356 gm.

Average preformed creatinine elimination for three days, 1.126 gm.

**Case XVIII., H. S.** *Psychosis; Manic Depressive, Imbecilic Basis.* — Age, nineteen years. Body weight, 58 kilos. Subject in fairly good physical condition, but mentally much depressed and very inactive. No pathological findings in urine.

1906.	March 25.	March 26.	March 27.
Quantity . . . . .	488 c.c.	475 c.c.	455 c.c.
Specific gravity . . . .	1.028	1.030	1.028
Preformed creatinine . .	0.565 gm.	0.665 gm.	0.670 gm.

Average preformed creatinine elimination for three days, 0.633 gm.

**Case XIX., M.G.** *Psychosis; Manic Depressive, Circular Form.* — Age, sixty-five years. Body weight, 46 kilos. Subject physically fairly normal, active, mildly excited, undersized. Muscular activity above the normal. No pathological findings in the urine.

1906.	May 22.	May 23.	May 24.
Quantity . . . . .	2820 c.c.	2250 c.c.	2125 c.c.
Specific gravity . . . .	1.015	1.015	1.013
Preformed creatinine . .	0.716 gm.	0.599 gm.	0.545 gm.

Average preformed creatinine elimination for three days, 0.620 gm.

**Case XX., C. W.** *Psychosis; Dementia Præcox, Paranoid Form.* — Age, fifty-two years. Body weight, 76 kilos. Subject in good physical condition, mentally perfectly clear, rather stout, practically a normal individual. Clinical examination of the urine revealed nothing pathological.

1906.	May 22.	May 23.	May 24.
Quantity . . . . .	1575 c.c.	1925 c.c.	1370 c.c.
Specific gravity . . . .	1.016	1.015	1.018
Preformed creatinine . .	1.090 gm.	1.137 gm.	1.233 gm.
Urine lost . . . . .	25 c.c.	25 c.c.	none

Average preformed creatinine elimination for three days, 1.153 gm.

**Case XXI., A. H.** *Psychosis; Imbecility.* — Age, thirty-three years. Body weight, 68 kilos. Subject a normal individual except for slight mental defect.

1906.	May 22.	May 23.	May 24.
Quantity . . . . .	2490 c.c.	1753 c.c.	1350 c.c.
Specific gravity . . . .	1.015	1.016	1.017
Preformed creatinine . .	1.310 gm.	1.242 gm.	1.257 gm.

Average preformed creatinine elimination for three days, 1.269 gm.

**Case XXII., A. Q.** *Psychosis; Dementia Præcox, Hebephrenic Form.* — Age, forty-five years. Body weight, 49 kilos. Subject rather inert as a rule.

and suffering from a mild attack of erysipelas; at time of experiment she was in bed. Clinical examination of the urine revealed nothing pathological.

1906.	May 22.	May 23.	May 24.
Quantity . . . . .	1815 c.c.	1600 c.c.	1995 c.c.
Specific gravity . . . .	1.013	1.016	1.012
Preformed creatinine . .	0.826 gm.	0.997 gm.	0.833 gm.
Urine lost . . . . .	450 c.c.	720 c.c.	320 c.c.

Average preformed creatinine elimination for three days, 0.885 gm.

**Case XXIII., Miss R.** *A member of Nurses' Training School.* — Age, twenty-five years. Body weight, 52 kilos. Subject was just convalescing from typhoid fever and was considerably under normal body weight. Her muscular activity was very slight. The blood had previously given a good Widal reaction, and the urine had given a strong Diazo reaction. During the early stages of the fever the patient's urine had contained a large amount of albumen and many granular casts, but at the time of experiment there was only a faint trace of albumen and an occasional granular cast. During the second day of experiment patient's temperature was 40° C., due to an abscess on arm. This abscess was found not to contain *B. typhosus*.

1906.	Oct. 21.	Oct. 22.	Oct. 23.
Quantity . . . . .	1000 c.c.	750 c.c.	570 c.c.
Specific gravity . . . .	1.015	1.018	1.017
Preformed creatinine . .	0.667 gm.	0.583 gm.	0.543 gm.

Average preformed creatinine elimination for three days, 0.598 gm.

**Case XXIV., M. P.** *Psychosis; Melancholia.* — Age, fifty-nine years. Body weight, 40 kilos. Subject poorly nourished, mentally very much agitated, occasionally becoming so excited that she has to be locked in her room; spends much of the time walking. Clinical examination of the urine frequently revealed the presence of uric acid crystals in the sediment, and on one occasion several hyaline casts. Patient subjected to experiment during two different periods.

1906.	Nov. 2.	Nov. 3.	Nov. 4.
Quantity . . . . .	590 c.c.	630 c.c.	300 c.c.
Specific gravity . . . .	1.022	1.030	1.034
Preformed creatinine . .	0.424 gm.	0.558 gm.	0.436 gm.

Average preformed creatinine elimination for three days, 0.473 gm.

1906.	Nov. 17.	Nov. 18.	Nov. 20.	Nov. 21.
Quantity . . . . .	375 c.c.	430 c.c.	300 c.c.	190+ c.c. <sup>1</sup>
Specific gravity . . . .	1.030	1.037	1.036	1.036
Performed creatinine .	0.423 gm.	0.593 gm.	0.513 gm.	0.358 gm.

Average performed creatinine elimination for the four different days, 0.472 gm.

**Case XXV., S. S.** *Psychosis; Melancholia.* — Age, fifty-seven years. Body weight, 59 kilos. Subject poorly nourished, goes about wringing her hands and moaning, very much agitated. Clinical examination of urine revealed nothing pathological.

1906.	Nov. 19.	Nov. 20.	Nov. 21.
Quantity . . . . .	1100 c.c.	790 c.c.	650 c.c.
Specific gravity . . . .	1.018	1.022	1.021
Performed creatinine .	0.800 gm.	0.721 gm.	0.506 gm.

Average performed creatinine elimination for three days, 0.676 gm.

**Case XXVI., M. W.** *Psychosis; Melancholia.* — Age, forty-five years. Body weight, 48 kilos. Subject considerably agitated during observation, and spent much time walking about the halls. Her physical condition is as a rule excellent, but during this depression it dropped below the normal. Clinical examination of the urine revealed nothing pathological.

1906.	Nov. 6.	Nov. 7.	Nov. 8.
Quantity . . . . .	450 c.c.	700 c.c.	540 c.c.
Specific gravity . . . .	1.017	1.014	1.017
Performed creatinine .	0.439 gm.	0.436 gm.	0.554 gm.

Average performed creatinine elimination for three days, 0.476 gm.

The earlier view that the creatinine elimination is in large measure proportional to the body weight has been supplemented by more recent views, one of which assumes that the creatinine elimination is proportional to the active mass of protoplasmic tissue, and a still more recent modification of this view which has been advocated by Shaffer to the effect that the creatinine elimination is affected by the general tonus of the muscles. Obviously, cases where body weight, muscular activity, and general physical condition varied as widely as did the

<sup>1</sup> Patient very much excited on the fourth day, and a small amount of urine was lost. The urine for the 16th was also lost.



cases here reported furnish unusual opportunity for a comparison of the different factors possibly influencing the elimination of creatinine.

To render the results more comparable, they have all been collected in the following table:

	Age.		Body weight.	Height.	Girth.			Creatinine.	Creatinine coefficient.
	years.	kilos.			Shoulders.	Waist.	Hips.		
			metres.	metres.	metres.	metres.			
1	85	39.3	1.65	0.81	0.74	0.89	0.292	7.4	
*2	66	40.1	1.65	0.92	0.61	0.81	0.505	12.6	
*3	52	64.9	1.45	1.00	0.88	1.17	0.698	10.8	
4	45	48.3	1.65	0.98	0.75	0.96	0.786	15.8	
5	32	52.9	1.58	0.97	0.81	1.02	0.732	13.9	
6 a	39	61.2	1.65	1.06	0.81	1.07	0.863	14.1	
6 b	..	78.0	....	....	....	....	0.926	11.9	
6 c	..	74.0	....	....	....	....	0.843	11.3	
*7	30	41.7	1.50	0.92	0.69	0.95	0.565	13.5	
8 a	57	49.0	1.55	0.89	0.64	0.84	0.627	12.8	
8 b	..	48.0	....	....	....	....	0.776	16.2	
9	45	42.5	1.49	0.88	0.57	0.81	0.447	10.5	
10	63	64.3	1.68	0.96	0.70	1.09	0.748	11.6	
*11	31	51.1	1.60	0.89	0.57	0.97	0.765	15.1	
*12	47	43.8	1.57	0.95	0.69	0.89	0.823	18.8	
*13	50	46.5	1.64	0.92	0.69	0.89	0.973	20.9	
14	70	68.5	1.65	0.93	0.72	1.07	0.963	14.0	
*15	51	59.0	1.65	0.89	0.68	0.98	0.754	12.8	
16	92	62.6	1.50	1.03	0.99	1.08	0.464	7.4	
17	25	85.0	1.51	0.99	0.71	1.52	1.126	13.2	
18	19	58.0	1.71	0.91	0.76	0.99	0.633	10.9	
19	65	45.8	1.28	0.92	0.76	0.99	0.620	13.5	
20	52	75.9	1.50	1.01	0.85	1.20	1.153	15.2	
21	33	67.6	1.75	1.00	0.76	0.97	1.269	18.8	
22	45	49.0	1.59	0.81	0.69	0.94	0.885	21.8	
23	25	52.2	1.65	....	....	....	0.598	11.5	
24	59	39.9	1.16	0.92	0.66	0.92	0.473	11.9	
25	57	59.4	1.68	1.04	0.72	1.12	0.676	11.4	
26	45	47.6	1.50	0.95	0.75	0.98	0.476	10.0	
Average . . . . .							0.739	13.4	
* Reported as total creatinine.									

To the data for the creatinine elimination are added several body measurements, for not only were the body weights of these subjects taken, but with a view of giving mathematical expression to the general physical condition, the height and the girth about the shoulders, waist, and hips were recorded. In all instances the measurements were taken over a thin cotton wrapper, with no clothing underneath. They represent, therefore, approximately the measurements of the naked body.

In the last column of the table has been recorded the "creatinine coefficient." This is determined by dividing the weight of creatinine in milligrams by the body weight, and it corresponds, therefore, to the number of milligrams of creatinine excreted per kilo of body weight. In the table no attempt was made to classify the patients according to age, weight, height, or creatinine elimination. They are recorded chronologically as they were studied in the laboratory.

**Creatinine coefficient.** — The creatinine coefficients which have been commonly observed in men by Folin and others range from 18 to 30 mgm., while Shaffer has pointed out<sup>1</sup> that with women in poor physical condition the creatinine coefficient may be very much lower than this. With the subjects here reported, the coefficients are unusually low, in some instances remarkably low, and in but two instances are they above 20. The previously reported<sup>2</sup> creatinine coefficients of women vary from 9.4 to 21.7 and these, indeed, were all with insane patients. Of these coefficients the lowest, 9.4, was obtained with a woman patient weighing 80.3 kilos, and the highest, 21.7, was obtained on a patient weighing 50.83 kilos. Unfortunately, the body measurements are not reported, and hence the general physical condition regarding the proportion of adipose tissue cannot well be estimated. The large amount of subcutaneous fat present in women would result in a materially smaller proportion of active protoplasmic tissue per kilo of body weight, and hence we would expect that if the creatinine elimination is proportional to the active protoplasmic tissue, the coefficients would all be smaller than with men of equal body weight by virtue of the large amount of inert adipose tissue which tends to increase the body weight and decrease the proportion of active protoplasmic tissue. Thus the general observation that women with relatively low body weights and larger proportions of adipose tissue excrete relatively low amounts of creatinine would be

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

<sup>2</sup> See p. 378.

in harmony with the view that the creatinine elimination is directly proportional to the active mass of protoplasmic tissue. A subsequent observation made in this series of experiments, however, is not in harmony with this hypothesis. By reference to the figures in the table, it will be seen that from the body weight, height, and girths an estimate as regards the approximate amount of adipose tissue can be made. Thus in Case 17, where the body weight was 85 kilos, it is seen that the height was but 1.5 metres, and consequently there must have been a large amount of adipose tissue. This large body weight results in a low creatinine coefficient, 13.2, although there was one of the largest twenty-four-hour excretions of creatinine observed in any of the cases. A comparison of the body weights and the creatinine excreted shows that in nearly all instances where there were large body weights the creatinine excretion was large. Conversely, where there was a small body weight, the creatinine is, as a rule, small.

The smallest average amount was that of Case 1, namely, 0.292 gm. We believe this to be the smallest daily average excretion of preformed creatinine thus far observed. In this case the body weight was extremely low, 39.3 kilos, and the height 1.65 metres. In Case 24, where the body weight was essentially the same, 39.9 kilos, and the height was 1.16 metres, the creatinine excretion was, however, over 60 per cent greater. Furthermore, in the second case there must have been a larger proportion of adipose tissue.

Taking the girth at the waist as a general index of the proportion of adipose tissue, it is seen that those patients with the largest girth, namely, Cases 16 and 3, had low creatinine coefficients; but here, again, this measurement alone will not suffice for a true index of the physical condition of the subject, and small body weight may likewise result in an extremely low coefficient, as is shown clearly in Case 1.

Muscular development independent of the adipose tissue is in general greater the taller the individual, and hence the height rather than the body weight might be taken as perhaps more nearly an index of the mass of muscle tissue, and yet the figures in the table show no uniformity regarding the ratio between height and total creatinine excretion.

It is evident, then, that the factors influencing the creatinine output are not clearly known, and we can but examine the results and study the influence of the various possible factors.

**Influence of age.** — Two of the cases, namely, 1 and 16, were of advanced age, eighty-five and ninety-two years respectively, and both of

these cases are significant as having unusually low coefficients. Compared with women of like body weight but younger, the excretion of creatinine is invariably low. Case 16, with a body weight of 62.6 kilos, excreted considerably less creatinine (0.464) than the younger woman, Case 18, who with a smaller body weight, 58.0, excreted considerably more (0.633).

That we can actually ascribe an influence of age as such is hardly probable, for with advancing years, especially over seventy years, there is a loss of adipose tissue (which according to one hypothesis should raise the creatinine coefficient) and a loss of muscular tonus which according to another hypothesis should decrease the creatinine output. Although there were marked differences in body weight in the two cases of aged patients (Nos. 1 and 16), there were likewise marked differences in the height and other measurements, since Case 1, although 0.15 metre taller than Case 16, was a much smaller woman so far as all the girths are concerned.

From the measurements of girths, it would appear that in these two cases, at least, there is but a slight influence of the adipose tissue on the creatinine elimination, for, in all probability, there was a larger proportion of active protoplasmic tissue in Case 1, while in the other case there was a considerable amount of fat in addition. An examination of the report of the physical condition of these two patients shows that in Case 1 the subject was in bed, feeble, withered, and very inactive, while Case 16, although distinctly fat, flabby, and decrepit, was partly paralyzed and also spent most of the time in bed. However, Case 16 was somewhat more active physically, and could be fairly stated to have a greater muscular tonus than did Case 1. This would imply, then, that the hypothesis of Shaffer to the effect that the creatinine is a measurement, not only of the active protoplasmic tissue, but also of the tonus or activity of muscles, is true.

**Creatinine coefficients in the same individual with marked differences in body weight.** — The problem of the influence of body weight upon the creatinine excretion, and consequently the creatinine coefficient, is studied only with difficulty where different individuals of different body weights are compared, for the proportion of fat, skeleton, and muscular or protoplasmic tissue varies widely in individuals. In one of the cases here studied, it was possible to note the creatinine excretion at different times of the year when the subject had a markedly different body weight. Thus, with Case 6, the body weight at one test was 61.1 kilos, and the average excretion of preformed creatinine

0.86 gram. During another test the body weight was 78 kilos and the average excretion 0.93, while in another it was 74 kilos with an average excretion of 0.84. In an earlier experiment made with this same subject and reported previously,<sup>1</sup> the creatinine excretion of this subject was determined with a body weight of 47.7 kilos. At this weight the excretion was 0.57 gm. of preformed creatinine. The creatinine coefficients for these various tests were, with a body weight of 47.7, 12.0; with a body weight of 61.1, 14.1; with a body weight of 74, 11.3; with a body weight of 78, 11.9.

This subject periodically fasts as a result of religious delusions, and, as has been shown by one of us,<sup>2</sup> after a period of inanition, there may be a very considerable storage of nitrogenous material, indeed, a storage considerably greater than that actually lost during the period of inanition, yet it is highly probable that the major portion of the fluctuations in weight observed in this patient were due to fat and water and not to protoplasmic tissue. On this assumption, therefore, it is seen that so far as this case is concerned, there is little evidence to show that the active mass of protoplasmic tissue determines the creatinine output, since the fat and water stored resulted in an apparent increase of the creatinine elimination, which was almost in proportion to the increment in body weight.

So far as we are aware, no instance of the creatinine determination of the same subject with such a wide difference in body weight has thus far been reported.

So far as the question of muscular tonus or general strength of the body is concerned, but little evidence is presented by this case. During the four periods of observation here reported, the general muscular strength did not vary markedly. Even after the prolonged fast the patient was able to walk about the ward and sit up. It was her common custom to sit for the greater portion of the day sewing and reading. Furthermore, the values taken for the creatinine elimination at the body weight of 47.7 kilos were taken after the period of fasting had ceased, and hence, in all probability, did not represent the maximum condition of fatigue. It has been found by a number of experiments during fasting that strength rapidly returns after the ingestion of food. It would appear, then, that it is necessary to assume that the muscular activity and tonus were very much less when the body weight was low than when high, in order to account for the con-

<sup>1</sup> BENEDICT and DIEFENDORF: This journal, 1907, xviii, p. 369.

<sup>2</sup> Carnegie Institution of Washington, Publication No. 77, 1907.

stancy in the creatinine coefficient. This is distinctly contrary to the evidence here presented, and judging from this one case, but little stress can be laid upon muscular tonus as a factor influencing creatinine excretion in persons not suffering from distinct pathological lesions. It should be stated, however, that the second experiment with Case 8, where the creatinine coefficient was much higher than in the first, the patient was possibly in somewhat better physical condition.

#### SUMMARY.

The conclusions reached in these experiments may then be summarized as follows:

1. The creatinine excretion of women is, in general, much lower than that of men.
2. While the excretion is, in general, proportional to the body weight, this is not always the case.
3. Age appears to play an important rôle in the excretion of creatinine, since elderly people excrete less creatinine than younger people, with essentially the same body weight.
4. The evidence furnished by the subject whose body weight varied considerably at times implies that the creatinine excretion is proportional to the body weight and not to the active mass of protoplasmic tissue.

## THE DETERMINATION OF CREATINE AND CREATININE.<sup>1</sup>

BY FRANCIS GANO BENEDICT AND VICTOR CARYL MYERS.

[From the Chemical Laboratory of Wesleyan University.]

THE physiological problems involved in the study of creatine and creatinine have resulted in the development of the colorimetric method for the determination of these two bodies. Based upon the Jaffé reaction, Folin<sup>2</sup> has described a simple colorimetric method for the determination of preformed creatinine. Creatine does not give this reaction, but by heating the urine with hydrochloric acid for a considerable length of time, the creatine may be in large part converted to creatinine, and thus the colorimetric method may be applied for determining creatine. As Folin recommends, the urine must be heated three hours on the water bath with hydrochloric acid to insure the conversion of the creatine to creatinine. Where a large number of determinations are to be made, the length of time required for this conversion is a serious factor, and hence it seemed desirable to so modify the method of procedure as to hasten if possible this conversion.

It is the purpose of this article to describe a modification of the Folin method which will enable the complete conversion of creatine to creatinine in a much shorter time than the usual three hours.

In connection with the series of experiments which are reported in the two papers accompanying this article, much evidence was secured regarding the reverse chemical transformation, *i. e.*, the conversion of creatinine to creatine, and the second part of this paper has to deal with the experimental evidence throwing light on this point.

### THE CONVERSION OF CREATINE TO CREATININE.

The long time required for the conversion of creatine to creatinine when heated with hydrochloric acid on the water bath suggested the

<sup>1</sup> This research was carried out with the aid of a grant from the Carnegie Institution of Washington. The analyses were made in the laboratory of the Connecticut Hospital for the Insane.

<sup>2</sup> FOLIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223.

desirability of increasing the temperature of the reaction by heating the liquid in an autoclave. Since an increase in temperature hastens chemical action, it was thought that the time of conversion could be materially shortened by this procedure. A preliminary series of experiments was made with some extract of beef, which contains both creatine and creatinine. A dilute solution of extract of beef was made, and the preformed creatinine determined by the Folin method, *i. e.*, without the preliminary heating with hydrochloric acid. A large number of samples of this dilute solution were then treated with hydrochloric acid and heated in the autoclave at a temperature of about  $117^{\circ}$  C. for periods varying from fifteen minutes to three hours. In this preliminary series of experiments it appeared that the maximum creatinine formation in the dilute beef extract was obtained in heating for fifteen minutes in the autoclave.

A comparative test made with the same beef extract solution in which the heating was carried out on the water bath showed that at least two hours' heating was necessary for the reaction to approach completion. At the end of three hours the colorimetric readings were exactly the same as when the solution was heated in the autoclave.

**Experiments with pure creatine.**—Dr. Folin kindly furnished us with a small amount of pure creatine, and a series of experiments was made with a solution of this product. Crystallized creatine contains one molecule of water of crystallization, and the relation between the molecular weights of crystallized creatine and creatinine are such that 0.5 gm. of crystallized creatine corresponds to 0.3794 gm. of creatinine. A solution was prepared containing 500 mgm. of crystallized creatine in 750 c.c. of distilled water; several 10 c.c. portions of this solution were heated each with 10 c.c. of normal hydrochloric acid in the autoclave. The results are as follows:

*Fifteen minutes.*—At the end of fifteen minutes three flasks were removed from the autoclave and tested in the usual manner.

1. The 10 c.c. of original creatine solution, to which 10 c.c. of acid had been added, was diluted to 350 c.c., after 15 c.c. of saturated picric acid solution and 10 c.c. of a 10 per cent sodium hydroxide solution had been added, and the color allowed to develop for three and one-half minutes. The average of a large number of readings with the colorimeter was 11.2 millimetres. This corresponds to 0.3798 gm. of creatinine in the solution instead of 0.3794 gm.

2. The 10 c.c. were diluted to 250 c.c., and the several readings averaged 8.2 mm., corresponding to 0.3704 gm. of creatinine.



3. The contents of this flask were likewise diluted to 250 c.c., and the average reading was 8.1 mm., equivalent to 0.3750 gram of creatinine.

*Thirty minutes.* — At the end of thirty minutes three flasks were diluted each to 250 c.c., and the averages of the numerous readings were 8.3, 8.2, and 8.2 mm. on the three flasks respectively.

*One hour.* — The three flasks were diluted to 250 c.c., and the average of the readings was 8.2 mm. on each of the three flasks.

*One and a half hours.* — Two flasks were removed at this period and diluted to 250 c.c. The average reading on both flasks was 8.2 mm.

*Two hours.* — The three flasks removed at the end of two hours were likewise diluted 10 c.c. to 250 c.c., and the average reading on all three flasks was 8.2 mm.

No color reaction was obtained before heating with acid, thus indicating the absence of creatinine.

The results therefore show that heating fifteen minutes in the autoclave at 117° C. resulted in as complete conversion of creatine to creatinine as did heating under the same conditions for two hours.

A comparative test was made by heating the creatine solution with acid on the water bath, as is ordinarily done with the Folin method of determining creatinine in urine. The results are as follows:

*Thirty minutes.* — At the end of thirty minutes three flasks were diluted 10 to 250 c.c., and the averages of the readings on the three flasks were 11.2, 11 and 11.0, 2 mm. A reading of 11.2 mm. corresponds to 0.2712 gm. of creatinine instead of 0.3794 required.

*One hour.* — Three flasks diluted at the end of an hour from 10 to 250 c.c. gave an average for the three flasks of 8.8 mm., corresponding to 0.3452 gm. of creatinine.

*One and a half hours.* — The three flasks removed at the end of one and a half hours gave average readings of 8.7, 8.7, and 9.00 mm. respectively.

*Two hours.* — At the end of two hours the averages of all the readings on the three flasks were 8.3, 8.3, and 8.5 respectively.

*Three hours.* — The three flasks gave average readings of 8.4, 8.4, and 8.5 mm. respectively, the reading 8.4 corresponding to 0.3616 gm. creatinine.

A comparison of the readings obtained when heating the creatine solution on the water bath with those obtained when the solution was heated in the autoclave shows that the reaction apparently is not absolutely complete at the end of three hours when heated in the water bath, although it approaches completeness in about two hours. On the other hand, it is complete at the end of fifteen minutes when heated in the autoclave.

No explanation is at present apparent as to why the conversion of the creatine in aqueous solution is not as perfect at the end of three hours when compared with the liquid heated in the autoclave, as was the conversion of the creatine in the beef extract solution, which, it will be remembered, was completely converted by heating three hours on the water bath.

It is conceivable that some of the normal urinary constituents might interfere with or delay the conversion of creatine to creatinine, and hence, as a final test of the efficiency of this method, 200 c.c. of normal urine from a healthy man was mixed with 100 c.c. of the creatine solution, and then the total creatinine determination after heating with acid in the autoclave was made.

The amount of preformed creatinine in the urine was first determined and found to be 0.103 gm. The amount of creatinine that would result from the conversion of the creatine in 100 c.c. of the creatine solution is 0.049 gm., or a total in the mixture (300 c.c.) of 0.152 gm. The creatinine in the mixture before heating with acid was found to be 0.107 gm., as against 0.103 found in the undiluted urine. After conversion, the total creatinine in the mixture was found to be 0.152 gm., or the sum of the preformed creatinine plus the creatine added in the solution.

The mixture of normal urine and creatine solution was tested to note the rate of conversion of the creatine to creatinine when heated in the autoclave. Nine flasks were placed in the autoclave; three were removed at the end of fifteen minutes, three at the end of thirty minutes, and three at the end of forty-five minutes. The flasks when diluted 10 c.c. to 250 c.c. all gave the same average reading, *i. e.*, 8.0 mm.

It is thus apparent that when either pure creatine solution, dilute beef extract solution, or normal urine to which a pure creatine solution has been added, is heated with hydrochloric acid at 117° C. in the autoclave, there is a complete conversion of the creatine to creatinine. Furthermore, there is no apparent influence of the normal urinary constituents on the degree or rate of this conversion.

The tests with pure creatine solution would imply that the method used in this laboratory is correct, and that the values for creatine and creatinine reported in the papers on the Elimination of Creatinine in Women<sup>1</sup> and the Elimination of Creatine<sup>2</sup> are based upon standard solutions which yield theoretical results with pure crystallized creatine.

<sup>1</sup> BENEDICT and MYERS: This journal, 1907, xviii, p. 377.

<sup>2</sup> *Ibid.* p. 406.

**The conversion of creatine to creatinine on a large scale.** — The success attending the use of the autoclave for the conversion of small quantities of creatine to creatinine lead to a series of experiments to convert larger amounts of creatine to creatinine. The commercial preparation of extract of beef contains considerable amounts of creatine, and accordingly 22 gm. of extract of beef were dissolved in water, 27 c.c. of concentrated hydrochloric acid added, and the whole diluted with water to 130 c.c. The solution was then heated to 117° C. in the autoclave for forty-five minutes.

A determination of the total creatinine showed that the 22 gm. of the extract contained 1.1 gm. of total creatinine, a portion of which had been present in the extract as creatine.

When 5 c.c. portions of the digested solution were again heated with 10 c.c. of normal hydrochloric acid in the autoclave, it was found that there was no further increase in the amount of creatinine, and hence that all the creatine had been converted. A preliminary test had shown that there was present in the beef extract twice as much creatine as creatinine.<sup>1</sup>

Thus, from this experiment and from the general action of creatine with hydrochloric acid in the autoclave, it would appear that not only are the small quantities of creatine converted to creatinine, but that the method is applicable for the preparation of creatine on a large scale.

Grindley and Woods<sup>2</sup> have found that when meat juice, containing practically no creatinine, is allowed to stand in the presence of picric acid and sodium hydroxide, a red color develops indicating a conversion of creatine to creatinine. This experiment was tried with a solution of chemically pure creatine and the same phenomenon observed, the depth of the color at the end of a week indicating that over half of the creatine had been changed to creatinine.

**The conversion of creatinine to creatine.** — It has frequently been observed in certain samples of urine, especially when the reaction was alkaline, that there is a tendency for creatinine to become converted to creatine. While not definitely proven, it has been commonly supposed that this action is due to bacteria, and hence it is customary to preserve the urine by the addition of chloroform or by the use of a solution of thymol in chloroform, 1 : 10.

The procedure that has thus far given the best satisfaction is as follows :

<sup>1</sup> See GRINDLEY and WOODS: *Journal of biological chemistry*, 1907, ii, p. 309.

<sup>2</sup> *Ibid.*

The urine should be placed in a bottle containing 5 c.c. of the thymol solution for each 1000 c.c. of urine. The urine should be, if possible, excreted directly into the bottle with the preservative. While the chloroform solution of thymol retards the destruction of the creatinine and its conversion to creatine, it nevertheless happens that frequently even with this preservative there may be a loss of creatinine after standing. Hence for the most accurate work analyses should be made as soon as possible after the urine is collected. The tendency for creatinine to change to creatine, and the tendency for both materials to undergo decomposition are well shown by the following series of experiments.

The urine from a healthy man was collected for six hours, and the creatine and creatinine determinations made. In the 700 c.c. of slightly acid urine with a specific gravity of 1.015 there was found 0.481 gm. of creatinine, but no creatine was present. Five portions of this urine were treated in the following ways :

- a. 80 c.c. of urine + 2 c.c. of concentrated hydrochloric acid.
- b. 130 c.c. of urine + 2 c.c. of concentrated hydrochloric acid + 5 c.c. of chloroform.
- c. 120 c.c. of urine + 5 c.c. of ammonium hydroxide.
- d. 120 c.c. of urine + 5 c.c. of chloroform.
- e. No preservative was added.

The samples were allowed to stand in the laboratory at average room temperature. One week later, creatinine determinations were again made, and no loss of creatinine or conversion to creatine was noted. In the urines to which concentrated hydrochloric acid had been added uric acid crystals were precipitated. A white precipitate (phosphates) was found in the urine to which ammonium hydroxide had been added. The urine to which no preservative had been added was fermented, but there was no evidence of a disintegration of the creatinine.

Nine weeks later determinations were again made with the following results:

a. Urine + hydrochloric acid.	
Creatinine 0.431 gm.	90.4 per cent of the total.
Creatine 0.050 gm.	9.6 " "

The creatine is expressed in terms of creatinine, and it is thus apparent that at the end of this time about 10 per cent of the preformed creatinine had been converted to creatine. The sum of the

creatinine and creatine showed that there was no absolute loss of material.

*b.* Urine + hydrochloric acid + chloroform.

Creatinine 0.446 gm.	93 per cent.
Creatine 0.35 gm.	7 " "

Here, again, there was a conversion of a small amount of the creatinine to creatine, although the total quantity of creatinine remained unchanged.

*c.* Urine + ammonium hydroxide.

Creatinine 0.383 gm.	80 per cent.
Creatine 0.035 gm.	7 " "
Lost . . 0.063	13 " "

In the strongly alkaline urine, therefore, four fifths of the original amount of preformed creatinine was found in the urine. 7 per cent of the original amount was in the form of creatine, and 13 per cent was lost.

*d.* Urine + chloroform.

Creatinine 0.418 gm.	87 per cent.
Creatine 0.049 gm.	8 " "
Lost . . 0.014	5 " "

The urine was found to be neutral in reaction.

*e.* No preservative added.

Creatinine 0.077 gm.	16 per cent.
Creatine 0.017 gm.	4 " "
Lost . . 0.387	80 " "

The urine was found very strongly alkaline, and the creatinine was in large part destroyed. It is of interest that but a small amount of creatine was found in the solution. A Gram's stain of the bacteria in the urine showed many varieties, both Gram positive and Gram negative, cocci and bacilli. In a hanging drop preparation many forms were still found to be motile. A number of very large refractile cocci, frequently in chains, were also noted. In the stained specimen there were many large Gram cocci, and it is probable that these were micrococci ureae, the chief agent in the ammoniacal fermentation of the urine and in the production of ammonium carbonate from urea.

Several samples of urine which had been found to contain preformed creatine were allowed to stand for several weeks, and then determinations of creatine and creatinine again made.

Two samples from Mrs. M. P., both preserved with chloroform, gave results as follows :

	Nov. 2, 1906.	Feb. 27, 1907.
Preformed creatinine . . .	0.424	0.292
“ creatine . . .	<u>0.152</u>	<u>0.245</u>
Total creatinine . . .	0.576	0.537 = 0.039 gm. lost.
	Nov. 3, 1906.	Feb. 27, 1907.
Preformed creatinine . . .	0.558	0.359
“ creatine . . .	<u>0.225</u>	<u>0.264</u>
Total creatinine . . .	0.783	0.623 = 0.150 gm. lost.

Three specimens of urine which had been preserved with the thymol-chloroform solution gave results as follows :

Mr. F. R.	Dec. 22, 1906.	Feb. 27, 1907.
Preformed creatinine . . .	0.810	0.810
“ creatine . . .	<u>0.170</u>	<u>0.396</u>
Total creatinine . . .	1.206	1.206

Preserved with thymol-chloroform.

Mrs. M. P.	Dec. 27, 1906.	Feb. 27, 1907.
Preformed creatinine . . .	0.441	0.302
“ creatine . . .	<u>0.088</u>	<u>0.227</u>
Total creatinine . . .	0.529	0.529

Preserved with thymol-chloroform.

Mrs. McL.	
Total creatinine	Dec. 20, 1906 = 1.006
“ “	Feb. 27, 1907 = <u>0.901</u>
Creatinine lost . . . . .	= 0.105

Chloroform is unsatisfactory as a preservative, and even the use of thymol-chloroform does not insure the absence of the conversion of creatinine to creatine, or indeed the loss of creatinine, although all the samples of urine constantly gave an acid reaction.

But little regularity can be observed in the properties of creatine and creatinine in human urine. In general the conversion of creatinine to creatine seems to be due to chemical action, or possibly to the action of enzymes in the urine. When a urine is allowed to stand, alkaline fermentation takes place, and the creatinine sooner or later almost completely disappears. Furthermore the evidence from alkaline urines is not conclusive to show that creatine is an intermediate

product. The conversion of creatinine to creatine even in the presence of chloroform and thymol shows that this conversion is not a bacteriological process. It is obvious that much experimenting must be carried out before the problem is wholly clarified. In general it would appear that there is more likely to be a complete loss of creatinine in a urine that is alkaline. Whatever the cause of the conversion of creatinine to creatine, in any determination of either of these compounds, a factor which must never be left out of consideration is that of the influence of bacteria. Consequently emphasis cannot be too strongly laid upon the importance of making determinations of creatine and creatinine in urine as soon as possible after it has been voided. A preservative may delay action, but accuracy demands immediate analysis.

## THE ELIMINATION OF CREATINE.<sup>1</sup>

BY FRANCIS GANO BENEDICT AND VICTOR CARYL MYERS.

[From the Chemical Laboratory of Wesleyan University.]

WITH the appearance of the colorimetric method for determining creatinine described by Folin<sup>2</sup> the investigation of the relation of creatinine to metabolism has received a great impetus.

This method not only permits the determination of creatinine, but since, as Folin has shown, the small quantities of preformed creatine existing in the urine may readily be wholly converted to creatinine by heating with hydrochloric acid on a water bath for three hours, the method may also be employed for determining the creatine. Thus one sample of urine is examined directly for the quantitative amount of preformed creatinine. A second sample is heated with acid, the creatinine converted to creatine, and then the total creatinine determined. The difference is obviously the amount of creatine measured in terms of creatinine, of which 1 mgm. corresponds to 1.16 mgm. of creatine.

In his original discussion of the subject Folin pointed out that in normal urine there was frequently no creatine, although occasionally measurable amounts may be found. Of the several 10 c.c. samples of urine that he analyzed, one contained 5.3 mgm. of creatinine and 99 mgm. of creatine, another 20.25 mgm. of creatinine and 1.1 mgm. of creatine, and another 17.4 mgm. of creatinine and 0.7 mgm. of creatine. Usually the quantity of preformed creatine in the urine has been neglected by observers when using the Folin method.

In connection with the fasting experiments made in this laboratory<sup>3</sup> it was found that during inanition considerable quantities of

<sup>1</sup> This research was carried out with the aid of a grant from the Carnegie Institution of Washington. The analyses were made in the Laboratory of the Connecticut Hospital for the Insane.

<sup>2</sup> FOLIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223.

<sup>3</sup> BENEDICT: Carnegie Institution of Washington, 1907, Publication No. 77.



performed creatine appeared in the urine. This performed creatine was explained on the assumption that during fasting there was a material amount of "flesh" katabolized, and in the katabolism of the flesh the creatine which had formerly been held in the flesh was liberated and excreted as such in the urine. In the fasting experiment with the fasting woman reported by Benedict and Diefendorf,<sup>1</sup> an excretion of creatine was likewise observed.

Shaffer in a private communication has stated that he has observed the excretion of performed creatine in a number of pathological cases.

Folin in a private communication also reports creatine in the urine of a fasting man.

In connection with an extended study of the creatinine elimination in women,<sup>2</sup> considerable amounts of creatine were occasionally observed in the urine. It is the purpose of this article to report the cases in which the excretion of performed creatine occurred. In addition to the experiments with women several were made with men.

**Methods.**—The analytical methods employed in this study were those used in the preceding paper. Pressure of other work prevented the complete analyses of the urine, including the partition of the nitrogen so successfully carried out by Folin.

#### EXPERIMENTS.

The experiments included the study of twenty-four-hour quantity of urine from a number of women subsisting on a creatine-free and creatinine-free diet. In general the urine for three consecutive days was examined. The studies were further supplemented by an examination of the urine of several patients in which considerable amounts of performed creatine were found. Unfortunately the mental or physical states of these patients in many instances precluded the accurate collection of the twenty-four-hour urine.<sup>3</sup>

Unless otherwise specified, the diets used were creatine-free and creatinine-free. The subjects were always on the diet for one day prior to taking the first sample.

<sup>1</sup> BENEDICT and DIEFENDORF: This journal, p. 362.

<sup>2</sup> BENEDICT and MYERS: This journal, p. 377.

<sup>3</sup> The subjects of these experiments were patients of Drs. W. E. FISHER, A. B. COLEBURN, J. H. MORRISON, and A. C. THOMAS, of the Connecticut Hospital for the Insane at Middletown, Connecticut. We are under obligations to these gentlemen for their kind co-operation in the investigation.

1. **Mrs. D. H.**<sup>1</sup>— Eighty-five years old. Weight, 39 kilos ; height, 1.65 metres. Very inactive, in bed. Exclusive milk diet.

This patient excreted 64, 53, 65, 96, 77, and 40 mgm. of creatine on six consecutive days. The average daily creatinine excretion was 292 mgm.

2. **Mrs. M. N.**<sup>2</sup>— Ninety-two years old. Weight, 63 kilos ; height, 1.50 metres. Partially paralyzed, in bed most of the time. This patient excreted 67, 53, 57, 139, and 64 mgm. of creatine on five consecutive days. The average daily creatinine excretion was 464 mgm.

3. **Miss R.**<sup>3</sup>— Twenty-five years old. Weight, 52 kilos ; height, 1.65 metres. Convalescing from typhoid fever ; muscularly inactive. Trace of albumen in the urine.

This subject excreted 80, 66, and 82 mgm. of creatine on three successive days. The average daily creatinine excretion was 598 mgm.

4. **Mrs. M. P.**<sup>4</sup>— Fifty-nine years old. Weight, 40 kilos ; height, 1.16 metres. Poorly nourished ; much agitated.

This subject in one experiment excreted 176, 261, and 88 mgm. of creatine on three successive days. The average daily creatinine excretion was 473 mgm.

In a later experiment the creatine excretion was 133, 132, 188, and 81 mgm. on four days. During this second experiment the average daily preformed creatinine excretion was 473 mgm. Owing to the excited state of the patient during the second experiment, the urine was not collected on consecutive days.

5. **Mrs. S. G.**<sup>5</sup>— Fifty-seven years old. Weight, 59 kilos ; height, 1.68 metres. Rather poorly nourished and very much agitated.

In a three-day experiment this subject excreted 126, 68, and 50 mgm. of creatine on three consecutive days. The average daily creatinine excretion was 676 mgm.

6. **Mrs. M. W.**<sup>6</sup>— Forty-five years old. Weight, 48 kilos ; height, 1.5 metres. Very much agitated. On the three days of this experiment the creatine excretion amounted to 220, 167, and 116 mgm. The average daily creatinine excretion was 476 mgm.

7. **Mrs. H. S.** *Psychosis ; Dementis Præcox, Catatonic Form.*— Thirty years old. Weight, 42 kilos ; height, 1.82 metres. During the first observation patient was in an almost complete catatonic stupor. Very much

<sup>1</sup> See Case I., this journal, p. 381.

<sup>2</sup> See Case XVI., *ibid.*

<sup>3</sup> See Case XXIII., *ibid.*

<sup>4</sup> See Case XXIV., *ibid.*

<sup>5</sup> See Case XXV., *ibid.*

<sup>6</sup> See Case XXVI., *ibid.*

under nourished; activity practically nil. Clinical findings in urine are reported in the table.

1906	Dec. 8.	Dec. 9.	Dec. 10.
Volume . . . . .	365 c.c.	400 c.c.*	470 c.c.
Specific gravity . . . .	1.015	1.020	1.010
Reaction . . . . .	alkaline	acid	alkaline
Sugar . . . . .	trace	faint trace	none
Albumen . . . . .	trace	trace	about 1 per cent.
Preformed creatinine	0.072 gm.	0.239 gm.	0.029 gm.
Creatine . . . . .	0.088 gm.	0.118 gm.	0.019 gm.

Of especial significance is the fact that in the alkaline urines there are but small amounts of both creatinine and creatine, while on the day when the urine was acid both constituents are present in considerable amounts.

In the second experiment the body weight was 49 kilos.

1907.	Feb. 8.	Feb. 9	Feb. 10.
Volume . . . . .	220 c.c.	315 c.c.	220 c.c.
Specific gravity . . . .	1.032	1.033	1.030
Sugar . . . . .	faint trace	none	none
Preformed creatinine . .	0.330 gm.	0.607 gm.	0.400 gm.
Creatine . . . . .	0.122 gm.	0.065 gm.	0.073 gm.

In this second period the urine was filled with amorphous urates and peculiar needle-like uric acid crystals. No albumen was found.

8. **Miss A. McI.** *Psychosis; Dementia Præcox, Catatonic Form.* — Eighteen years old. Weight, 44 kilos; height, 1.58 metres. Well nourished, but very inactive.

On the three days of this experiment the subject excreted 153, 77, and 155 mgm. of creatine. The average creatinine excretion for the three days was 689 mgm.

9. **Mr. F. R.** *Psychosis; Dementia Paralytica.* — Nineteen years old (!). Weight, 61 kilos; height, 1.64 metres.

The creatine excretion for three days was 110, 197, and 80 mgm. respectively. The average daily creatinine elimination was 947 mgm.

10. **Mr. H. G. M.** *Psychosis; Dementia Præcox, Catatonic Form.* — Twenty-six years old. Weight, 28.6 kilos (!); height, 1.79 metres. On admission to the hospital (January, 1905), his weight was 51 kilos. The patient died on the third day of the experiment, apparently from inanition. Though he had been suffering from pulmonary tuberculosis, the disease was not sufficiently advanced to have been the sole cause of death.

From the height and body weight it is apparent that the patient was greatly emaciated. At the time of entering the hospital and at

death the patient was suffering from chronic nephritis. At the autopsy chronic passive congestion was found in the kidneys, and at that time a specimen of urine was drawn from the bladder. All the samples of urine were acid. Segal's test for acetone was negative.

On the first day of the experiment 150 c.c. of urine were collected, and it was estimated that this was one fourth of the total for the day. The excretion of creatine for the day was 376 mgm. The creatinine excretion was 324 mgm.

On the second day there were 415 c.c. (one half the twenty-four-hour quantity) collected. The creatine for the day was 469 mgm., and the creatinine excretion amounted to 369 mgm.

On the third day 90 c.c. of urine was removed from the bladder at the autopsy. In this amount there was found 61 mgm. of creatine and 51 mgm. of creatinine.

The striking feature of this case is the enormous relative amount of creatine eliminated. It was not possible to obtain the total twenty-four-hour quantities, but he was under the charge of a nurse of long experience, and the total quantities were probably quite closely estimated.

11. **Miss K. O'C.** *Psychosis; Dementia Præcox, Catatonic Form.*—Sixteen years old. Weight, 43 kilos; height, 1.55 metres. Patient was in an almost complete catatonic stupor, and so filthy that it was entirely impossible to save the twenty-four-hour quantities. In consequence of her condition patient was very inactive. She declined food, and had to be tube-fed a portion of the time, in consequence of which she was poorly nourished. One day 155 c.c. of urine, specific gravity 1.028, were collected. In this specimen 80 mgm. of creatine and 330 mgm. of creatinine were found.
12. **Miss B. D.** *Psychosis; Toxic Delirium.*—Thirty-six years old. Weight, 49 kilos; height, 1.65 metres. The patient was poorly nourished, and spent a considerable portion of the time in bed. On account of her poor physical condition, she was allowed a small quantity of beefsteak for dinner on the four different days she was on diet. At noon on the day before the experiment she also had a small amount of chicken. Nothing pathological was found in the clinical examination of the urine.

	1907.	Feb. 16.	Feb. 17.	Feb. 18.
Volume . . . . .		636 c.c.	695 c.c.	420 c.c.
Specific gravity . . . .		1.025	1.022	1.028
Preformed creatinine . .		0.696 gm.	0.722 gm.	0.671 gm.
Creatine . . . . .		0.154 gm.	0.133 gm.	0.139 gm.

13. **Mr. G. M.** *Psychosis ; Dementia Præcox, Paranoid Form, 2d Group.*— Fifty-seven years old. Weight, 50 kilos ; height, 1.63 metres. Considerable amounts of albumen and a few granular casts were found in the urine of each day. On the three days of the experiment there were found 155, 97, and 114 mgm. of creatine respectively. The average daily amount of creatinine was 559 mgm.
14. **Mr. M. L.** *Psychosis ; Alcoholic Delusional Insanity.*— Forty-six years old. Weight, 53 kilos ; height, 1.75 metres. On all three days the urine contained a considerable amount of albumen and many granular casts. On the three days of the experiment the patient excreted 155.9, and 85 mgm. of creatine respectively. The average daily amount of creatinine was 697 mgm.
15. **Mr. M. S.** *Psychosis ; Alcoholic Delusional Insanity.*— Sixty-nine years old. Weight, 58 kilos ; height, 1.65 metres. The urine contained a faint trace of albumen and a few hyaline casts. On the three days of the experiment there were excreted 102, 116, and 123 mgm. of creatine respectively. The average daily amount of creatinine was 827 mgm.

In regard to the last three cases it may be said in general that they were all suffering from phthisis and also from chronic nephritis to a greater or less extent. During the past two years the body weights of these three patients had fluctuated within 5 or 6 kilos, but they had not lost flesh to any considerable extent. Their diet previous to and during the experiment consisted of eggs, toast, milk, and bread and butter. They were all poorly nourished and very inactive.

It should be stated that three other phthisis patients, also suffering from chronic nephritis, who were living under exactly the same conditions, were experimented upon, and practically no creatine found in their urines.

The absolute amount of creatine found in the majority of these cases is not large, the largest amount being 469 mgm. on the second day of the experiment with Mr. H. G. M. The important points developed in this study are (1) that creatine in the urine is in all probability independent of the creatinine, and (2) that while creatinine is a normal constituent of the urine the experiments on fasting individuals and the pathological cases here reported indicate that the presence of creatine in the urine is pathological.

As yet too little experimental evidence is at hand to show clearly the relation between the creatine output and disease, but such information as is now available would imply that creatine is excreted in wasting diseases where flesh is broken down. Indeed an hypothesis

suggested by one of us<sup>1</sup> considers that the creatine excretion is an index of the flesh katabolized during fasting.

If creatine is an index of a disintegration of muscular and glandular tissue, the clinical significance would certainly warrant estimations of the preformed creatine elimination in many pathological cases, and it is not beyond the bounds of reasonable speculation to conceive of the creatine determination as of a distinct diagnostic value.

It is furthermore of interest to note that Cases 4, 5, and 6, all of which showed relatively large amounts of preformed creatine in the urine, were at the time of the experiment very much agitated.<sup>2</sup>

<sup>1</sup> BENEDICT: Carnegie Institution of Washington, Publication No. 77, 1907.

<sup>2</sup> MAXWELL has recently found that creatine acts as a brain stimulant. *Journal of biological chemistry*, 1907, iii, p. 25.

CONCERNING THE EFFECT OF CHANGES OF BLOOD  
PRESSURE PRODUCED BY TEMPORARY OCCLUSION  
OF THE AORTA UPON RESPIRATORY ACTIVITY.

By J. A. E. EYSTER, C. R. AUSTRIAN, AND C. R. KINGSLEY.

*[From the Physiological Laboratory of the Johns Hopkins University.]*

THE effect of sudden increase and decrease of the blood pressure upon respiratory activity has been recently investigated by Guthrie and Pike.<sup>1</sup> The literature is given in the paper by these authors and will not be repeated here. As a result of their experiments, these observers conclude that an increase in blood pressure, produced by temporary occlusion of the thoracic or abdominal aorta in cats and dogs, is accompanied by an increased rate of respirations together with diminished amplitude. A fall of pressure, produced by release of such occlusion, is accompanied by decreased rate and increased amplitude. Their experiments led them to no conclusion as to how the increased blood pressure effects the result described. They offer, however, two suggestions, — one proposed by Hill, that the increase may act directly upon the centre, or the other, that the increased blood pressure may lead to increased metabolism accompanied by a greater carbon dioxide output with consequent increase of the respiratory rate.

In a previous paper<sup>2</sup> one of the present authors has shown that a certain amount of blood supply to the respiratory centre is necessary for its activity. The necessary amount of blood is apparently very small, and increased respiratory activity accompanies as a rule a reduction of the blood supply to the respiratory centre until this limit is reached. Subsequent (unpublished) experiments by the same author, in which the blood pressure in the Circle of Willis was recorded from the peripheral end of one internal carotid artery at a

<sup>1</sup> GUTHRIE, C. C., and PIKE, F. H.: This journal, 1906, xvi, p. 475.

<sup>2</sup> EYSTER, J. A. E.: Journal of experimental medicine. 1906, viii, p. 565.

time when the cerebral arteries were ligated, have brought out more clearly this result. Following the ligation of each cerebral artery there is a fall of pressure in the Circle of Willis, and following the ligation of several arteries (in dogs, usually two carotids and one or both vertebrals) there is an increase in respiratory activity. If the cerebral anæmia is now carried further (in dogs, for example, by the ligation of the subclavians, cervical and spinal anastomoses), respirations cease quite suddenly, and this effect is associated with an extremely low pressure (5 to 10 mm. of mercury) in the Circle of Willis. In experiments in which the cerebral anæmia was produced by increase of intracranial tension to a point greater than blood pressure, similar phenomena were observed. In these experiments there were numerous examples of a sudden increase of blood supply to a partially anæmic centre, in which however the amount of blood had not been reduced to the low point at which cessation of respiratory activity occurs, with the result that there was a marked decrease of respiration or even a complete apnoea of short duration. The interpretation of this decrease of respiration under such conditions was the sudden reduction of the respiratory stimulus by an increase of the blood supply to the centre, the increased amount of blood allowing an increased diffusion and hence a lessened accumulation of carbon dioxide in the cells of the centre.

The accumulation of carbon dioxide in the cells of the respiratory centre according to prevalent theories determines its activity. The amount of carbon dioxide discharged from the cells depends upon the relative tension of this gas in the cells and the surrounding blood, as well as upon the amount of blood present. A small amount of blood with a carbon dioxide tension lower than that of the cells of the centre would have its tension more rapidly raised than would a larger amount of blood under the same conditions. In other words, the stimulus to the respiratory centre depends not only upon the tension of the carbon dioxide in the blood supply, but also upon the amount of blood flowing through the centre. A decrease in blood supply, the other factor remaining constant, should result therefore in an increase of the stimulus, an increase in the blood supply to a decrease of the stimulus.

From the above considerations, and in view of the importance of the conclusions of Guthrie and Pike, if confirmed, the present series of experiments were undertaken.



#### METHODS.

The animals used were dogs and cats. Sudden rises and falls of blood pressure were produced by temporary occlusion of the thoracic aorta. This occlusion was obtained by several methods. In our early experiments, a medium-sized curved aneurism needle was passed into the left side of the thorax between the eighth and ninth ribs close to the vertebral column, and the aorta compressed against the vertebræ by traction upon this needle. Later the method employed by Guthrie and Pike was used. A ligature was passed around the aorta and vertebral column by means of a large aneurism needle, the ligature entering and leaving close to the vertebræ upon each side. Traction upon this ligature occluded the aorta by drawing it tightly against the vertebral column. Finally, in animals with partially open thorax, breathing by means of the apparatus of slight positive intra-pulmonary pressure devised by Brauer,<sup>1</sup> the first part of the descending thoracic aorta was occluded by direct temporary clamping.

The animals were anæsthetized, and in certain of the experiments with dogs, morphia had been given previously. The respiratory exchange was measured in some experiments. The blood pressure was recorded from the central end of one common carotid artery. Respiratory tracings were obtained by a tube connecting the ether bottle with the recording tambour, by a similar connection between the recording tambour and the outflow of the gasometer, or by a transmitting tambour on the thorax of the animal. Careful autopsies to verify the position of the ligatures were performed at the conclusion of all experiments in which these were employed.

#### EXPERIMENTAL RESULTS.

In our early experiments, in which a rise of blood pressure was produced by occlusion of the aorta by means of a curved aneurism needle introduced into the thorax, the results obtained were exactly opposite to those of Guthrie and Pike. The rise of blood pressure so obtained was always associated with decreased respiratory activity, either a decrease in both rate and depth or decrease in depth with unchanged rate. Measurement of the respiratory exchange in these cases showed a marked diminution accompanying the rise in blood

<sup>1</sup> BRAUER: Mittheilungen aus den Grenzgebieten der Medizin und Chirurgie, 1904, xiii, Art. xviii.

pressure. This was true for both dogs and cats. The method of occlusion by means of a ligature as employed by Guthrie and Pike was now tried, and the results described by them were obtained in certain cases; in others our previous results were repeated. In a number of experiments several ligatures were passed around the vertebral column and aorta in the same animal, and others were passed similarly but not including the aorta. Occlusion of the aorta by traction upon certain of these ligatures caused an increase in respiration; in others a marked decrease was observed. Moreover, in direct contradiction to the statement of Guthrie and Pike, a ligature passed around the vertebral column in the usual region but not including the aorta may cause upon traction marked changes in respiratory activity. There may result from tightening such a ligature an increased rate of respiration with decrease in depth (Fig. 1), an increased depth with constant (see the table), or decreased rate, or a decrease in rate or depth, or both. Autopsy upon those animals in which several ligatures were employed, showed that it was those ligatures which entered and left very close to the vertebral column which caused an increase of respiration upon traction. On the other hand, those entering and leaving further out caused quite constantly a decrease in respiration associated with the rise of blood pressure.

In the case of those ligatures not including the aorta and which gave upon traction increase or decrease of respiration, slight changes of blood pressure were present. The similarity of such records to the tracings obtained from stimulation of the central end of a sensory nerve was very suggestive.<sup>1</sup> Fig. 1 shows the effect of traction upon

<sup>1</sup> Stimulation of the central end of a sensory nerve, as, for example, the saphenous, may affect respiration and blood pressure in various ways, depending upon the depth of anæsthesia and the condition of the animal. The blood pressure may show a rise or a fall. The respirations may be variously affected, — the rate increased with decreased depth, both depth and rate increased, increased depth with normal rate, decreased depth with normal rate, or both depth and rate decreased. A decrease in respiration is usually associated with a fall of blood pressure in such stimulation, an increase with a rise. The former is more common late in an experiment and under deep anæsthesia. These different effects upon respiration and blood pressure have all been obtained by ligatures passed around the vertebral column in the usual position but not including the aorta. There has been noted, moreover, a striking correspondence between the effects of stimulation of the saphenous, on one hand, and tightening such a ligature on the other, at any particular time. When tightening of a ligature caused, for example, a decrease in respiration with a fall of blood pressure, stimulation of the saphenous caused a similar result. An example of this is given in Fig. 2.

a ligature passed around the vertebral column but not including the aorta. There is a slight rise of blood pressure and marked increase of respiratory rate with decreased depth. The respiratory exchange

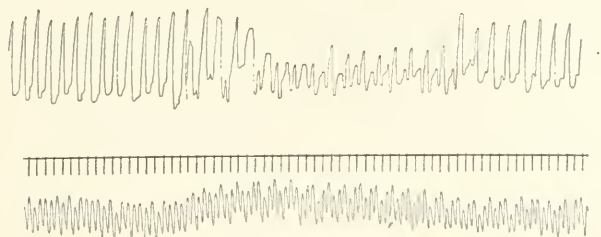


FIGURE 1.—Increase of respiratory rate with decrease in depth from traction upon a ligature passed around vertebral column but not including aorta. Uppermost line respirations (up-stroke inspiration), middle line time in one-second intervals, lowest line blood pressure from carotid. The zero line for blood pressure is not shown. Blood pressure before occlusion, 84 mm. of mercury; during occlusion, 88 mm. This and all following tracings are to be read from left to right.

was measured in this case, and it was found that there was an increase during the traction of the ligature. The first slowing of the respirations with slight fall of blood pressure in Fig. 2 followed the tighten-

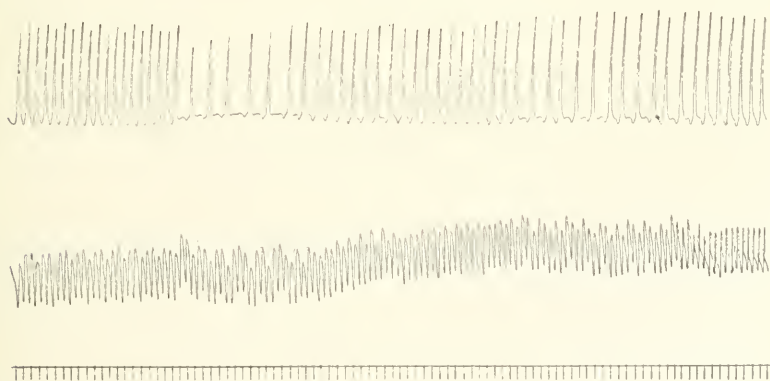


FIGURE 2.—The first slowing of respirations in this figure is the result of tightening a ligature passed around the vertebral column but not including the aorta. The second slowing is due to stimulation of the central end of one saphenous nerve. Curves as in last figure. Blood pressure before occlusion, 122 mm.

ing of a similar ligature toward the end of another experiment. The second slowing and fall of blood pressure in the same tracing is due to the stimulation of the central end of the cut saphenous nerve.

Experiments were now performed in which the condition of anæsthesia was varied and the depth carefully noted. It was found in these cases that certain ligatures which included the aorta gave different effects on respiratory activity upon traction, and that this difference could be shown to depend upon the depth of anæsthesia. Thus in deep anæsthesia the rise of blood pressure accompanying

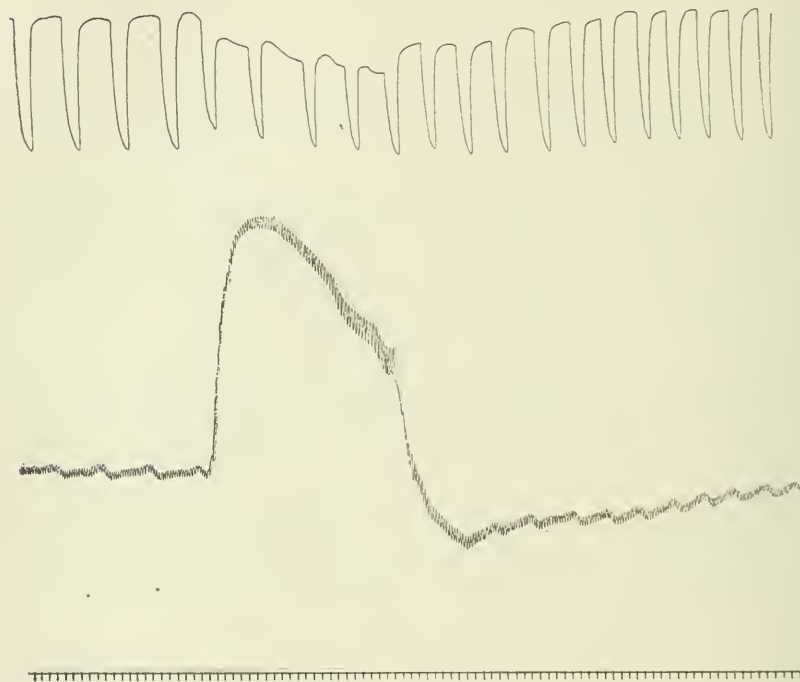


FIGURE 3.— Decrease in respiration as a result of occluding the aorta by ligature in a cat while in a condition of deep anæsthesia. Uppermost line respiration, middle line carotid blood pressure, lowest line time in one-second intervals. Base line of blood pressure not shown. Blood pressure before occlusion 134 mm., during 204 to 164 mm., after 116 mm.

traction of such a ligature would be associated with a marked decrease in respiration. Traction upon the same ligature within a short time in a condition of light anæsthesia would be associated with an increased rate or depth or both. Figs. 3 and 4 are from such an experiment. One minute after the tracing shown in Fig. 3, in which the animal (a cat) was deeply anæsthetized, the ether was entirely removed. Two and a half minutes later occlusion gave the result represented by Fig. 4.

The following table gives the results of an experiment in which three ligatures were employed and the depth of anæsthesia varied. Autopsy showed that ligatures 1 and 2 passed around the aorta ; ligature 3, on the other hand, did not, but simply included the vertebral column and contiguous structures. Ligatures 1 and 3 entered and left the thorax close to the vertebral column, ligature 2 entered and left further out. In the first part of the experiment, in a condition of light anæsthesia, traction upon ligature 1 caused marked increase of rate and depth of respirations with increase of respiratory exchange (Occlusion 1 of the table). In deep anæsthesia traction upon this ligature caused decrease in rate and depth (Occlusion 2 and 3) with marked decrease of respiratory exchange. In moderate anæsthesia traction upon this ligature (Occlusion 4) caused increase in rate but decrease in depth of the respirations to such an extent that the respiratory exchange was decreased. Traction upon ligature 2 caused decrease in rate and depth in both light and deep anæsthesia. Traction upon ligature 3 (which did not include the aorta) caused first (Traction 9 of the table) increased respiratory activity (decrease in rate,

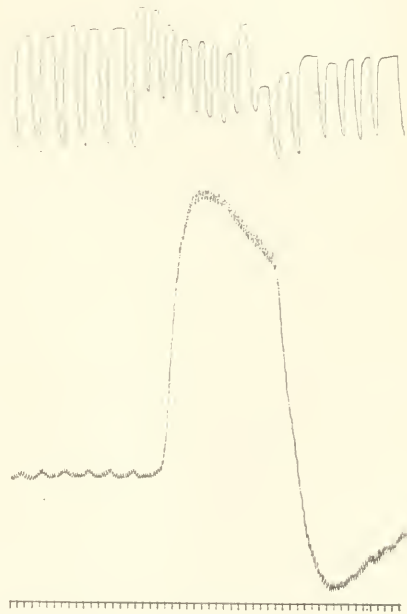


FIGURE 4. — Increase in respiration as a result of occlusion of aorta by the same ligature in the same animal as the tracing shown in Fig. 3, three and one-half minutes later and in a condition of light anæsthesia. Tracing is to be interpreted as Fig. 3. Blood pressure before occlusion 140 mm., during 232 to 210 mm., after 104 mm.

increase in depth and increase of respiratory exchange); later it gave a decrease of respiration. Traction upon ligature 1 toward the end of the experiment in light anæsthesia was not attended by an increase of respiration, as was obtained previously, but a considerable decrease, and stimulation of the central end of the saphenous at this time showed a similar though not so great decrease. The animal at this time was thus in such a condition that stimulation of a sensory

No. of occlusion.	Ligature.	State of anaesthesia.	Length of occlusion.	Blood pressure.			Respiratory rate.			Respiratory depth.			Respiratory exchange.		
				Before occlus.	During occlus.	After occlus.	Before occlus.	During occlus.	After occlus.	Before occlus.	During occlus.	After occlus.	Before occlus.	During occlus.	After occlus.
1	1	Light	secs. 30	mm. Hg. 130	mm. Hg. 184-144	mm. Hg. 72-110	per min. 56	per min. 78	per min. 56	mm. 20.0	mm. 25.0	mm. 18.0	0.58	0.80	0.46
2	1	Deep	30	94	144-126	62-76	74	60	62	4.5	3.0	5.5	0.15	0.09	0.12
3	1	Deep	60	96	142-134	62-90	66	59	70	....	....	....	0.46	0.23	0.49
4	1	Mod.	60	126	178-156	70-110	72	74	68	19.0	11.0	12.0	1.43	0.94	0.96
5	2	Deep	30	120	170-136	76-105	52	26	50	22.0	12.0	28.0	....	....	....
6	1	Deep	23	118	170-160	80-100	72	32	60	34.0	28.0	38.0	....	....	....
7	2	Light	10	120	164-150	76-90	84	36	72	18.0	13.0	21.0	....	....	....
8	2	Mod.	15	128	186-180	80-100	78	36	....	22.0	18.0	....	....	....	....
9	3	Mod.	30	140	130	138	87	81	96	14.0	15.0	17.0	0.80	0.94	1.03
10	3	Deep	60	140	135	146	102	48	71	21.0	20-28	25.0	2.20	1.60	2.19
11	2	Deep	30	130	180-170	64-90	92	50	36	18.0	12.0	13.0	1.00	0.36	0.59
12	3	Light	30	132	132	132-140	114	66	96	15.0	16.19	21.0	1.12	0.82	1.51
13	1	Light	20	134	190-180	80-100	111	60	96	15.0	10.0	15.0	....	....	....
14	....	Deep	....	130	120	130	57	51	69	....	....	....	....	....	....
15	....	Light	....	....	....	....	....	....	....	....	....	....	0.80	0.62	....
16	....	Mod.	....	....	....	....	....	....	....	....	....	....	0.60	0.49	0.54

In Experiments 14, 15, and 16, the central end of the saphenous nerve was stimulated.

nerve instead of causing the usual rise of blood pressure and increase of respirations caused decrease of both. Fig. 1 is an example of a rise of blood pressure and increase of respiration from a ligature not including the aorta.

Finally, direct stimulation of the thoracic sympathetic chain by a faradic current may cause marked increase of respiratory activity

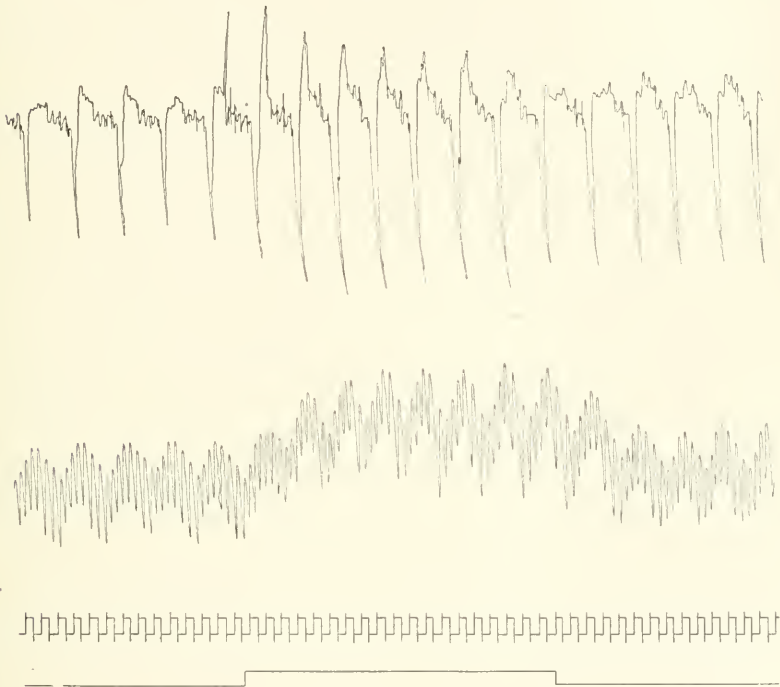


FIGURE 5.—Stimulation of thoracic sympathetic chain by faradic current in a dog. Uppermost line respirations (down-stroke inspiration), second line from top carotid pressure, third line time in one-second intervals, lowermost line base line for blood pressure. Rise of base line represents beginning of stimulation, the fall cessation of stimulus.

with a rise of blood pressure. An example of this is given in Fig. 5. The animal's thorax was opened upon the left side sufficiently wide to allow dissection of the thoracic sympathetic chain for a distance of several centimetres and the application to it of a shielded electrode. Natural respiratory activity was maintained by the method of Brauer. The opposite result from such stimulation has also been observed, namely, a fall of blood pressure with decrease of respiration. Direct

ligation of the aorta in these experiments leads to a rise of blood pressure with decrease in respiration.

#### DISCUSSION OF RESULTS.

In the interpretation of their results Guthrie and Pike took into account two factors, the changes in blood pressure and the changes in carbon dioxide contents of the blood. In the first place, upon ligating the aorta, the brain was separated from the blood flowing from that portion of the body which consumes the most oxygen and gives off the most carbon dioxide. One would expect, according to these authors, a decrease in respiration as a result; on the contrary, an increased rate was the usual occurrence. They therefore concluded that this increased rate was due to the rise of blood pressure. With the fall in blood pressure coincident with the release of the ligature, the blood returning from the posterior part of the body must be surcharged with carbon dioxide and at first cause an increase in respiratory activity. They observed a constant augmentation of respiration when the blood pressure fell. It is not stated whether this augmentation is simply over that observed during the occlusion or over that before the occlusion. Our experiments show that the respiratory depth following the occlusion is usually greater than that observed during occlusion. This however is not always true, as the first occlusion of the table shows. Occlusion of the aorta by the ligature method of Guthrie and Pike may affect the respiration during the occlusion in a number of different ways, and the state of respiratory activity following the occlusion may no doubt be influenced by this. During occlusion, both rate and depth may be increased (first occlusion of the table), rate increased and depth decreased (fourth occlusion of the table), rate normal or decreased and depth increased (ninth occlusion of the table), or, finally, decrease of both rate and depth. A decrease of respiratory activity, usually both rate and depth, is the condition that always accompanies occlusion with rise of blood pressure in our experiments, when care was taken to prevent nervous stimulation. The other effects upon respiration produced by occlusion by means of ligature, we believe to be due to stimulation of the respiratory centre through afferent nerves lying in the neighborhood of the ligature and pressed upon by traction of the same. The most prominent nervous structure in this region is the thoracic sympathetic chain, and it is almost impossible to pass a ligature around the vertebral column in this region,



when the ligature enters and leaves the thorax close to the vertebræ, without, upon traction of the same, causing compression of this chain or some of its branches on at least one side. Fig. 5 shows that stimulation of the thoracic sympathetic may markedly affect respiration.

We therefore believe that the results of Guthrie and Pike upon which their conclusions were based were due, not to the rise of blood pressure, but to stimulation of the respiratory centre through afferent nerves as a result of traction upon the ligatures. We base this belief upon, (1) the constancy of our results when this factor is excluded by ligatures placed some distance from the vertebral column, occlusion by traction upon a hook, direct ligation of aorta with open thorax, (2) the effects upon respiration of ligatures passed around the vertebral column in the usual region and not including the aorta, (3) the varying effect of ligatures occluding the aorta under different degrees of anæsthesia, (4) the position of the ligature in regard to its effect upon respiration (thus a ligature entering and leaving the thorax close to the vertebral column nearly always causes a stimulation upon traction in light anæsthesia, one further out may not).

Returning to the condition of respiratory activity accompanying the fall of blood pressure following the occlusion, it may be seen from the table and figures that this shows considerable variation. When the centre is stimulated by traction upon the ligature, the increased respiratory activity during the occlusion may be followed by a decrease in rate and depth accompanying the release of the traction and the cessation of the stimulus (first occlusion of the table). Under certain conditions the latent period of such stimulation may be so long as to cause the principal effect upon respiration to occur after the release of the ligature (ninth line of the table; a ligature that did not include the aorta).<sup>1</sup> Following occlusions of the aorta in which nervous stimulation is excluded, the decrease in respiratory rate and depth associated with the rise of blood pressure is always followed by an increase in rate and depth (over that during the occlusion) occurring immediately after release of the ligature. This sudden increase is too soon for the blood surcharged with carbon dioxide from the posterior part of the body to reach the brain, as it has to pass through the heart and lesser circulation, and the first part of the increase is most probably due to increase of the respiratory stimulus as a result of the

<sup>1</sup> A similar effect upon stimulation of the saphenous is shown in the fourteenth line of the table.

sudden fall of pressure (sudden reduction of blood supply). Soon the effect of the blood laden with carbon dioxide is observed, there is a still further increase in respiration which in the majority of cases leads to an activity greater than that present before occlusion.

Guthrie and Pike explain their anomalous results by one occlusion following quickly upon previous occlusion and release. In such a case they believe that the sudden checking of the flow of blood to the brain from the posterior part of the body which is surcharged with

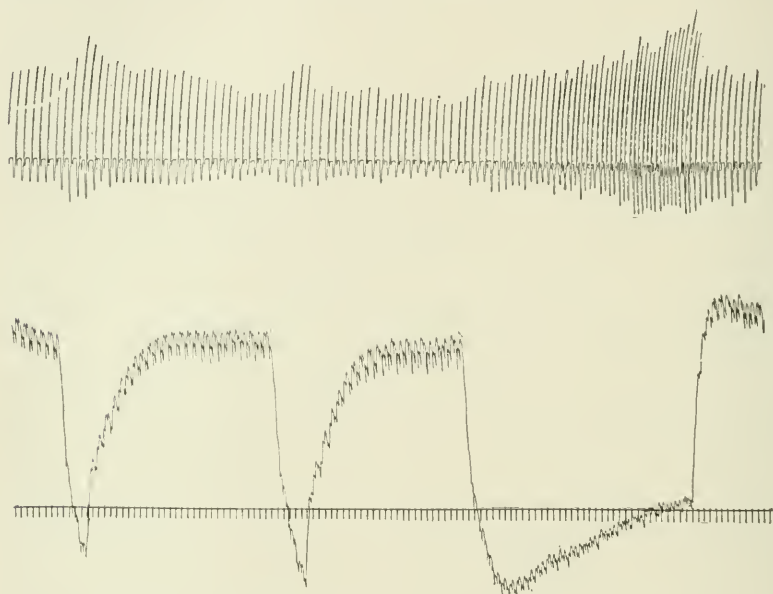


FIGURE 6. — Results of several occlusions separated by short intervals. The figure shows the last part of the first, the second, third, and part of the fourth of the six occlusions of the aorta in this series.

carbon dioxide, may lead to such a decrease of the respiratory stimulus as to counteract the stimulating effect upon the respiratory centre of the rise of blood pressure. The former factor then masks the latter and a decrease of respiratory activity results. Care was used in our experiments to have the occlusions so far separated as to avoid this possibility. Fig. 6 shows some points of interest in this connection. Here a number of rises of blood pressure follow in succession. Each rise of blood pressure from occlusion of the aorta is accompanied by a decrease of respiration, each fall by an increase. The second and third occlusions follow the first immediately upon the

fall of the blood pressure to its lowest points. Each fall of blood pressure in these cases is associated with increased respiratory activity over that during the rise, not however over that before the rise. After the third rise an interval of about forty seconds intervenes before the fourth rise, and just before this the rate and depth have increased over that before the first rise (the normal). The fifth rise follows in about twelve seconds and is similar to the second and third rises. The sixth rise follows in sixty-five seconds and is similar to the third. This shows that there are two factors tending to cause an increase of respiration with the fall of blood pressure ; the one first acting is the increase of stimulus as a result of the decrease in the blood supply to the centre, and, secondly, the somewhat later effect of the surcharged carbon dioxide blood from the posterior part of the body. A rise of blood pressure occurring at either stage is sufficient to cause a marked decrease of respiration.

A number of experiments were performed after section of both vagi with results similar in every way to those described for intact vagi.

#### CONCLUSIONS.

1. There is no evidence to show that a rise of blood pressure produced in an animal by occlusion of the aorta affects the respiratory centre *per se* when it is already receiving an amount of blood sufficient for its normal activity. The results of such a rise when other factors are excluded is to cause a decrease of respiratory activity, probably as a result of the decrease of the respiratory stimulus following upon an increased blood supply to the centre. If the rise of blood pressure *per se* stimulates the centre, its effect is masked by the latter factor.

2. There is likewise no evidence from these experiments to show that a fall of pressure causes a decreased activity of the centre when the supply of blood is not too greatly reduced. On the contrary, the increase of the stimulus following a reduced blood supply causes an increased respiratory activity. If the centre is affected otherwise by the fall, the effect is masked by the increase of stimulus.

3. The opposite conclusions of Guthrie and Pike we believe to be due to another factor entering into their experiments, namely, the stimulation of the respiratory centre through afferent nerves as a result of their method of occlusion. We believe that our experiments explain the many anomalous results reported in their paper.

# PHARMACOLOGIC INVESTIGATIONS ON THORIUM.<sup>1</sup>

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[From the Pharmacological Laboratory of Western Reserve University, Cleveland, Ohio.]

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### I. QUALITATIVE REACTIONS OF THORIUM.

IN order to evolve a practical method for the quantitative estimation of thorium, and to prepare a compound which would not precipitate proteids, we felt obliged to make a rather extensive study of the principal precipitation reactions of the metal, of the solubility of the precipitates, and of the inhibitory action of various substances on the precipitations. We quote our results below, although we are aware that many of them are not new. The experiments were all made with chemically pure crystallized thorium nitrate.<sup>2</sup>

1. The aqueous solution of this salt is distinctly acid to litmus.
2. **NaOH**, **KOH**, and **NH<sub>4</sub>OH** precipitate the solution completely.<sup>3</sup>

The precipitate occurs as heavy white flakes (Th(OH)<sub>4</sub>). It retains

<sup>1</sup> The introduction to the paper of CHACE and GIES makes a special introduction to our paper superfluous. Our interest in thorium was kindled by the same general consideration which prompted these authors to investigate the actions of the metal. As we were in entire ignorance of this previous and unpublished work, our experiments were chiefly along somewhat different lines.

<sup>2</sup> We are indebted to Dr. M. Metzenbaum for the earlier samples. Those used for the later experiments were purchased from Merck. We did not concern ourselves with the question whether this thorium constitutes a single substance, since our primary object was the investigation of the thorium as actually found in the market.

<sup>3</sup> The absence of thorium was confirmed by acidulating the filtrate strongly with HCl and adding oxalic acid.

the alkali with great tenacity, nine washings by centrifugalization being required before the fluid was neutral to litmus.

The precipitate was *insoluble* in water (several days); in excess of the reagents; in 10 per cent NaCl; 25 per cent sodium citrate or sodium potassium tartrate.

The complete insolubility of this and all other precipitates in the citrate and tartrate is especially interesting, because these organic salts prevent the precipitation when they are added to the thorium before the precipitant. Neutral oxalates also tend to prevent precipitation and they also dissolve the precipitates.

The thorium hydrate is *soluble* in ammonium oxalate and in dilute acids. The solution is instantaneous in concentrated HCl, but rather slow in weak acids; the solubility decreasing from hydrochloric to acetic, to citric, to tartaric acid. The solubility is increased by heating, diminished by prolonged washing and standing. Boiling the suspended hydrate, or drying it for fifteen hours at 100° C., does not diminish its solubility.

The precipitation is *prevented* by citrates, tartrates, egg albumen, alkali albumen, blood, and Witte's peptone,<sup>4</sup> *not* by sodium carbonate or ammonium oxalate. The citrate mixture, however, precipitates on boiling or on prolonged standing, the precipitate being readily soluble in dilute HCl. The tartaric mixture does not precipitate on heating or standing.

3. Thorium oxid ( $\text{ThO}_2$ ) has some rather peculiar solubility characters. Fresenius states that when it is prepared by gentle calcination of the oxalate, moistened with HCl or  $\text{HNO}_3$  and evaporated, it forms the corresponding salts, which dissolve readily in water, but which are again precipitated by the further addition of the acid.

When strongly calcined, the oxid is insoluble in acids, dissolving only when heated with a mixture of equal parts of strong sulphuric acid and water (Fresenius). In our hands the solution was generally incomplete. We obtained the best results by a new method, by fusion with  $\text{NaHSO}_4$ . The resulting mass is perfectly soluble in water. The attempt to obtain a soluble salt by fusing the oxalate with sodium nitrate was unsuccessful.

4.  $\text{Na}_2\text{CO}_3$  and  $\text{K}_2\text{CO}_3$  precipitate the carbonate  $\text{Th}(\text{CO}_3)_2$ . The precipitate is *soluble* in ammonium oxalate, hot or cold. It dissolves

<sup>1</sup> These prevention experiments were made by adding the citrate, etc. to a thorium nitrate solution until the resulting precipitate was redissolved, and then adding the reagent hydrate, etc.

also in concentrated solution of the alkali carbonate in the cold, but is re-precipitated completely on boiling. Some precipitation occurs also at ordinary temperature after a time; this is quite marked in two hours, but is not complete even after four days.

The precipitate which occurs on heating the carbonate solution does not dissolve on cooling. It is *insoluble* in sodium citrate or tartrate. It is *soluble* in dilute acids, even tartaric, and with some difficulty in hot ammonium oxalate.

The precipitation of the carbonate is *prevented* by citrate and tartrate, *not* by ammonium oxalate. The citrate mixture precipitates completely on boiling, incompletely on standing (two days). The tartrate mixture does not precipitate by boiling or standing.

The *solution of thorium carbonate in sodium carbonate is precipitated* at once and at room temperature by NaOH, *not* by  $\text{Na}_2\text{HPO}_4$ , NaF, or proteids.

5.  $\text{NaHCO}_3$  causes a precipitate which is difficultly *soluble* in hot ammonium oxalate. It also dissolves sparingly in an excess of  $\text{NaHCO}_3$ . This solution re-precipitates completely on standing or boiling, the precipitate dissolving readily in dilute acetic acid.

6.  $\text{K}_2\text{SO}_4$  in saturated solution, precipitates immediately and completely (double sulphate,  $\text{Th}(\text{SO}_4)_2 \cdot 2\text{K}_2\text{SO}_4 + 2\text{H}_2\text{O}$  and  $\text{Th}(\text{SO}_4)_2 \cdot 4\text{K}_2\text{SO}_4 + 2\text{H}_2\text{O}$ ).<sup>1</sup> The precipitate is *insoluble* in excess of the reagents, and in 25 per cent sodium citrate, cold or boiling, even after prolonged washing. It dissolves very slowly in cold water. It is *soluble* in dilute acids, even tartaric, the solution being hastened by heating; also in boiling water and in hot ammonium oxalate.<sup>2</sup>

7.  $\text{Na}_2\text{HPO}_4$  causes complete precipitation ( $\text{Th}_3(\text{PO}_4)_4 + 4\text{H}_2\text{O}$ ). The precipitate is *insoluble* in dilute hydrochloric acid and in acetic, citric, and tartaric acid, and in sodium citrate and tartrate. These acids also do not prevent the precipitation of the phosphate. When an equal volume of 5 per cent HCl is added to a suspension of the phosphate, and the mixture heated or allowed to stand, the precipitate becomes gelatinous, but does not dissolve. When an equal volume of 30 per cent HCl is added to a suspension, the precipitate *dissolves*, and remains in solution on diluting. The phosphate is also soluble in hot ammonium oxalate.

<sup>1</sup> P. E. BROWNING: Introduction to the rarer metals. J. Wiley & Sons, 1904.

<sup>2</sup> The neutral thorium sulphate ( $\text{Th}(\text{SO}_4)_2$ ) is said to be soluble in ice-water, but to precipitate at room temperature, re-dissolving in ice-water only after calcination.

The precipitation of the phosphate is *prevented* by citrate, carbonate or oxalate, *not* by tartrate. The citrate mixture precipitates completely on boiling or prolonged standing, or by adding HCl, but it is not affected by citric or tartaric acid or NaOH.

8. **NaF** causes complete precipitation ( $\text{ThF}_4 + 4\text{H}_2\text{O}$ . Double salts may also form, Browning). The precipitate is *insoluble* in HCl, even when concentrated and boiling; in acetic or citric acid or sodium citrate. It *dissolves* in hot ammonium oxalate.

The precipitation is *prevented* by sodium citrate or carbonate or ammonium oxalate, *not* by tartrate. The citrate mixture does not precipitate by boiling or standing (difference from phosphate). HCl gives a precipitate, insoluble in excess. The precipitate in the tartrate mixture is soluble in 5 per cent HCl.

9. **Oxalic acid** precipitates the thorium almost, but not quite completely (the filtrate giving a slight turbidity with NaOH). The precipitate  $\text{Th}(\text{C}_2\text{O}_4)_2 + 2\text{H}_2\text{O}$  is practically *insoluble* in oxalic acid and in citrates and tartrates. When a suspension of thorium oxalate is heated with one-third volume of concentrated HCl, a trace dissolves, precipitating again on cooling.

The thorium oxalate is readily and completely *soluble* when heated with fairly concentrated solutions of oxalate of ammonium, potassium, or sodium, remaining in solution on cooling. Warm or even cold ammonium oxalate solutions also dissolve the thorium oxalate, but quite slowly.

The solution of thorium oxalate in ammonium oxalate is precipitated by HCl, and also somewhat by excess of oxalic acid. It is precipitated quantitatively by NaOH or  $\text{NH}_4\text{OH}$ , redissolving on neutralization. (This may be utilized for the quantitative estimation.)

The precipitation of thorium nitrate by oxalic acid is *not prevented* by citrate or tartrate, if only sufficient of these salts have been added to the thorium to redissolve the thorium citrate or tartrate precipitate. The precipitation in this case is complete. If, however, a large excess of citrate or tartrate is used (equal parts of 25 per cent solution of sodium citrate or tartrate, and of 1 per cent thorium nitrate), the precipitation is *prevented*, even on boiling. The addition of HCl to this solution causes complete precipitation.

10. **Ammonium oxalate** produces with thorium nitrate a precipitate, which is *insoluble* in excess of thorium nitrate; and in dilute HCl even on boiling. It *dissolves* readily in an excess of ammonium oxalate on heating, especially if the solution is concentrated; it is

also soluble in concentrated HCl, being precipitated by further addition of oxalic acid. It dissolves also in 25 per cent sodium potassium tartrate.

The solution in excess of ammonium oxalate is *precipitated* by NaOH,  $\text{Na}_2\text{CO}_3$ , or oxalic acid; it is *not precipitated* by  $\text{Na}_2\text{HPO}_4$ , NaF, egg-white, blood, alkali albumen, sodium caseinate, or Witte's peptone.

The precipitation of thorium nitrate by ammonium oxalate is *prevented* by citrates and tartrates, cold or hot. With a thorium-sodium carbonate ammonium oxalate mixture, precipitation occurs only on boiling.

11. Citric and tartaric acid, and acid sodium citrates and tartrates produce precipitates with thorium nitrate. The precipitates are *insoluble* in excess of thorium and in acetic acid. They are *soluble* in excess of the precipitants, in excess of dilute HCl, and in NaOH (being converted by the last into the more soluble neutral salts). The precipitation is *prevented* by HCl, *not* by acetic acid.

The solutions in excess of the precipitants tend to precipitate on standing or heating, if the proportion of thorium exceeds a certain maximum. The heat-precipitates tend to redissolve on cooling. This will be discussed more fully below.

12. Neutral citrates or tartrates cause precipitation. The precipitates are *insoluble* in excess of thorium, in acetic acid, and in NaOH. They are *soluble* in excess of the citrate or tartrate and in excess of HCl. The precipitation is *prevented* by HCl, *not* by acetic acid.

The solutions in excess of citrate or tartrate do not precipitate on standing or heating. The cautious addition of HCl precipitates the less soluble acid citrate or tartrate, which dissolves readily in an excess of the acid. Acetic acid does not produce any precipitate itself, nor does it dissolve the precipitate caused by HCl.

The solution of thorium citrate in sodium citrate is *precipitated* at once by oxalic acid. It is not affected at once, but precipitates completely *on boiling or prolonged standing*, by  $\text{NH}_4\text{OH}$  or NaOH,  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{HPO}_4$ . It is not *precipitated* by NaF, ammonium oxalate, albumen, alkali albumen, blood, Witte's peptone or sodium caseinate. The proteid mixtures are precipitated by acetic acid.

The solution of thorium tartrate in sodium tartrate is *precipitated* by  $\text{Na}_2\text{HPO}_4$ , NaF, and oxalic acid; it is not *precipitated* (even on boiling or standing) by NaOH,  $\text{NH}_4\text{OH}$ ,  $\text{Na}_2\text{CO}_3$ , ammonium oxalate, or the proteids mentioned in the last paragraph.



13. **Butyric acid** gives a precipitate which is *insoluble* in excess of butyric acid, *soluble* in excess of thorium and in HCl. The solution in thorium nitrate is precipitated by NaOH before neutralization is reached. It is also precipitated by butyric acid. (The presence of thorium in the  $\text{Th}(\text{NO}_3)_4$  — butyric precipitate was shown by washing and testing with HCl and oxalic acid.)

**Sodium butyrate** (neutral) precipitates  $\text{Th}(\text{NO}_3)_4$ ; the precipitate is insoluble in NaOH, soluble in butyric and other acids.

14. **Nitric, hydrochloric, formic, lactic, or acetic acids, and their sodium salts; glycerine, urea, saccharose or dextrose, or acid albumen** (prepared from egg-white) do *not precipitate* thorium, nor do they prevent the precipitation by the other reagents.

15. **Egg albumen** produces a white, curdy precipitate, *soluble* in excess of albumen or thorium, in sodium citrate, in NaOH, and in ammonium oxalate.

Acetic acid gives a slight turbidity in the solution in excess of thorium nitrate, not in excess of albumen,

The solution in NaOH, although strongly alkaline, does not precipitate thorium nitrate, unless the latter is in great excess. More thorium goes into solution when the excess is added in the order:

Albumen, Thorium, NaOH,

than when it is in the order:

Albumen, NaOH, Thorium.

The precipitation of albumen by thorium is *prevented* by citrates, tartrates, carbonate, and oxalate.

16. **Alkali albumen** (prepared from egg-white by heating with NaOH and neutralizing the excess of alkali) causes a curdy precipitate, apparently *insoluble* in excess of thorium, *soluble* in excess of alkali albumen, in NaOH, and difficultly in ammonium oxalate or sodium citrate (much more readily in the oxalate than in the citrate). The NaOH solution behaves towards thorium as in the case of albumen.

The precipitation is *prevented* by the same reagents as in the case of albumen.

17. **Blood** (defibrinated, dog's, diluted with four volumes of water), causes a precipitate (see below), *soluble* in excess of blood, sodium citrate and ammonium oxalate (see remarks under alkali albumen)

and NaOH (see under albumen). The solubility in excess of thorium could not be observed because of the precipitation of acid hematin.

The *prevention* of the precipitation is as for albumen.

18. **Caseinate of sodium** (casein dissolved by the aid of NaOH) produces a precipitate, *insoluble* in excess of casein or thorium and in NaOH; sparingly soluble in ammonium oxalate; solution in sodium citrate doubtful.

19. **Peptone Witte** gives a precipitate, readily *soluble* in excess of thorium, difficultly in excess of peptone, sparingly in ammonium oxalate, sodium citrate doubtful; also soluble in 5 per-cent HCl and in NaOH. The solution in thorium is precipitated in NaOH; the solution in NaOH by thorium.

**Discussion of the thorium reactions.** — The resemblance of the thorium reactions to those of aluminium and cerium, which belong to the same group, is very striking. The general analogy holds true for the principal precipitation reactions, for the solubility of the precipitates, and for the prevention of precipitation by the presence of citrates, tartrates, etc. through the formation of strong double salts, although there are differences in the details.

The *principal precipitants* of thorium may be classified as follows:

1. Oxalic acid, fluorid, and phosphate.
2. Alkalies: OH, CO<sub>3</sub>, and HCO<sub>3</sub>.
3. Citric acid and citrates, tartaric acid and tartrates, ammonium oxalate; *not* acetic, lactic, or formic acids.
4. Proteids: albumen and alkali albumen, blood, casein, and peptone, *not* acid albumen. Casein really constitutes a class by itself, however, the precipitates being much less soluble than those of other proteids.

As regards the *solubility* of the precipitates, the first class is generally insoluble in *acids*, whilst the other classes are generally soluble. To this, however, there are some exceptions: Of Class 1, the oxalate is sparingly soluble in concentrated hydrochloric acid, the phosphate freely so; but the latter forms a peculiar gelatinous precipitate in the presence of dilute hydrochloric acid.

In Class 2 (alkalies) the decreasing solubility in organic acids is worthy of notice; of these, acetic acid is the most powerful solvent, whereas it fails entirely to dissolve the precipitates of Class 3 (citrates, etc.). NaOH dissolves only the precipitates of the fourth class (proteids) with the exception of casein. This and all the precipitates of the first three classes are insoluble in NaOH.

The most remarkable character of the thorium precipitates is their universal solubility in concentrated *ammonium oxalate* solution, especially on heating. *Citrates and tartrates*, although they prevent precipitation in many instances, do not dissolve the formed precipitates, except in Class 4. The casein precipitate is again insoluble.

**Double salts : solubility in excess of precipitant.** — Through the tendency to the formation of double salts, many precipitates are soluble in an excess of the reagents, as follows :

The precipitates are

1. **Insoluble in both precipitant and thorium:** oxalic acid, fluorid, phosphate, sulphate, hydroxid, casein.

2. **Soluble in the precipitant, insoluble in thorium:** carbonate, bicarbonate, citrate, tartrate, ammonium oxalate, alkali albumen.

3. **Soluble in both precipitant and thorium:** egg-white, blood, peptone.

These double salts or solutions of thorium in excess of precipitant generally *resist precipitation by other reagents*. The double thorium sodium citrate is not precipitated at once by any of the precipitants, although a large excess of the citric acid is needed to prevent the precipitation by oxalic acid. The double tartrate is precipitated only by the fluorid and phosphate, and oxalic acid; the double carbonate and the double oxalate are precipitated by these, and by sodium hydrate, and the oxalate also by sodium carbonate. The solution of thorium in proteids is not precipitated by sodium hydrate.

The *addition of acid* appears to disrupt these double salts, so that the thorium again becomes precipitable. Thus the mixture of sodium phosphate or fluorid with thorium sodium citrate is precipitated at once on the addition of dilute hydrochloric acid. *Alkalies* do not cause this decomposition, with citrates or tartrates, so that there is no change; but they do precipitate the double carbonate or oxalate.

The double salts seem to be rather unstable in solution, tending to precipitation, on heating or prolonged standing, perhaps by hydrolysis, even when no reagent is added.

The phenomenon is seen most strikingly with the carbonate and bicarbonate. It is absent with the double oxalate and with the neutral citrate and tartrate. Acid citrates and tartrates tend to precipitate on prolonged standing, unless a fair excess of the organic acid or its salt is present. These acid double salts present another peculiar phenomenon, namely, that they require a much greater quantity of the organic acid at the boiling temperature than in the cold. A bal-

anced mixture of thorium and citric or tartaric acid, or their acid salts, prepared at room temperature, will precipitate on boiling, and tend to redissolve on cooling. The completeness of the re-resolution on cooling depends on the preponderance of the citrate or tartrate over the thorium. This phenomenon will be discussed in greater detail below.

*Heating or prolonged standing also affects in a similar manner the resistance of the double salts to other precipitants.* Next to the double carbonate, the double citrate (neutral) is the most subject to this decomposition, whilst the double tartrate is not affected, and the oxalate combination is even more firm.

The double thorium-sodium-citrate, which is not affected immediately in the cold by any of the common precipitants, is precipitated at once on boiling, or slowly on prolonged standing, by:  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaOH}$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{NaHCO}_3$ . It is not precipitated, even under these conditions, by oxalic acid, sodium fluorid, ammonium oxalate, citrate or tartrates, or any of the proteids.

**Detailed study of the citrate and tartrate reactions.** — The re-resolution of the thorium precipitate in an excess of the precipitant was studied more intimately for these two reagents. When a solution of  $\text{Th}(\text{NO}_3)_4$  is added to a solution of neutral sodium citrate or tartrate, a dense precipitate occurs, which re-dissolves readily in an excess of the citrate or tartrate. The solutions still show an acid reaction to litmus. A definite quantity of citrate or tartrate is required to re-dissolve a given amount of thorium, independent of the quantity of solvent; for instance, 1 gram-molecule of  $\text{Th}(\text{NO}_3)_4$  requires 1.3 gram-molecules of  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ , no matter whether they are mixed in 1 per cent or in 10 per cent solution.

The end reaction is not quite sharp, since the precipitation and resolution require a little time; the sharpness can be improved by heating.

Acid citrates or tartrates, and the free citric and tartaric acid, behave similarly; but a much larger quantity is required to re-dissolve a gram-molecule of thorium, the larger the less alkali is present (see below).

When thorium is dissolved in a just sufficient quantity of the acids or acid salts, a small quantity is deposited slowly on standing, and a much larger quantity precipitates immediately on boiling, re-dissolving on cooling. These precipitates are absent if the citrates or tartrates are added in large excess.

Quantity of citrate or tartrate required to dissolve one gram-molecule of thorium. — To test this point a 1 per cent thorium nitrate solution was added to a mixture of citric or tartaric acid and sodium hydrate, until the first appearance of a permanent turbidity. As noted above, the results are not quite accurate, since the precipitation and solution are not completed at once; but a fair approximation was secured by averaging repeated experiments under somewhat varying conditions. The following results were obtained:

0.520 gm. of <i>citric acid</i> ( $H_3C_6H_5O_7 \cdot H_2O$ ) in 10 c.c. of water		plus c.c. $\frac{1}{1}$ NaOH. plus c.c. 1 % $Th(NO_3)_4 \cdot 12 H_2O$ .	
o		10	= no precipitate in cold, even on standing.
o		12.7	= beginning precipitation. immediate.
o		1.0	= no precipitation on boiling.
o		1.8	= precipitates " "
2.67 (= $NaH_2C_6H_5O_7$ )	32.2		= precipitation begins on boiling.
2.67	38.5		= " ended " "
5.3 (= $Na_2HC_6H_5O_7$ )	48.0		= " begins on boiling.
8.0 (= $Na_3C_6H_5O_7$ )	88.0		= " " " "
0.5136 gm. of <i>tartaric acid</i> ( $H_2C_4H_4O_6$ ) in 10 c.c. of water.			
o		50	= only turbidity in cold.
o		5	= no precipitate in cold on standing.
o		10	= slight " " "
o		1	= no precipitate on boiling.
o		2.8	= beginning precipitation on boiling.
3.45 (= $NaHC_4H_4O_6$ )	45.3		= " " " "
3.45	53.9		= end of " " "
6.9 (= $Na_2C_4H_4O_6$ )	84.5		= beginning " " "
6.9	100.0		= end of " " "

Using the numbers in heavy type, one gram-molecule of  $Th(NO_3)_4$  requires for re-solution:

Immediately or on standing, in cold	9.52 + gm. mol. }	$H_3C_6H_5O_7$
Boiling	68.7 + " " }	
"	3.84 + " " }	
"	2.58 + " " }	
"	1.31 + " " }	$NaH_2C_6H_5O_7$
"		$Na_2HC_6H_5O_7$
"		$Na_3C_6H_5O_7$
Immediately in cold	4.278 + " " }	$H_2C_4H_4O_6$
Standing " "	8.555 + " " }	
Boiling	61.09 + " " }	
"	3.777 + " " }	
"	2.024 + " " }	$NaHC_4H_4O_6$
"		$Na_2C_4H_4O_6$

To complete precipitation there are required about :

3.210	gm. mol. of	$\text{NaH}_2\text{C}_6\text{H}_5\text{O}_7$
3.174	“ “ “	$\text{NaHC}_4\text{H}_4\text{O}_6$
1.711	“ “ “	$\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$

We had hoped that the determination of these molecular equivalents would enable us to establish formulas for these double salts; but it appears that the conditions are too complex. For instance, the simplest compound which could be harmonized with the ratio of thorium to sodium tartrate would be:  $20 \text{Th}(\text{Ta})_2 + \text{Na}_2\text{Ta}$ ; for the sodium citrate,  $5 \text{Th}_3(\text{Ci})_4 + \text{Na}_3\text{Ci}$ . With the acid salts such speculation appears quite hopeless.

We also attempted to isolate the double salts by *crystallization*, but with no better success. The spontaneous evaporation of all the balanced mixtures yielded large feathery or rosette-formed crystals, but solutions containing 1, 5, 10, and 20 c.c. of 1 per cent thorium to 0.520 gm. of citric or 0.514 gm. of tartaric acid yielded identical uniform crystals on evaporation.

*Effect of prolonged standing on citrate and tartrate mixtures.* — When the amount of citrate or tartrate in a mixture is just barely sufficient to re-dissolve the thorium precipitate, a small part of the thorium is gradually deposited, but with extreme slowness. Thus, in an  $\text{NaH}_2\text{Ci} - \text{Th}$  solution, the first turbidity occurred after eleven days, and recurred each time after filtering (four filtrations at weekly intervals). In other cases the precipitation (of a portion of the thorium) was completed in five days, so that the turbidity did not recur on filtering.

*Effect of boiling on the mixtures.* — Solutions in  $\text{Na}_3\text{Ci}$ ,  $\text{Na}_2\text{HCl}$ , and  $\text{Na}_2\text{Ta}$  do not precipitate on boiling, even when saturated with thorium. Solutions in  $\text{NaH}_2\text{Ci}$ ,  $\text{H}_3\text{Ci}$ ,  $\text{NaHTa}$ , and  $\text{H}_2\text{Ta}$  precipitate on heating when the quantity of thorium exceeds a certain limit, which is very near the saturation limit for the acid salts, but which for the free acids lies considerably below the saturation limit in the cold.

For instance, 0.520 gm. of citric acid will re-dissolve 0.127 gm. of thorium nitrate; but a solution containing only 0.018 gm. will precipitate on boiling, whilst one containing only 0.01 gm. will not precipitate.

0.5136 gm. of tartaric acid re-dissolves 0.10 gm. of thorium nitrate; 0.028 precipitates on boiling; 0.01 does not.

The heat precipitates tend to re-dissolve on cooling, but this solution is often very slow and imperfect, according to the excess of thorium.

A rather peculiar phenomenon, for which we can offer no explanation, is that crystallization of the compounds lessens their liability to heat precipitation when they are re-dissolved; mixtures containing only a moderate excess of thorium may not precipitate at all under these conditions, as, for instance, the tartaric acid mixture, containing 0.05 gm. of thorium nitrate.

*Effect of acidulation.* — The cautious addition of dilute hydrochloric acid to a sodium citrate solution converts it into citric acid, in which thorium is less soluble; there is consequently precipitation proportional to the quantity of thorium present, and hence, in a balanced solution, proportional to the alkalinity of the original citrate. In citric acid mixtures the hydrochloric acid produces no precipitate, nor in any of the tartrates.

Dilute acetic acid does not decompose the citrate, so that there is no precipitation. Concentrated acetic acid precipitates only in the neutral citrate.

The precipitated thorium citrate is readily soluble in dilute hydrochloric acid, insoluble in acetic acid. Adding an excess of HCl therefore prevents precipitation by excess of thorium, whilst acetic acid does not.

## II. THE QUANTITATIVE ESTIMATION OF THORIUM.

The precipitation of thorium by oxalic acid, and the ready solubility of the precipitate in hot concentrated ammonium oxalate solution, distinguishes this metal from all others, and furnishes a convenient method of isolation and of quantitative estimation. If the precipitation is made in an acid medium, the presence of citrates, etc., does not interfere. The simplest quantitative method is therefore as follows:

*Method A.* — The solution is strongly acidulated with hydrochloric acid, and treated with an excess of oxalic acid solution. The mixture is heated and filtered, and the precipitate is washed with a small quantity of distilled water, and dissolved in a boiling saturated solution of ammonium oxalate. The solution is filtered and again precipitated by acidulating strongly with hydrochloric acid. The precipitate, consisting of thorium oxalate and oxalic acid, is washed,

calcined, and weighed as thorium oxid: 0.3796 gm. of  $\text{ThO}_2 = 1$  gm.  $\text{Th}(\text{NO}_3)_4 \cdot 12 \text{H}_2\text{O}$ .

This method is not quite accurate, since thorium oxalate is slightly soluble in hydrochloric acid. This fallacy can be avoided by either method B or C.

*Method B.*—All the filtrates of method A are evaporated to a small bulk, and this concentrated solution is again put through the method A, the result being added to the former.

*Method C.*—The ammonium oxalate solution in method A is precipitated by ammonium hydrate instead of hydrochloric acid; the hydrate of thorium being less soluble than the oxalate.

This method contains another source of error, since the precipitated hydrate entrains other matter, and retains this so tenaciously that it is not quite pure even after prolonged washing, so that the results are generally rather high. This is also true of the oxalate, but to a less degree.

Notwithstanding these criticisms, these methods yield fairly satisfactory results, not only in pure solutions, but also in the presence of urine and of citrates, as shown in Table I.

With urines, however, it was found that the organic matter was rather disturbing, since it discolored the filtrates and adhered to every precipitate. It was therefore destroyed by incineration. When this is not too prolonged, the residue dissolves very readily in dilute hydrochloric acid; but for safety we resorted to fusion with acid sodium sulphate.

*The method finally adopted for urines* is therefore as follows:

The urine is rendered strongly acid with concentrated hydrochloric acid and heated. This dissolves the precipitates which are commonly present in the urine, and darkens its color. This solution is filtered, and to the filtrate and washings a saturated solution of oxalic acid is added in excess, whilst the solution is still hot, and it is then set aside for a few hours, until it has cooled and the precipitate has well settled. The solution is then filtered. The minute traces of thorium which could escape into the filtrate are disregarded. The filter and precipitate are dried and calcined, and this residue is fused in a porcelain crucible with some acid sodium sulphate. The fused mass is treated with distilled water and filtered. The solution was then treated according to methods A and B.

*For the determination in feces*, we followed at first the same method as for urines. Later, however, we adopted the plan of first incin-



TABLE I.  
THORIUM DETERMINATIONS; CONTROL EXPERIMENTS.

Method.	Detail.	Th(NO <sub>3</sub> ) <sub>4</sub> used.	Added to	ThO <sub>2</sub> found	ThO <sub>2</sub> used	Difference	Error per cent	
A OH Method. Incineration.	{ Boiling with HCl . . Filtrate incinerated . Boiling with HCl . . Filtrate incinerated . { Boiling with HCl . . Filtrate incinerated . Boiling with HCl . . Filtrate incinerated . Boiling with HCl . . Filtrate incinerated . { Boiling with HCl . . Filtrate incinerated . Primary incineration . Primary incineration . Prim. Incin. fused with NaHSO <sub>4</sub>	HCl(COOH) <sub>2</sub> Am. Ox. HCl	0.1 gm.	50 c.c. human urine	0.0346	{ 0.0380	0.0034-	8.9
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	....	....	.0	{ ....	.0	....
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	0.01 gm.	50 c.c. human urine	0.0039	{ 0.0038	0.0001+	0.2
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	....	....	.0	{ ....	.0	....
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	0.01 gm.	50 c.c. human urine	0.0030	{ 0.0038	0.0008-	2.1
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	....	....	.0	{ ....	.0	....
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	0.22 gm.	50 c.c. rabbit urine	0.1035	{ 0.0835	0.0200+	23.9
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	0.1 gm.	50 c.c. rabbit urine	0.0348	{ 0.0380	{ 0.0030+	7.9
		HCl(COOH) <sub>2</sub> Am. Ox. HCl	....	....	0.0062	{ ....	{ 0.0030+	3.1
		Li <sub>2</sub> SO <sub>4</sub> (COOH) <sub>2</sub> Am. Ox. HCl	0.1 gm.	50 c.c. rabbit urine	0.0392	{ 0.0380	0.0012+	5.0
C OH Method. Incineration.	{ Prim. Incin. fused with NaHSO <sub>4</sub>	H <sub>2</sub> SO <sub>4</sub> (COOH) <sub>2</sub> Am. Ox. Ox. HCl	0.2 gm.	50 c.c. rabbit urine	0.0800	{ 0.0760	0.0040+	10.0
		(COOH) <sub>2</sub> Am. Ox. HCl	0.2 gm.	50 c.c. rabbit urine	0.0840	{ 0.0760	0.0080+	5.0
		(COOH) <sub>2</sub> NH <sub>4</sub> OH	0.1 gm.	Water	0.0360	{ 0.0380	0.0020-	21.1
		HCl(COOH) <sub>2</sub> Am. Ox. NH <sub>4</sub> OH	0.25 in cit.	Water	0.11510	{ 0.0950	0.0201+	6.1
		(COOH) <sub>2</sub> Am. Ox. HCl	0.25 in cit.	10 c.c. th. sod. cit. sol.	0.1015	{ 0.0950	0.0065+	5.0
A OH Method. Incineration.	{ Prim. Incin. fused with NaHSO <sub>4</sub>	(COOH) <sub>2</sub> Am. Ox. HCl	0.125 in cit.	5 c.c. th. sod. cit. sol.	0.0500	{ 0.0475	0.0025+	6.9
		(COOH) <sub>2</sub> Am. Ox. HCl	0.0995 ThO <sub>2</sub>	Water	0.0926	{ 0.0995	0.0069-	

erating the feces by placing them on a piece of fine wire gauze, and applying the Bunsen flame beneath. This ash was then fused with the acid sodium sulphate, dissolved in distilled water, filtered, and treated according to methods A and B.

*For the determination in tissues*, the minced material was first digested by the Fresenius-Babo method. The filtrate was treated by methods A and B; the insoluble residue was incinerated and fused with the acid sodium sulphate and then treated by A and B.

This method, however, gave negative results, as shown by the following protocols:

Guinea pig No. 1, weighing 400 gm., was injected subcutaneously with 5 c.c. of a thorium sodium citrate solution (= 0.125 gm. of thorium nitrate). The animal was placed in a porcelain dish and covered with an open bell-jar. It was killed with chloroform after two days, and the whole body treated as just described. No thorium was recovered. The urine, treated by method B, was also negative.

Guinea pig No. 2, 365 gm., was injected with the same quantity of thorium sodium citrate. It was killed immediately, and the body digested by the Fresenius-Babo method. The filtrate was precipitated by oxalic acid. This precipitate was incinerated lightly and dissolved in hydrochloric acid, in which it was completely soluble. This solution was treated by methods A and B.

The filtrate from the original oxalic precipitation was evaporated to dryness, incinerated, dissolved in hydrochloric acid, and treated by methods A and B.

The residue containing the undigested tissue and fat was incinerated and fused with acid sodium sulphate. This was not completely soluble. The solution was treated by A and B.

None of the solutions yielded any thorium.

Our method, therefore, although satisfactory for pure solutions and for thorium added to urines and feces, failed completely with tissues. We have no explanation to offer for this failure. It naturally arouses a doubt whether our method is always reliable for urine and feces.

### III. THE ABSORPTION AND EXCRETION OF THORIUM.

We administered thorium, either in the form of nitrate or of the double citrate, to a series of animals, by various channels, and estimated the quantity excreted in the urine and feces. The results are summarized in Table II.

TABLE II.  
AMOUNT OF THORIUM GIVEN AND AMOUNT RECOVERED, CALCULATED AS THORIUM OXID.

Oral Administration.			Days collected after administration and Amount recovered.									
Animal.	No.	Wt. Kg. Amount	Salt used	Where found	1	2	3	4	5	Total	Per cent.	Method
Rabbit	4	2.0	0.3796	Th (NO <sub>3</sub> ) <sub>4</sub>	Urine Feces	0 0.0331	0	0 0	0	0.0331	8.71	HCl + Heat + (COOH) <sub>2</sub> B " " " " " "
"	4	2.0	0.3796	"	Urine Feces	0 0.0252	0	0 0.0283	0	0.0535	14.09	Fresenius liabo + Incin. of residue
"	4	2.5	0.4745	"	Urine Feces	0.0397	0.0670	0	0.0215	0.1282	27.01	Incin. + NaHSO <sub>4</sub> Fused
Dog	12		0.4745	"	Urine Feces	0	0	0.0447	0	0.0447	9.42	HCl + Heat + (COOH) <sub>2</sub> B Incin. + HCl + (COOH) <sub>2</sub>
INTRAMUSCULAR INJECTION.												
Rabbit	6	1.85	0.136656	Th (NO <sub>3</sub> ) <sub>4</sub>	Urine		0				0.	{ HCl + Heat + (COOH) <sub>2</sub>
"	4	2.75	0.217925	Th cit.	Urine				0.0360	0.0360	16.51	{ Incin. + NaHSO <sub>4</sub>
INTRAVENOUS INJECTION.												
Dog	3	2.5	0.23725	Th cit.	{ Urine Feces	0	0	0	0		0.	Incin. + HCl + (COOH) <sub>2</sub>
"	18	5.5	0.52195	"	Urine	0.0755		0	0	0.0755	14.46	HCl + Heat + (COOH) <sub>2</sub> Incin. + NaHSO <sub>4</sub>
"	11	6.0	0.5694	"	{ Urine Feces	0.03196 <sup>1</sup>	0	0	0	0.03196	5.41	HCl + Heat + (COOH) <sub>2</sub> B Incin. + HCl + (COOH) <sub>2</sub>
"	19	4.0	0.7592	"	Urine	0	0	0	0		0.	HCl + Heat + COOH + Incin. + NaHSO <sub>4</sub>
"	10	8.4	0.79716	"	{ Urine Feces	0	0.0442	0	0	0.0442	5.54	HCl + Heat + (COOH) <sub>2</sub> B
"	15	6.8	1.1388	"	Urine	0.0495		0		0.0495	43.46	Incin. + HCl + (COOH) <sub>2</sub>
"	14	7.5	1.3286	"	Urine		0					" " " "

<sup>1</sup> To 20 Hours = 0.03181; 20 to 24 Hr. = 0.00015.

When the thorium was administered by stomach, it was found in the feces, but not in the urine. When it was injected into the muscles or veins, none was found in the feces, but a considerable proportion could generally be recovered from the urine.

The excretion by the urine was always completed by the second day; by the feces, by the fourth day, and in half of the animals by the second day; or rather our method failed to reveal any thorium after this time.

Only a portion of the thorium was recovered, ranging from 0 to 43 per cent. The apparent loss does not seem to be influenced by the method of administration or by the dose.

The loss could be accounted for either by retention of a part of the thorium in the tissues or by the faults of our methods. The organs were examined for thorium in a number of our animals: Liver (Dogs 10, 11, 14, and 15); kidneys (Dogs 10, 11, 15); lungs (Dog 14); muscle, brain, spleen, blood serum, blood clot (Dog 11); bones (Dog 15); after intravenous administration, also the intestine and contents (Dog 11) and stomach (Dog 14). The result was uniformly negative. In view, however, of the uncertainties of our method when applied to tissues, we are unable to state whether or not the thorium is retained in the tissues.

The fact that thorium was invariably found in the feces and never in the urine, when it was administered by the stomach, whilst the reverse obtained when it was injected intramuscularly or intravenously, indicates that thorium is neither absorbed nor excreted through the alimentary canal. This conclusion, although very probable, cannot be positive as long as we are ignorant of the fate of the thorium which escaped detection.

#### IV. THE PHARMACOLOGIC ACTIONS OF THORIUM.

**Solutions used.** — The first attempts to study the actions and toxicity of thorium emphasized strongly the necessity of distinguishing between the local and systemic actions of the salts. Thorium nitrate, by its metallic ion and by the acidity of its solutions, is a powerful precipitant of proteids, and hence a strong astringent and irritant. This precipitant action can be avoided by the addition of an excess of sodium citrate or tartrate, or proteids, etc., as described in the first section of this paper. We employed mainly the double citrate, our

*standard thorium sodium citrate solution* being prepared by adding 40 c.c. of a 10 per cent thorium nitrate solution to 110 c.c. of a sodium citrate solution, of the freezing-point of a 1 per cent sodium chlorid solution, and containing 2.737 per cent of the anhydrous salt; and making up the total volume to 160 c.c. Each cubic centimetre of this solution therefore, represents 0.025 gm. of  $\text{Th}(\text{NO}_3)_4 \cdot 12 \text{H}_2\text{O}$  and 0.0188 gm. of  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ .

*Precipitation of defibrinated dog's blood and change of hemoglobin.* — The addition of thorium nitrate solutions to defibrinated blood causes a dense precipitate, curd or clot, and changes a part of the hemoglobin into acid hematin. This conversion proceeds rather slowly. (The acid hematin was identified by the change of color to a darker brown, giving a spectroscopic band in the red, remaining unchanged by hydrocyanic acid.) A stronger acid hematin spectrum was obtained in a mixture containing 10 per cent of blood than with 90 per cent of blood, to 0.6 per cent of thorium nitrate, the acidity of the salt being partly neutralized by the blood. A large excess of blood dissolves the thorium precipitate; a mixture containing 0.06 per cent of thorium nitrate to 10 per cent of blood did not precipitate or change in color.

The precipitation and the formation of acid hematin was prevented by sodium citrate, even in mixtures containing 2 per cent of thorium nitrate to 10 per cent of blood, although the thorium citrate mixture was distinctly acid to litmus.

Solutions of thorium nitrate in alkali albumen, tartrates, sodium carbonate, or ammonium oxalate also do not precipitate blood and do not change its color.

*Effect on blood corpuscles.* — A 10 per cent thorium nitrate solution crenates the corpuscles of a frog; a 5 per cent solution causes little, if any, change.

A mixture of 3.5 c.c. of the standard thorium sodium citrate solution and 1.5 c.c. of water does not lake rabbits' corpuscles. A mixture of 2 c.c. of the solution and 3 c.c. of water caused noticeable laking in three hours.

*Clotting of oxalate plasma.* — 3 c.c. of 2 per cent potassium oxalate are added to 47 c.c. of fresh dog's blood, and the mixture centrifugalized until a clear plasma is obtained. A sample coagulates into a firm clot within five minutes after adding a little calcium chlorid. Thorium citrate or the double citrate do not cause clotting, but form a precipitate (of thorium oxalate).

*Astringent action.*—Thorium nitrate solutions have a markedly astringent taste; this is almost absent in the double citrate or tartrate.

*Application to vessels of the web of the frogs' foot, or to the mesentery.*—A 5 per cent solution of the nitrate constricts the smaller vessels, not the larger; the marginal space is obliterated; the arterial stream appears quickened. A 10 per cent solution causes a marked slowing, and eventually intravascular clotting.

*Action on cilia.*—Thorium sodium citrate solution appears to cause a marked slowing of ciliary action in the frog's œsophagus.

*Skeletal muscle, frog.*—Muscle teased in 5 per cent thorium nitrate shrivels; in 10 per cent it becomes more contracted and granular.

The whole muscle laid in the 5 per cent solution becomes whitened and hardened. No spontaneous rhythmic contractions were observed. The standard thorium sodium citrate solution also hardens the muscle, slowly but firmly.

The excitability of the muscle, nerve or endings, is not apparently reduced when the muscle is laid into 10 per cent thorium nitrate for thirty minutes. Thorium sodium citrate also did not diminish the excitability, whereas sodium citrate lessened it noticeably. It appears, therefore, that thorium lessens the toxicity of the sodium citrate.

The contractility of the muscle (height of contraction), on the other hand, is diminished, both by thorium nitrate (5 per cent) and by thorium sodium citrate, more rapidly than by sodium citrate.

The sodium citrate had a considerable veratrin action, which, however, was exhausted so rapidly that a second stimulation generally produced an ordinary, short, and lower contraction. Thorium sodium citrate did not have this effect.

*Effects on the frog's heart.*—Thorium nitrate has very little, if any, effect on the frog's heart, either when injected into the lymph sac, or when applied to the exposed heart, in 10 per cent solution. Thorium sodium citrate appears to be less depressant than sodium citrate.

*Effects on excised intestine.*—The behavior of pieces of excised rabbit's intestines in thorium nitrate and thorium sodium citrate solutions was compared with other solutions; all the reagents (unless otherwise stated) being made of such a strength as to depress the freezing-point by the same degree as a 0.9 per cent sodium chlorid solution. A  $\frac{1}{10}$  solution means that 1 volume of the isotonic solution of the salt was added to 9 volumes of 0.9 per cent sodium chlorid. The intestines were removed immediately after the death of the ani-

mal, cut in pieces about 10 cm. long, and placed in Locke's fluid at 38° C. Oxygen was passed through these solutions during the sojourn of the intestine.

Three sets of experiments lead to the following conclusions:

1. Better and more lasting movements were obtained with the intestines of chloralized rabbits, placed in oxygenated Locke's fluid with the addition of blood, than with those of morphinized rabbits when placed in non-oxygenated solutions. With the former, vermicular movements continued over two hours. In the absence of oxygen they are quickly arrested.
2. Oxygen greatly improves the movements.
3. Vermicular movements were obtained in sodium citrate, sulphate, chlorid, and Locke's fluid, in this order (the movements being strongest and most persistent in Locke's solution, least in the citrate). 2½ per cent NaCl also causes vermicular movements.

In some cases the movements in the citrate and sulphate were arrested in a short time, the intestine becoming relaxed and responding poorly to pinching. Replacement in Locke's solution caused recovery.

4. Tetanic contracture, preceded by vermicular movement, was observed in 1 per cent thorium nitrate in sodium citrate, made isotonic with NaCl; in  $\frac{1}{10}$  per cent BaCl<sub>2</sub>; and in 1 per cent thorium nitrate made isotonic with NaCl.

The effect was weakest with thorium sodium citrate, strongest with the thorium nitrate; barium being intermediate.

The pieces which had been in the barium or thorium sodium citrate, when replaced in Locke's fluid, relaxed and resumed the vermicular movements. Those which had been in thorium nitrate remained contracted in Locke's fluid, being apparently coagulated.

5. The vermicular movements provoked by Locke's fluid, sodium chlorid, sulphate or citrate, or 2½ per cent sodium chlorid; as also the tonic contracture produced by barium chlorid or thorium sodium citrate, are arrested when the pieces are transferred to  $\frac{1}{10}$  CaCl<sub>2</sub>;  $\frac{1}{10}$  MgCl<sub>2</sub>; isotonic MgCl<sub>2</sub>.

The movements resume when the pieces are placed in Locke's fluid.

The contraction produced by thorium nitrate is not relieved by these solutions.

6. Urea causes at first vermicular movement, then tetanic contraction, then relaxation and stoppage of movements. These resume in Locke's fluid.
7. Distilled water arrests the movements, and these do not resume in Locke's fluid.

It appears from these results that sodium citrate produces vermicular movements; thorium sodium citrate, vermicular movements





cardiac end of the femoral vein (twelve experiments). Five animals were used.

The *arterial injections* produced either no effect, or slight increase of heart rate, blood pressure and respiration. In one case there was also some diuresis, in the others not. Even very large doses (two injections, each of 0.084 gm. per kg., Dog 3) did not lower the blood pressure or result in other deleterious effects within several hours.

The *intravenous injections* either produced effects entirely analogous to those of the arterial injections, *i. e.*, mainly negative; or prompt arrest of the heart, followed closely by arrest of respiration. The quantity necessary for this result was variable, but generally in inverse ratio to the concentration and to the rapidity of injection. The smallest fatal quantity is recorded in Dog 3, namely 0.0084 gm. per kg., injected as 0.6 per cent solution, in one-fourth minute. A previous injection of the same dose of the same solution, but extended over four minutes, had no effect. The largest non-fatal intravenous injection was observed in Dog 4, namely, 0.014 gm. per kg., as 6 per cent solution, extended over one minute. The autopsies showed a very dark appearance of the blood, intestinal congestion, and acute congestive nephritis.

The fatal result is to be explained by intravascular clotting, which generally involves the heart when the injection is made into the veins. This will not occur if dilute solutions are used, or if the injection is made slowly, since thorium is soluble in excess of blood. The arterial injection also causes coagulation, but this is confined to unimportant areas.

*Immediate effects of the intravenous injection of thorium-sodium citrate and of sodium citrate.*—The injections were made into the cardiac end of the femoral vein of anesthetized dogs. The solution used consisted of the standard thorium sodium citrate (2.5 per cent thorium nitrate, 1.88 per cent of anhydrous sodium citrate), and sodium citrate solution isotonic with 1 per cent NaCl (2.737 per cent of anhydrous citrate); in some of the experiments a sodium citrate solution of 1.88 per cent was used. The number of the experiments and the dosage will be found in Table IV.

The effects were practically identical for the two solutions, so that it may be concluded that thorium has no effect on the circulation or respiration.

Both the thorium and the sodium citrate, in sublethal doses, cause a moderate fall of blood pressure, with rapid recovery and rise above

TABLE III.  
EFFECT OF THORIUM NITRATE ON THE FROG.

No.	How given	Amount (as Thorium Nitrate)		Symptoms	Death	Autopsy
1	Hypoderm.	0.02	2% Sol.	Respiration increased	2 Days	
2	"	0.1	10% "	" labored	1 Day	
17	"	0.1	10% "	Lies limp	49 Min.	
8	By mouth	0.005	Crystals	Not observed	2 Days	Hemorrhage in intestines
9	"	0.005	"	None	8 "	No hemorrhage or congestion of intestines
10	"	0.005	"	"	6 "	Intestines slightly congested
11	"	....	(NaCl) 0.005 gm.	Control	6 "	" more congested than No. 10
3	"	0.01	Crystals	Respiration increased	5 "	
4	"	0.01	"	Not observed	10 "	
5	"	0.01	"	"	13 "	Hemorrhage in stomach and intestines
THORIUM CITRATE SOLUTION. 1 c.c. = 0.025 Th(NO <sub>3</sub> ) <sub>4</sub> · 12 H <sub>2</sub> O.						
13	Hypoderm.	0.00625	....	None		Alive at end of seven days
14	"	0.0125	....	"		" " " "
15	"	0.01875	....	"	2 Days	" " " "
16	"	....	NaCl	Control	2 "	" " " "

normal. The heart rate was somewhat quickened. The respiration was increased, either in rate or depth, then depressed. Muscular twitchings occurred.

No acute fatality occurred with the thorium solution. Indeed, the results indicate that it lessens the toxicity of the sodium citrate; but the latter appears so variable, depending on the rate of injection, that the results are not absolutely conclusive. Fatal doses of sodium citrate cause arrest of respiration, followed promptly by stoppage of the heart.

The autopsy, with both solutions, showed strong congestion and hemorrhages in the intestine. This cannot therefore be referred to the thorium.

The usual results are illustrated by the following protocols:

#### THORIUM SODIUM CITRATE INJECTIONS.

**Dog 7.** — Weight, 7.5 kg. The animal exhibits convulsive movements.

9.41 A. M. Pressure, 117 mm. of Hg.; heart, 120 per minute.

9.43 to 9.46. *First injection* of thorium sodium citrate, 20 c.c. = 0.5 gm. of thorium nitrate.

The pressure falls 25 mm. during the injection, but recovers rapidly. Pressure at 9.47 = 107; at 9.50 = 123.

9.50 to 9.53. *Second injection.* 29 c.c. = 0.725 gm.

The pressure again falls rapidly by 18 mm., and shows little tendency to recover.

9.57. Pressure, 95; heart, 120.

10.09. Pressure, 81; heart, 140.

10.11 to 10.13. *Third injection.* 50 c.c. = 1.25 gm.

The same phenomena are repeated.

10.12. Pressure, 64.

10.14½ to 10.16½. *Fourth injection,* 50 c.c. = 1.25 gm.

This causes a slight rise of pressure.

10.16. Pressure, 78.

10.17 to 10.18½. *Fifth injection.* 51 c.c. = 1.275 gm.

This produces practically no effect; it is followed by a slight rise.

10.30. Pressure, 88; heart, 112.

10.30 to 10.32. *Sixth injection.* 50 c.c. = 1.25 gm.

The result agrees with the fifth injection.

10.44. Pressure, 107; heart, 140.

10.44 to 10.47. *Seventh injection,* 50 c.c. = 1.25 gm.

The result agrees with the fifth and sixth injections.

The convulsive movements were increased by the injections, but

readily controlled by ether. The respirations increased during each injection.

The animal received in all 300 c.c. of solution = 7.5 gm. of thorium nitrate, or 1 gm. per kg., and 0.74 gm. per kg. of sodium citrate, without any material effect.

The wound was sutured, but the animal died during the night. The intestines were found hemorrhagic; but little significance can be attached to this, since dogs 8 and 9, injected with sodium citrate alone, showed similar appearances. These may have been associated with the presence of animal parasites.

#### SODIUM CITRATE INJECTION.

**Dog 9.** Weight 8 kg. 9.27 A. M.: Pressure, 108; heart, 110; respiration, 20; at 9.30, respiration, 16.

9.30 to 9.32½. *First injection*, 50 c.c. of sodium citrate solution, 2.737 per cent. The blood pressure at first rose, then descended during the injection; after the injection it showed a further rise. The heart was markedly quickened.

9.31½. Heart, 160.

9.35. Pressure, 127; respiration, 15.

9.35½ to 9.40. *Second injection*, 50 c.c. The pressure fell to 70; pulse waves lowered.

9.41. Pressure, 88; respiration, 10, regular, very deep.

9.43. Pressure, 94; rhythmical twitchings of muscles at rate of 120 per min.

9.45. Respiration, 16, deep.

9.46½ to 9.51½. *Third injection*, 150 c.c. This caused a moderate fall of blood pressure; the respiration was at first slowed, but quickened during the injection. The pressure recovered promptly after the injection was stopped; the respiration remained quickened.

9.47½. Respiration, 10, very deep.

9.49. Heart, 120; twitchings have partly disappeared.

9.51½. Respiration, 20.

10.00. Pressure, 94; heart, 124; respiration, 20, very deep. Fibrillary twitching of muscles.

10.01½ to 10.05½. *Fourth injection*, 200 c.c. The injection had at first very little effect; but soon the respiration became irregular, then slowed, then stopped (10.6½). The pressure showed a strong asphyxial rise and then the fall to zero. Artificial respiration, inaugurated at 10.7, did not save the animal.

Including the third injection, the animal had received 6.843 gm. of sodium citrate (= 0.855 gm. per kg.) without serious effect. The fourth injection, making a total of 1.540 gm. per kg., proved fatal.

**Late effects of the intravenous injection of thorium sodium citrate and of sodium citrate.**—Although the injection of thorium sodium citrate is practically devoid of any immediate serious results, the animals are commonly severely depressed for some hours or days after the injection, and many succumb with ill-defined symptoms within the first twenty-four hours; but those which recover and survive for a period of three days live for an indefinite time. They are, however, subject to disturbances of nutrition, frequently connected with severe ulceration of the mouth, which greatly aggravates the condition. The animals became excessively emaciated and very miserable, and had to be killed on this account.

The autopsy of the survived animals is practically negative for doses corresponding to less than 0.5 gm. of thorium nitrate = 0.376 gm. of sodium citrate per kg. This dose is generally fatal; but of the three animals which survived, two (Nos. 14 and 19) showed a remarkable deposition of calcareous material, described in detail under Dog 14. These dogs exhibited the buccal ulceration in extreme degree. Attempts to reproduce these conditions failed on account of the great acute mortality; the only animal which survived (No. 23) did not show the effects. We cannot, therefore, be certain whether the ulceration and calcification were caused by the injections or whether they were merely coincidences. We are also uncertain as to how far the effects were attributable to the thorium, and how far to the citrate. Attempts were made to execute control experiments with sodium citrate alone, but most of the animals died during the injection. Only three survived, a number too small for definite conclusions. Their behavior and symptoms, however, impressed us with the opinion that the effects of the thorium sodium citrate injections are referable mainly, if not solely, to the sodium citrate rather than to the thorium.

The *fatality* of the injections may be gathered from Table IV. With the sodium thorium citrate solution, 1 gm. of thorium nitrate and 0.74 gm. of sodium citrate did not cause death within some hours. The smallest dose which proved fatal within one day contained, per kg, 0.33 gm. of thorium nitrate and 0.28 gm. of sodium citrate; the largest dose which was not fatal in a day, contained 0.5 gm. of thorium nitrate and 0.376 gm. of sodium citrate. With pure sodium citrate, the smallest acutely fatal dose equalled 0.064 gm. The largest dose which did not prove acutely fatal was 0.855 gm.; 0.376 gm. was survived for 33 days. These doses are calculated per kilogram of body weight.

TABLE IV.

FATALITY FROM INTRAVENOUS INJECTIONS. ARRANGED BY ASCENDING DOSES  
(GRAMS PER KG.).

*Thorium Sodium Citrate.*

Animal:	Dose per kg. Thorium Nitrate	Sodium Citrate	Death	Killed	Calcification	Buccal Ulcers
Dog 10	0.25	0.188	....	6 days	none	none
" 11	"	"	....	50 "	"	present
" 13	"	"	....	35 "	"	none
" 18	"	"	....	27 "	"	"
" 30	0.33	0.28	....	27 "	"	"
" 31	"	"	1 day	....		
" 15	0.44	0.32	2 days			
" 19	0.5	0.376	....	11 days	present	present
" 14	"	"	....	24 "	"	"
" 24	"	"	....	23 "	none	none
" 23	"	"	1 day			
" 27	"	"	"			
" 28	"	"	"			
" 29	"	"	"			
Cat 1	"	"	2 days			
Dog 7	1.0	0.74	1 day			

*Sodium Citrate*

Animal	Dose per kg.	Death	Killed	Calcification	Buccal Ulcers
Dog 22	0.064	immediate			
" 26	0.158	"			
" 17	0.188	....	45 days	none	none
" 20	"	....	7 "	"	present
" 8	0.2	Not immediately			
" 21	0.248	immediate			
" 16	0.3	"			
" 25	0.376	....	33 days	"	none
" 8	0.391	immediate			
" 9	0.855	Not immediately			
" 9	1.540	immediately			

*The calcification and buccal ulceration.*—This was most conspicuous in Dog 14; the protocol of the experiment follows:

**Protocol of experiment on Dog 14** (showing the calcification).—Received 0.5 gm. of thorium nitrate and 0.376 gm. of sodium citrate per kg. in-

travenously. Two and one-half hours later the animal appears very sick and vomits.

Next (second) day, very sick; respiration hurried (62), with expiratory grunt. Heart, 128; temperature, 37.8. The urine dribbles constantly.

Animal is hardly able to stand and refuses food.

Third to fifth days. Progressive improvement. Urine controlled, takes first water, later food, with relish. Occasional retching.

Seventh day. Has eaten nothing since fifth. Nauseated and retching.

Eleventh day. Eats again, vomiting and retching present, but less frequent.

Twenty-first day. Foul ulcer on lips and gums, which becomes worse.

Twenty-fourth day. Killed.

*Autopsy.*—White, hard, apparently calcareous deposits are widely distributed. They occur under the parietal and visceral peritoneum, including the under surface of the diaphragm; beneath the serosa of the intestines and appendix, following the course of the vessels; also on the gall-bladder, none on the liver. The mucous folds of the stomach are filled with this material, contained especially in the submucous layer, the walls being very much thickened, especially at the pyloric end and appearing as if filled with earthworms. Four small erosions extend through the mucosa to the muscularis. The mucosa of the intestine appeared normal, perhaps slightly thickened. The deposit was abundant in both lungs, and extended over the vena cava, pericardium, and parietal pleura; in the heart it follows the course of the coronary arteries. It is absent from the valves.

The mouth exhibits large necrotic areas on both lips, and numerous foul ulcers on the tongue.

*The chemical examination of the deposits* shows the absence of thorium and the presence of an abundance of calcium.

Specimens were saved for microscopic examination, but were lost through an unfortunate accident.

*The introduction of thorium nitrate by stomach tube* into dogs (two experiments) does not produce vomiting or any other immediate or late effects, even in doses of 1.25 gm. In rabbits (two animals) it is similarly ineffective, with a dose of 1 gm. per kg., or with 0.5 gm. per kg., repeated four times at intervals.

*The hypodermic injection of thorium nitrate* into a rabbit causes severe local sloughing and slowly healing ulcers, but no systemic effects, in the dose of 0.2 gm. per kg. Another animal died thirty-six days after the injection of 1 gm. per kg.; but in view of the general negative results, the death can scarcely be referred to the systemic action of the thorium.

The intraperitoneal injection of thorium nitrate into two rabbits, in doses of 0.5 and 1 gm. per kg., kills in three to seven days, with the phenomena of peritonitis. This is the probable cause of death rather than by any systemic actions.

The intramuscular injection of thorium sodium citrate. — A dose corresponding to 0.25 gm. of thorium nitrate per kg. produced severe diarrhoea, but no other effect for fourteen days, when the rabbit sickened, dying on the sixteenth day. Since the animal had been kept in the laboratory for over four months, and used during this time for many experiments, the death cannot with certainty be ascribed to the thorium.

The toxicity of thorium. — Deaths resulting from the administration of thorium cannot readily be referred to the metal; for when the nitrate is used, the strictly local actions must be considered; whilst the double citrate, which is free from this objection, raises the question of the apparently greater toxicity of sodium citrate.

In fact we did not encounter a single instance in which we felt justified in referring death to the systemic action of thorium. The toxic dose of thorium can therefore only be stated as greater than the largest quantity which we administered, and which did not cause death, namely (calculated as thorium nitrate, gm. per kg. of body weight):

Thorium Nitrate.	Thorium Sodium Citrate.
Frog, stomach . . . . 0.34	Frog, lymph sac 0.6
Lymph sac . . . . 0.6	
Dog, artery . . . . 0.984	Dog, vein . . . . 1.0 gm. (immediately)
Vein . . . . 0.014	0.5 gm. (after a day)
Stomach . . . . 0.25	
Rabbit, stomach . . . . 1.0	
Muscles . . . . 0.25	

## V. CONCLUSIONS.

Thorium resembles aluminum closely in its chemical and pharmacological actions.

The precipitation reactions, the solubility of the precipitates, and the prevention of the precipitation by various substances, are detailed in the text. The formation of double, non-precipitable salts with certain organic salts and proteids is particularly interesting, and was investigated in detail for citrates and tartrates.

Methods for the quantitative estimation of thorium were elaborated



and found satisfactory for pure solutions, and for thorium added to urine; but they proved unsatisfactory when applied to the recovery of administered thorium.

When thorium is given by mouth, it can be discovered in the feces, but not in the urine; when it is injected into the tissues or into the circulation, it can be demonstrated in the urine, but not in the feces, indicating that it is neither absorbed nor excreted by the alimentary canal.

The excretion by the urine begins promptly; none could be demonstrated after the second day.

The failure of the quantitative method to recover more than a fraction of the thorium renders the conclusions as to absorption and excretion insecure.

Thorium nitrate has the properties of an astringent irritant. Its solutions have an acid reaction and convert hemoglobin into hematin. It precipitates proteids and blood, the precipitate being soluble in an excess of these fluids. It does not cause clotting of oxalate plasma. It coagulates tissues, but this action is superficial, the excitability of frog's gastrocnemius or cardiac muscle being scarcely affected, even by 10 per cent solution. Excised intestine is at first stimulated to vermicular movements, then coagulated. Thorium nitrate is antiseptic. It has a pronounced astringent taste, and contracts the mesenteric vessels on local application. Its injection causes only local effects, according to the site of injection: subcutaneously, sloughing; intraperitoneally, peritonitis; intravenously, death by intravascular coagulation. Its toxicity is very low, rabbits surviving 1.0 gm. per kilo of body weight by stomach; dogs, 0.084 gm. per kg. by artery; frogs, 0.6 gm. per kg. by lymph sac.

The precipitant, astringent, and other irritant effects of thorium nitrate can be avoided by dissolving it in a solution of sodium citrate. This solution produces effects analogous to simple sodium citrate.

The thorium sodium citrate has little if any effect on bacteria or mould. It slows the action of cilia. It does not diminish the excitability of muscle or nerve (counteracting the citrate depression). It lessens the contractility of skeletal muscles (counteracting the veratrin action of the citrate). Its direct application to the heart does not cause marked depression (again counteracting the citrate). Its intravenous injection affects the blood pressure, heart, and respiration but slightly, and in the same direction as sodium citrate; but the toxicity of the latter appears to be largely decreased. Very large doses may

be given, corresponding to 1 gm. of thorium nitrate per kg. of body weight. With these large doses, however, the animal may die after some hours, with lesions of hemorrhagic enteritis, presumably referable to the citrate. Animals which survive the large doses are apt to suffer with disturbed nutrition, and perforating ulcers of the mouth and cornea, and in some cases exhibited a curious calcification of organs. It was not possible to decide in how far these late effects were attributable to the thorium or citrate, or accidental.

# PRELIMINARY OBSERVATIONS ON THE POISONOUS ACTION OF THORIUM.

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## INTRODUCTION.

SEVERAL years ago the senior author began in this laboratory a series of studies of the toxicological effects of rare elements. The first publications in this connection gave the results of an inquiry into the poisonous effects of tellurium.<sup>1</sup> Subsequent communications have presented data pertaining to the toxic influences of compounds of selenium,<sup>2</sup> radium,<sup>3</sup> cerium,<sup>4</sup> and several other rare elements.<sup>5</sup> Further studies are in progress.

<sup>1</sup> GIES: Philadelphia medical journal, 1901, vii, p. 566, and the Therapeutic monthly, 1902, ii, p. 144; MEAD and GIES: American journal of physiology, 1901, v, p. 104; GIES and collaborators: Biochemical researches, 1903, i, p. 40, and Reprints Nos. 20, 21, and 22.

<sup>2</sup> WOODRUFF and GIES: Proceedings of the American Physiological Society, 1901, American journal of physiology, 1902, vi, p. xxix; GIES and collaborators; Biochemical researches, 1903, i, p. 58.

<sup>3</sup> GIES and collaborators: Proceedings of the Society for Experimental Biology and Medicine, 1905, ii, p. 86; also Proceedings of the Section of Biological Chemistry of the American Chemical Society in affiliation with Section C (chemistry) of the American Association for the Advancement of Science, 1905; Science, 1906, xxiii, p. 331, and Proceedings of the American Association for the Advancement of Science, 1906, iv, p. 326; BERG and WELKER: Journal of biological chemistry, 1906, i, p. 371; BURTON-OPITZ and MEYER: Journal of experimental medicine, 1906, viii, p. 245; MEYER: Journal of biological chemistry, 1907, ii, p. 461; MEYER and SALANT: Proceedings of the Section of Biological Chemistry of the American Chemical Society in affiliation with Section C (chemistry) of the American Association for the Advancement of Science, 1906; Science, 1907, xxv, p. 459; GAGER, *Ibid.*, p. 462.

<sup>4</sup> BAEHR and WESSLER: Proceedings of the Section of Biological Chemistry of the American Chemical Society in affiliation with Section C (chemistry) of the American Association for the Advancement of Science, 1906; Science, 1907, xxv, p. 455. This communication includes comparative observations on neodymium, praseodymium, lanthanum, and thorium oxalates.

<sup>5</sup> KNOX and WELKER: *Ibid.*, p. 461. This report gives data pertaining to effects on the growth of seedlings. Compounds of the following rare elements

In January, 1901, shortly before the publication of the results obtained with tellurium, and immediately after the completion of our study of "ureine,"<sup>1</sup> we together began the experiments with thorium that are described below. Our attention was drawn to thorium at that time by various discussions of its radioactivity, which had been discovered about two years previously.<sup>2</sup> The possible importance of radioactivity and radioactive elements from a pharmacological standpoint stimulated our inclination to undertake this work, which seemed to be called for at that time especially, because little or no information had been acquired in this connection.

Our experiments were started while the thorium of Berzelius was still universally regarded as an individual element.<sup>3</sup> Our work in collaboration was discontinued in May, 1901, after learning of Baskerville's<sup>4</sup> and Brauner's<sup>5</sup> announcements that the thorium originally described by Berzelius was a *mixture* of elements. We planned, however, promptly to resume our work in this connection with a study of the toxicology of the new thorium and also of *berzelium* and *carolinium*, names that Baskerville<sup>6</sup> has applied to two elements associated with the new thorium in the old thorium, but thus far this intention has not been realized.<sup>7</sup> Because of this purpose, however, we withheld publication of our preliminary results with a chlorid of the old thorium.

Two years ago we received from Professor Sollmann, for presentation at the February meeting (1905) of the Society for Experimental Biology and Medicine, a brief statement of results then very recently obtained by him and Dr. Brown in a study of the pharmacology of

were used: Beryllium, cerium, cesium, "didymium," erbium, lanthanum, neodymium, praseodymium, selenium, tellurium, and yttrium.

<sup>1</sup> CHACE and GIES: Medical record, 1901, lix, p. 329; GIES and collaborators: Biochemical researches, 1903, i, Reprint No. 31.

<sup>2</sup> A résumé of literature in this connection was given several years ago by Baskerville: Journal of the American Chemical Society, 1904, xxvi, p. 923, also p. 926.

<sup>3</sup> References to preliminary doubts in this connection were made about the same time by Baskerville: *Loc. cit.*, p. 926.

<sup>4</sup> BASKERVILLE: Science, 1901, xiii, p. 827. Also, *loc. cit.*, 1901, xxiii, p. 761.

<sup>5</sup> BRAUNER: Chemisches Centralblatt, 1901, (1), p. 1036.

<sup>6</sup> BASKERVILLE: Journal of the American Chemical Society, 1904, xxvi, p. 922.

<sup>7</sup> Professor BASKERVILLE very kindly consented to give us chemically pure samples of compounds of these three new elements at his earliest opportunity. He has not yet been able to supply them.

thorium.<sup>1</sup> Professor Sollmann's communication induced us to present at that meeting, with his results, the data of our own work in this connection.<sup>2</sup> We thereupon suggested to Professor Sollmann that the detailed results of both researches be published simultaneously, and proposed to him not only that we accordingly delay publication of our own paper on the data obtained in 1901 until he should be ready with his, but also that further investigation of the action of the thorium of Berzelius be left entirely to him and Dr. Brown. The appearance of this and the preceding paper in this issue of the journal occurs in harmony with that understanding. Pursuant to that proposal we have made no additions to our experimental data, and publish them here as they stood when our work was discontinued in May, 1901.<sup>3</sup> When sufficient supplies of chemically pure preparations become available, we hope to take up, in this laboratory, the long-purposed study of the toxic effects of the elements of which the thorium of Berzelius appears to be composed.<sup>4</sup>

#### HISTORICAL.

When our work was started, a review of the literature on the toxicology of the heavy metals indicated that, with the exception of a few experiments by Bokorny, nothing had been done to determine the effects of thorium upon organisms. Bokorny<sup>5</sup> stated that the following forms of lower plants and animals "could remain over a week entirely unharmed in 0.1 per cent solution of thorium sulfate": *algæ*, such as *spirogyra*, *oscillaria*, *palmella*, and *diatoma*; various *zoöspores*; and *phanerogamic plants*, such as *Vicia cracca* (tufted vetch); also *amæba* and *infusoria*. Bokorny's references to his thorium experiments were very brief. Apparently only the strength of solution referred to above—0.1 per cent  $\text{Th}(\text{SO}_4)_2$ —was employed in his work.

In one of our comparative studies of connective tissue glucoproteins

<sup>1</sup> BROWN and SOLLMANN: Proceedings of the Society for Experimental Biology and Medicine, 1904-05, ii, p. 55.

<sup>2</sup> CHACE and GIES: *Ibid.*, p. 56.

<sup>3</sup> The results were described by Dr. CHACE in an unpublished thesis presented by him, in 1903, to the Faculty of Pure Science, at Columbia University, in partial fulfilment of the requirements for the degree of M.A. This thesis was deposited in the Columbia library.

<sup>4</sup> BASKERVILLE's observations on berzelium, carolinium, and the new thorium have not yet been confirmed by other investigators.

<sup>5</sup> BOKORNY: Chemiker-Zeitung, 1894, xviii, (2), p. 1739.

(1900),<sup>1</sup> we found that thorium chlorid in aqueous solution (5 per cent) precipitated mucoids<sup>2</sup> from neutral or alkaline aqueous solutions of mucoid (salts), or from opalescent, acid, mucoid suspensions in water. The precipitates thus produced were soluble in such mixtures, when treated with an excess of the reagent, or of neutral mucoid (salt) solution.<sup>3</sup>

In 1902, in our study with Loeb<sup>4</sup> on the antitoxic action of ions, thorium (nitrate) was used in this connection as a tetravalent element. We found that thorium nitrate exerted little if any antitoxic effects on fertilized *Fundulus* eggs in  $\frac{5}{8}$  *m* NaCl solution. We also observed strong precipitative effects of our thorium nitrate solutions on protoplasm, as well as distinct, though not very striking, toxic influences on various fishes; also on both fertilized and unfertilized *Fundulus* eggs in fresh water and in sea water. None of the latter facts were mentioned in the paper by Loeb and Gies,<sup>5</sup> because these observations had no special relation to the main results of their research.

The following data bearing on the action of thorium are quoted for the first time from the notes on the experiments by Loeb and Gies (1902):

1. Direct action of thorium nitrate on fertilized *Fundulus* eggs.

Nature of the solution.	Percentage of eggs that developed embryos. <sup>5</sup>
100 c.c. of distilled water (control) . . . . .	17
100 c.c. of distilled water + $\frac{m}{250}$ Th(NO <sub>3</sub> ) <sub>4</sub> — $\frac{1}{2}$ c.c. . . . .	24
100 c.c. " " " " 1 c.c. . . . .	23
100 c.c. " " " " 2 c.c. . . . .	31
100 c.c. " " " " 4 c.c. . . . .	14
100 c.c. " " " " 8 c.c. . . . .	4
100 c.c. " " " " 16 c.c. . . . .	0

<sup>1</sup> MEAD and GIES: Proceedings of the American Physiological Society, 1901; American journal of physiology, 1902, vi, p. xxviii. Also GIES and collaborators: Biochemical researches, 1903, i, p. 53.

<sup>2</sup> As thorium compounds, presumably. The results have not yet been published in detail.

<sup>3</sup> This fact indicates that any free HCl, that may have been present, as impurity, in our thorium chlorid preparation, was too slight in amount in these instances to decompose the neutral salt of the mucoid that was added in excess. The amount of free HCl as impurity could not have been sufficient to effect the solution noted.

<sup>4</sup> LOEB and GIES: Archiv für die gesammte Physiologie, 1902, xciii, p. 246; also GIES and collaborators: Biochemical researches, 1903, i, p. 47 and Reprint No. 29.

<sup>5</sup> LOEB and GIES: *Ibid.*

<sup>6</sup> After removal from the animal the eggs were immediately fertilized in sea water with fresh sperm. Half an hour later the fertilized eggs were lifted upon a small strainer and quickly transferred in portions to the series of solutions.

The eggs in the thorium nitrate solutions were surrounded by white, opaque, precipitated matter, the depth and density of which were most marked in the egg, the greater the concentration of surrounding thorium nitrate.

2. Possible antitoxic effects of thorium nitrate on fertilized *Fundulus* eggs in  $\frac{5}{8} m$  NaCl solution.

Nature of the solution.	Percentage of eggs that developed embryos.
A. 100 c.c. of sea water (control) . . . . .	15
100 c.c. of $\frac{5}{8} m$ NaCl (control) . . . . .	0
100 c.c. " " + $\frac{m}{30}$ Th(NO <sub>3</sub> ) <sub>4</sub> — 1 c.c. . . . .	0
100 c.c. " " " " 2 c.c. . . . .	0
100 c.c. " " " " 4 c.c. . . . .	0
100 c.c. " " " " 8 c.c. . . . .	0
B. 100 c.c. of distilled water (control) . . . . .	16
100 c.c. of $\frac{5}{8} m$ NaCl (control) . . . . .	0
100 c.c. " " + $\frac{m}{250}$ Th(NO <sub>3</sub> ) <sub>4</sub> — $\frac{1}{2}$ c.c. . . . .	0
100 c.c. " " " " 1 c.c. . . . .	0
100 c.c. " " " " 2 c.c. . . . .	0
100 c.c. " " " " 4 c.c. . . . .	0
C. 100 c.c. of distilled water (control) . . . . .	49
100 c.c. of $\frac{5}{8} m$ NaCl (control) . . . . .	0
100 c.c. " " + 2 c.c. $\frac{m}{100}$ Th(NO <sub>3</sub> ) <sub>4</sub> . . . . .	0
100 c.c. " " 2 c.c. $\frac{m}{250}$ " . . . . .	1 <sup>1</sup>
D. 100 c.c. of sea water (control) . . . . .	26
100 c.c. of $\frac{5}{8} m$ NaCl (control) . . . . .	0
100 c.c. " " + $\frac{m}{150}$ Th(NO <sub>3</sub> ) <sub>4</sub> — 2 c.c. . . . .	0
100 c.c. " " " " 4 c.c. . . . .	0
100 c.c. " " " " 8 c.c. . . . .	2 <sup>2</sup>
E. 100 c.c. of sea water (control) . . . . .	19
100 c.c. of $\frac{5}{8} m$ NaCl (control) . . . . .	0
100 c.c. " " + $\frac{m}{100}$ Th(NO <sub>3</sub> ) <sub>4</sub> — 2 c.c. . . . .	0
100 c.c. " " " " 4 c.c. . . . .	0
100 c.c. " " " " 8 c.c. . . . .	0

In each of the above solutions containing thorium nitrate, white precipitates surrounding the eggs were very conspicuous, and, as in the previous series, the depth and density of the precipitated layer

<sup>1</sup> This egg was 1 of 96. Nine days after the beginning of the experiment development was rapidly proceeding. As usual, this egg and those that did not develop were surrounded by white precipitates.

<sup>2</sup> Three eggs developed in a lot numbering 163. The embryonic growth did not reach the distinctly pigmented stage.

varied with the concentration of the thorium compound. The solution of thorium nitrate did not yield such turbidities or precipitates when added, even in excess, to distilled water or  $\frac{5}{8}$  *m* NaCl solution.

Aso<sup>1</sup> has recently shown that thorium nitrate, in proportions of from 10 to 100 mg. per kilo of soil, failed to manifest any marked action on phanerogamic plants.

#### EXPERIMENTAL.

*Thorium compound employed.*—We used thorium chlorid in all our experiments. The best product that could be conveniently purchased when we began our work was a "C. P." Schuchardt preparation, which seemed to have very little free HCl in it and apparently no free chlorin.<sup>2</sup> Aqueous solutions of the product were distinctly though not very strongly acid,—a condition due chiefly to hydrolytic dissociation and the consequent presence of the ions of HCl.

*Animals.*—Our experiments were carried out on frogs and dogs. A mouse was also used. The protocols of the experiments are given below.

#### FIRST GROUP OF EXPERIMENTS. ON COLD-BLOODED ANIMALS (FROGS).

Only lively, vigorous frogs were selected from a large number of healthy-looking ones that were available for these experiments. During an experiment the individual frog was kept, as a rule, in a tall glass jar containing a shallow layer of water. The latter was changed several times daily, so that the animal rested in comparatively fresh water, and could not be materially affected by its own excretory products. From time to time during an experiment the animal was removed to a wet glass plate under a very large, slightly tilted bell-jar, with aperture at the top, in order to afford particularly favorable opportunity for observations of the condition of the animal when its movements were relatively unrestricted. Each frog was

<sup>1</sup> Aso : *Chemisches Zentralblatt*, 1904, (2), p. 49.

<sup>2</sup> The amount of water of crystallization in thorium chlorid preparations differs considerably. There is decided disagreement among the figures for composition of thorium chlorid products that have been obtained under approximately equal conditions by different investigators. Our product probably had the composition indicated by the formula  $\text{ThCl}_4 \cdot 7\text{H}_2\text{O}$ , and was doubtless prepared by the method described by Krüss [*Chemisches Zentralblatt*, 1897, (2), p. 252].



kept under observation during a preliminary period for determination of its condition, peculiarities, if any, etc., before an experiment was begun.

Unless statements appear to the contrary, each dose of thorium chlorid was dissolved in 1 c.c. or less of water.

**Series I. Effects of subcutaneous injection.** — In all of these experiments the dose of thorium chlorid was injected, at a point high up the back, into a lymph sinus. It was thus impossible for any portion of the dose to leave the body through the minute opening made by the needle, whether the animal remained in a sitting position or hopped about.

*Experiment 1.* — Weight of the frog, 25 gm. Three doses, 5 mg. each, were injected at 10.35 A. M., 11.35 A. M., and 12.35 P. M., without eliciting any observable symptoms, except moderate excitement for a few minutes each time after the conclusion of the operation. The animal was under observation for a week afterward (see Experiment 8). *Total dose* (15 mg.) per gram of body weight was 0.5 mg.

*Experiment 2.* — Weight of the frog, 16 gm. One dose, 1 gm., at 2.30 P. M. The skin in the immediate vicinity of the point of injection rapidly assumed a shrunken appearance and soon became dry. Stupor appeared at once and the animal sat with eyes closed. 2.50. Irritability diminishing. Muscles set and weak. When the legs were pulled out, several minutes elapsed before they were drawn back again to their usual position. When turned upon its back, the frog could not right itself. Shrivelled and anhidrotic condition of skin increasing. 3.00. All power of co-ordinated voluntary movements lost. Tapping the nose failed to arouse. 3.30. Completely prostrated. Could be aroused only by introducing a probe deeply into a nostril. 3.50. Corneal reflex, as well as all other reflexes, abolished. 4.05. Dead. Lived one hour and thirty-five minutes after dosage. *Dose* (1 gm.) per gram of body weight was 62 mg. *Post mortem.* Subcutaneous tissue, wherever the thorium chlorid extended, was hard and yellowish white. Heart-beats could be induced by mechanical stimulation. All organs appeared to be normal.

*Experiment 3.* — Weight of the frog, 13 gm. One dose, 0.5 gm., at 3.25 P. M. 3.30. Marked loss of activity. When turned on its back, the animal remained in that position for some time before righting itself. Eyes closed. Occasionally attempted to jump, but moved only slightly. When on its back slight twitchings of the abdominal muscles were noticeable. All the muscles in a spastic condition. 3.50. When turned on its back was unable to right itself. Skin dry and shrunken about point of injection. 4.20. Complete loss of motor power. Conjunctival reflex

faintly elicited. Slight movement of legs when probe was placed in the nose. 4.45. Only sign of life was a slight movement of the legs when the nares were irritated. 4.50. Dead. Lived one hour and twenty-five minutes after dosage. *Dose* (0.5 gm.) per gram of body weight was 39 mg. *Post mortem*. Same results as those of Experiment 2.

*Experiment 4.*—Weight of the frog, 13 gm. One dose, 0.25 gm., at 10.15 A. M. Activity at once diminished. Eyes nearly closed. 10.25. Skin dry and shrunken about point of injection. When turned on its back had just enough strength to right itself, after great effort. 10.45. Sits with eyes open. When irritated, crawls, but cannot hop. Skin has changed from a bright green to a brown color. 10.50. Very weak; unable to draw up the hind legs when they were pulled out. Eyes closed; pupils contracted. 11.00. Complete loss of voluntary motion. Loss of all reflexes about the eyes. Shrivelled area of skin increased. 11.15. Nasal reflexes abolished. 11.35. Dead. Lived one hour and twenty minutes after dosage. *Dose* (0.25 gm.) per gram of body weight was 19 mg. *Post mortem*. Integument changed from green to brown. Modification of tissue about the point of injection somewhat less marked than in Experiments 2 and 3. Liver unusually dark. Gall-bladder greatly distended and filled with bile. Other organs seemingly normal.

*Experiment 5.*—Weight of the frog (female, with eggs), 18 gm. One dose, 0.1 gm., at 9.40 A. M. 9.50. General weakness, especially marked in legs. Front legs nearly useless. 9.55. Complete paralysis of fore legs. Hind legs very weak. Unable to assume sitting position when turned on her back. Eyes closed. Skin dry and shrunken about point of injection. 10.20. Completely prostrated. Remained in any unnatural position in which she was placed. Muscles stiff. 10.35. Dead. Lived fifty-five minutes after dosage. *Dose* (0.1 gm.) per gram of body weight was 6 mg. *Post mortem*. Slight destruction of tissue around point of injection. Organs apparently normal.

*Experiment 6.*—Weight of the frog, 13 gm. One dose, 0.05 gm., at 2.55 P. M. Observations were made during eight days.

First day. 11.00 P. M. No effect since.

Second to eighth days. During the following week nothing abnormal was noticed. (See Experiment 7; also Experiment 13, in which a similar dose produced effects when given by the mouth.)

*Dose* (0.05 gm.) per gram of body weight was 4 mg.

*Experiment 7.*—Weight of the frog, 14 gm. One dose, 0.05 gm., at 10.25 A. M. Observations were made during two days.

First day. 12.30 P. M. Skin dry. Signs of general weakness. 2.30. Torpid. When turned on its back did not attempt to get up.

Second day. 9 A. M. Very stupid. Unable to move. 5 P. M. Dead. Lived thirty hours and thirty-five minutes after dosage.

*Dose* (0.05 gm.) per gram of body weight was 4 mg. *Post mortem.*  
Congestion of gastro-intestinal tract. Otherwise normal.

**Series II. Effects of administration per os.** — In all of these experiments the dose of thorium chlorid was administered through a pipet with a long, narrow, blunt tip. The administration of the dose with this pipet could be very readily accomplished.

*Experiment 8.* — Weight of the frog, 25 gm. This animal had been used in Experiment 1, and had received doses of 5 mg. *subcutaneously* at 10.35 A. M., 11.35 A. M., and 12.35 P. M., without observable effects, except excitement, due probably to local irritation.

Three doses, 5 mg. each, were given per os. at 3, 4, and 5 P. M., without noticeable symptoms, except a few peculiar head movements that appeared to be due to irritation of the throat. The animal remained under observation for a week afterward without exhibiting any symptoms.

*Total dose* (15 mg.) per gram of body weight was 0.5 mg.

*Experiment 9.* — The frog (weight, 25 gm.) had been used in Experiments 1 and 8. This experiment was begun a week after the conclusion of Experiment 8.

Eight doses, 20 mg. each, were given at irregular intervals, beginning at 9.50 A. M.

First dose, 9.50 A. M. No effects except head movements due to irritation of the throat.

Second dose, 10.50 A. M. Movements sluggish. Hind legs flaccid and outstretched.

Third dose, 11.50 A. M. Weakness increasing.

Fourth dose, 12.50 P. M. Decidedly weak. Unable to hop. Moved sluggishly when stimulated vigorously. 1.30. Eyes half closed, fore feet turned under the body, hind legs outstretched, sides of abdomen drawn in.

Fifth dose, 2.10 P. M. Weakness still more marked. Movements lack co-ordination. Mouth open, eyes closed.

Sixth dose, 3.10 P. M. Falls sideways when attempting to crawl. Skin dry, respiration slower.

Seventh dose, 4.10 P. M. Weakness so great that the animal lies prostrate on its abdomen. Eyes closed. Aroused by mechanical stimulation.

Eighth dose,<sup>1</sup> 5.10 P. M. Fore legs do not respond to mechanical stimulation, but hind legs do. 7.30. Very inert. Aroused with difficulty. When placed on its back remained in that position without attempting to turn over. When subjected to vigorous mechanical stimulation attempted to rise, but failed to do so. Midnight, prostration complete.

<sup>1</sup> Variable, though only small proportions of most of the doses were ejected shortly after administration.

Almost insensible to stimulation. Next morning (7 o'clock), found dead, prostrate on abdomen.

Lived more than fourteen hours after the first administration.

*Total dose* (160 mg.) per gram of body weight was 6 mg. *Post mortem*. Nothing abnormal except congestion of the alimentary tract.

*Experiment 10.*—Weight of the frog, 18 gm. Three doses, 100 mg. each, were given at hour intervals, beginning at 9.50 A. M.

First dose, 9.50 A. M. Initial irritant effect. Animal jumped about for a few minutes, with mouth open, then assumed normal appearance.

Second dose. 10.50. For a time moved quickly backwards repeatedly as if endeavoring to get away from the irritant in the mouth and throat. Responded promptly to mechanical stimulation, though was obviously weaker and less active. Mouth open most of the time.

Third dose. 11.50. Muscular weakness more evident. Crawled when irritated, but could not hop. 12.30 P. M. Watery matter ejected from the stomach. Mouth open continuously. 12.50. Laid in an awkward position with eyes closed and failed to move when vigorously stimulated. 2.00. Skin dry. Fluid dribbled from the mouth. Laid on belly with limbs outstretched. When placed on its back was unable to turn over. Liquid ejected from stomach several times since 12.30. Mouth constantly open. Eyes remained closed even during moments of marked response to stimulation. 2.30. General prostration. 3.00. Dead.

Lived five hours and fifty minutes after the first administration.

*Total dose* (300 mg.) per gram of body weight was 17 mg. *Post mortem*. Distended stomach, full of yellowish mucus. Nothing noteworthy otherwise.

*Experiment 11.*—Weight of the frog, 25 gm. Two doses, 100 mg. each, on different days. Observations were made during four days.

First day. *First administration*, 3.30 P. M. Frequent head movements, evidently due to throat irritation. Swallowed repeatedly. Aside from these effects, which were exhibited frequently for several hours, and a certain degree of restlessness, there were no special symptoms. Skin remained moist. 11 P. M. Sat awkwardly. Not very susceptible to mechanical stimulation.

Second day. 1 P. M. Abundant deposit of white matter in throat, otherwise apparently normal. *Second administration*, 1 P. M. At 7 P. M. looked sleepy, but moved promptly when touched. Otherwise. nothing noteworthy.

Third day. 9 A. M. A large white mass, composed of epithelial cells and of connective tissue fibres, apparently ejected from the throat, was found in the water beside the animal. Otherwise, appeared to be normal. 9 P. M. Inactive.

Fourth day. 10 A. M. Bloated. Skin loose here and there; pieces of

it detached. When placed on its back, turned over with difficulty. Stupor. 1 P. M. Prostration general. 2 P. M. Dead.

Lived ninety-four hours and thirty minutes after the first administration.

*Total dose* (200 mg) per gram of body weight was 8 mg. *Post mortem.* Intestines inflamed. Stomach distended and full of a very thick yellowish mucus. Heart beats when touched. Blood-vessels under skin much distended. Liver very yellowish. Throat inflamed and lined with bloody mucus. Muscles normal. Blood corpuscles apparently unaffected. (See Experiments 13 and 15.)

*Experiment 12.*—Weight of the frog, 26 gm. One dose, 1 gm., at 10.50 A. M. Immediately ejected some of the solution. Then jumped about very strenuously, striking the sides of the confining vessel and falling backwards only to turn over quickly and jump again. Eyes closed. Sides drawn in. Rests head against the side of the jar. Gasps and froths at the mouth. 11.00. Unable to sit normally, but falls to one side; cannot move legs co-ordinately. 11.05. Toes doubled up. Legs in abnormal positions. When irritated, hind legs are thrown backwards without moving the body. Front legs are paralyzed. Eyes closed. Unable to move about. 11.15. Dead. Lived twenty-five minutes after dosage.

*Dose* (1 gm.) per gram of body weight was 38 mg. *Post mortem.* Stomach contracted and hard. A little reddish fluid in the abdominal cavity. Heart failed to beat when touched. (See Experiment 14.)

*Experiment 13* (begun as a repetition of Experiment 11).—Weight of the frog, 23 gm. One dose, 100 mg., at 11.25 A. M. No apparent effect for about two hours, except repeated swallowing and head movements due to irritation of the throat. 2 P. M. Sat stupidly with chin close to plate. Jumped when vigorously irritated. Defecated dark, greenish mucous material. 4.00. Lying quietly. Occasionally swallows in the usual peculiar manner. 4.25. Eyes closed. Not active when aroused. 6.15. Sprawling. Very weak. Seems unable to sit in a normal position. 7.30. Dead. Lived eight hours and five minutes after dosage.

*Dose* (100 mg.) per gram of body weight was 4 mg. *Post mortem.* Stomach widely distended and full of fluid. Other organs apparently normal. (See Experiment 15.)

*Experiment 14* (repetition of Experiment 12).—Weight of the frog, 27 gm. One dose, 1 gm., at 2.18 P. M. Very lively at once. Usual swallowing motions very pronounced. Gasps and froths at the mouth. Gradually prostrated, with eyes half closed and sides drawn in. 2.40. A little mucous matter exudes from the mouth. Lively when disturbed. 3.05. Sits upright and continues to froth at the mouth. 3.25. Legs weak. Fore legs turned under. 3.40. Almost lifeless. Gasping frequently. Unable to move. 3.45. Several convulsive movements of whole body.

Lies on belly, back arched and hind legs extended. 3.50. Unable to turn over when placed on its back. Remains in any position, however unnatural. 4.00. Completely prostrated. 4.20. Two series of convulsions in posterior parts. 4.25. Spasmodic movements when prodded. 4.35. Dead. Lived two hours and seventeen minutes after dosage.

*Dose* (1 gm.) per gram of body weight was 37 mg. *Post mortem*. Stomach very hard and full of brownish, thick mucus, which was also present in throat and mouth. Heart began to beat when touched.

*Experiment 15* (begun as a repetition of Experiment 11).—Weight of the frog, 23 gm. One dose, 100 mg., at 9.30 A. M. first day. Observations were made during nine days.

First day. 9.30 A. M. Peculiar swallowing movements were quite marked, from the moment of administration. 10.00. Sat quietly with eyes half closed, but lively when disturbed. 10.15. Sat with head lowered and back curved upwards. Eyes completely closed. 11.00. Occasional attempts at swallowing, but otherwise apparently normal. 12 M. Rested quietly with head down and eyes closed, but was active when disturbed. 2.30 P. M. Appeared sluggish; head lowered, skin dry and eyes open. 9.00. No particular change.

Second day. 11 A. M. Although resting in a shallow layer of water, the frog's skin above the water is dry. (Skin of normal frog alongside is wet all over.) Eyes closed, lids dry. Not easily disturbed. Very stupid looking.

Third day. 9 A. M. Much improved.

Fourth to ninth days. Apparently entirely well from the fourth day, until the morning of the ninth day when it was found dead. The associated normal frog remained entirely well.

Lived nearly nine days after dosage.

*Dose* (100 mg.) per gram of body weight was 4 mg. *Post mortem*. Stomach and intestines edematous and full of reddish mucus. The other organs appeared to be normal. (See Experiment 13.)

*Experiment 16* (carried out to ascertain whether the swallowing movements following each administration of thorium chlorid (Exps. 8-15) were due to the local action of a portion of the dose or were the results of irritation caused by the glass pipet used for the injections).—Weight of the frog, 20 gm. Three administrations of water, in 1 c.c. doses, in the usual manner, at 9.25 A. M., 11.00 A. M., and 12.00 M. There were no swallowing movements after the first administration. During the first five minutes after the second administration, one hour and thirty-five minutes later, however, the animal exhibited a few of the usual movements of the head. These movements did not recur in the interval before the third administration, after which they again ceased in a minute or two.

**Series III. Effects of introduction per rectum.** — Doses were introduced per rectum with the aid of the pipet used for administration per os.

*Experiment 17.* — Weight of the frog, 19 gm. One dose, 50 mg., at 9.30 A. M. No apparent effects for about an hour. 10.30. Signs of weakness. 11.00. Unable to sit up when turned on its back. Legs flabby and remain extended when pulled out. 11.10. Cannot move. 11.20. Dead. Lived one hour and fifty minutes after dosage. *Dose* (50 mg.) per gram of body weight was 3 mg. *Post mortem.* Edema in right leg. Under surface of body hyperemic. Lower part of intestine somewhat hardened. (See Experiment 18.)

*Experiment 18* (repetition of Experiment 17). — Weight of the frog, 17 gm. One dose, 50 mg., at 11.50 A. M. 12.30 P. M. Weaker. 12.40. Very weak. Skin dry. 1.00. Cannot turn over when placed on its back. 1.45. Paralysis complete. 3.00. Dead. Lived three hours and ten minutes after dosage. *Dose* (50 mg.) per gram of body weight was 3 mg. *Post mortem.* Nothing abnormal except hardening of the lower part of the intestine.

*Experiment 19.* — Weight of the frog, 25 gm. One dose, 100 mg., at 2.30 P. M. Immediately passed most of the dose. No effects for about fifty minutes, except signs of excitement; animal very lively until 3.20, when it began to weaken. 3.30. Very weak. Abdominal surface congested. 3.40. Dead. Lived one hour and ten minutes after dosage. *Dose* (100 mg.) per gram of body weight was 4 mg. *Post mortem.* Fluid in peritoneal cavity. Stomach distended and full of mucus. Much of intestinal tissue near rectum hardened and disintegrated.

*Experiment 20.* — Weight of the frog, 18 gm. One dose, 1 gm., at 11.52 A. M. Immediately jumped twice and fell backwards unable to right itself. Quivering of hind legs, gradually extending to entire body. 12 M. *Hind legs* outstretched, stiff and dry. Crawls with *fore legs*. Under surface of hind legs hyperemic. 12.05 P. M. Fore legs stiff. Whole body rigid. Only noticeable movement is a slight twitching of the skin under the lower jaw. Toes of fore feet turned under. Ventral surface of body drawn up as if abdominal muscles were strongly contracted. 12.15. Integument on under surface of body and hind legs red, dry, and shrunken. 12.20. Mouth red on inside. Only signs of life are reflex responses about the nose and eyes. 12.40. Dead. Lived forty-eight minutes after dosage. *Dose* (1 gm.) per gram of body weight was 56 mg. *Post mortem.* Much black fluid in peritoneal cavity. Edema of skin. Heart did not beat when stimulated. Blood very black. Whole alimentary tract hardened and contracted and lower part of intestine disintegrated. General blackening of superficial blood vessels.

*Experiment 21* (to ascertain the effects of water introduced per rectum by the method employed in Experiments 17-20). — Weight of the frog,

20 gm. Several administrations, 2 c.c. of water at a time, at intervals of about an hour, caused no observable symptoms.

## SECOND GROUP OF EXPERIMENTS. ON WARM-BLOODED ANIMALS (MOUSE AND DOGS).

The animals selected for these experiments appeared to be normal in all respects. After dosage, the mouse (Exp. 22) was kept under the bell-jar used in the preceding experiments. The dogs were selected after preliminary periods of observation had afforded ample opportunity for detection of individual peculiarities. During the experiments they were kept in cages similar to the one recently described.<sup>1</sup>

The injected doses of thorium chlorid were always dissolved in small volumes of water, which never exceeded 4 c.c. The subcutaneous injections were made on the right side in the lumbar region.

**Series I. Effects of subcutaneous injection [Mouse (22) and Dogs (23 and 24)].** *Experiment 22.* — Weight of mouse, 17 gm. One dose, 250 mg., at 9.55 A. M. No immediate effect except rapid and labored respiration. 10.10. Signs of slight irritation at the point of injection. 10.40. Walks awkwardly and shows signs of general muscular weakness. Has lost all co-ordinated use of the fore legs. 11.30. Looks stupid. There has been rapid and labored respiration since injection. 12.00 M. Increasing stupor. Has urinated once. 12.30 P. M. Eyes dull and nearly closed. Breathing very labored. Head lowered. Urinates frequently. 12.55. Retching movements, as if trying to vomit. Gasps occasionally. Great general weakness. 1.05. Difficulty in breathing. Gasping every few seconds. Mouth opened widely; moves with difficulty. 1 to 2. Gradual decline. Respiration slow and difficult; gasping repeatedly. When placed on its back is unable to rise. Cannot move. Prostration gradually becomes general. 2.15. Respiration slow. Eye reflex absent. 2.30. Breathing spasmodic. 2.35. Dead. Lived four hours and forty minutes after the injection.

*Total dose* (250 mg.) per gram of body weight was 15 mg. *Post mortem.* Organs normal. Tissue about point of injection hardened and blanched.

*Experiment 23.* — Weight of the dog, 6½ kilos. One dose, 2 gm., at 10 A. M. Observations were made during nine days.

First day. 10 A. M. Injection immediately caused marked local irritation. Hind leg on injected side was quickly weakened. 10.10. Appears to be sleepy. 10.20. Twitching of muscles all over the body and

<sup>1</sup> GIES : This journal, 1905, xiv, p. 403.



a slight tremor, increasing to a marked trembling of the whole body. 10.30. Ate proffered meat. 10.45. Nearly as lively as before injection. Twitching less marked. 11.00. Nose dry and warm. 11.15. Lies on its back in very unnatural position and trembles. 11.30. Walked about and appeared normal. 12.50. No change. 3.00. Recumbent and trembling most of the time. Weak. 5.00. Same as at 3. Somewhat sleepy. Ate a good-sized piece of meat, but was not especially anxious for it. 8.00. Sat up with great difficulty when aroused and drank water quite eagerly. 10.30. Nose temperature normal. Unable to jump out of cage. One hind leg very stiff. When taken from cage and put on floor, was able to move about, though awkwardly. Not fed yesterday. Only little food and water accepted to-day.

Second day. 12.30 A.M. Quite stupid. Trembling still marked. Appetite better.

Third day. 10.30 A.M. Hind leg very stiff and sore. Otherwise same. Nose cold. 7.15 P.M. Able to jump from cage. Appetite normal.

Fourth day, 10 A.M. Leg very stiff. Tissue hard around point of injection. Nose warm. Unable to rise. Walks quite well, however, when put on his feet.

Fifth day. Stiffness seems to be getting less marked.

Sixth day. Left hind leg edematous. Painful when touched. The overlying skin shows signs of breaking down. Jumps out of cage with difficulty.

Seventh day. Opening in skin at point of injection; flesh greenish-gray beneath. Dog jumps out of cage more easily.

Eighth day. Rapidly recovering. Skin opening is enlarging by sloughing.

Ninth day. 12.30 P.M. Killed under chloroform anesthesia. *Total dose* (2 gm.) per kilo of body weight was 0.31 gm. *Post mortem*. Marked degeneration of tissue about the point of injection. The surrounding tissues were indurated, proteins having been precipitated. All the organs appeared to be normal.

*Experiment 24.*—Weight of the dog, 15 kilos. One dose, 5 gm., at 10 A.M. Observations were made during eight days.

First day. Immediately after injection there was great uneasiness. The dog rolled over and remained some time on his back. There were peculiar movements of the jaws as if due to a bad taste. Rapid and labored breathing at first, but after lying down respiration became normal. Frequent stretching. Marked stiffening of the hind leg on side of injection. Limp when walking. Licks jaws and appears nauseated. Twitching of muscles and general trembling. 12 M. Sleepy. Twitchings continue. 1 P.M. Increase of muscular symptoms. 2.00. More stupid. Twitching becomes general. 3.00. Resting quietly with slight quivering of muscles.

4.00. Trembling continues, especially well marked in front legs. A hard swelling over the point of injection. 5.00. Very dull. No desire for food. Thirsty. Midnight, same.

Second day. Much brighter. Tissue about point of injection hard and painful to the touch.

Third day. Seems to be much better. Increasing induration at site of injection. Appetite normal; still very thirsty.

Fourth day. Not particularly ill. Lies quietly. Appears to be unable to rise. When put on all fours staggers very decidedly. Trembles on standing. Some fever. Breathes rapidly. Appetite and thirst normal.

Fifth day. Much better. Able to jump out of cage. When released, ran about in lively manner.

Sixth, seventh, and eighth days. With exception of the indurated mass about the point of injection the dog appeared to be normal. At the end of the eighth day the indurated mass referred to had degenerated considerably and the skin above it had opened.

*Dose* (5 gm.) per kilo of body weight was 0.33 gm.

(This animal was used on the ninth day in Experiment 28.)

**Series II. Effects of administration per os. Experiment 25.** — Weight of the dog, 6½ kilos. One dose, 2 gm., administered at 9.45 A. M. in one of two pieces of meat, each weighing 50 gm. Observations were made during a period of three days.

First day. No immediate effects. 10.20 A. M. Less lively. Moved jaws as if there were gastric disturbance. 11.00. Vomited both pieces of meat. Practically all the thorium chlorid had dissolved and disappeared from the meat. Soon ate the piece of meat which had not contained thorium. Tasted the other piece, but would not swallow it. Liquid vomit was flocculent, not slimy. 12 M. Ate nearly all of second piece of meat with its thorium chlorid. 12.30 P. M. Ate the rest of the meat. 12.45. Very thirsty. 2.10. Appetite seemed impaired. Very anxious for water, which was taken eagerly.

Second and third days. Apparently normal.

*Dose* (2 gm.) per kilo of body weight was 0.31 gm.

*Experiment 26.* — Repetition of Experiment 25 with a dog weighing 8 kilos yielded the same results.

*Dose* (2.5 gm.) per kilo of body weight was 0.31 gm.

**Series III. Effects of intravenous introduction. Experiment 27.** — Weight of the dog, 10 kilos. Light ether anesthesia. Death resulted during the process of slowly injecting into a jugular vein 1 c.c. of water containing 1 gm. of thorium chlorid. Death was so sudden that symptoms were not observed. *Post mortem.* The heart was distended and full of blood. The right auricle contained a black flocculent mass — evidently blood proteins precipitated by thorium chlorid. Such a precipitate

was obtained upon adding thorium chlorid solution to fresh blood.  
*Dose* (1 gm.) per kilo of body weight was 0.1 gm.

*Experiment 28.* — Weight of the dog, 15 kilos. (Had previously been used in Experiment 24.) 1 cc. of aqueous 0.8 per cent NaCl solution, containing 0.1 gm. of thorium chlorid, was injected slowly into a femoral vein. Almost immediately muscles in various parts of the body began to twitch, decided general tremor quickly followed, and tetanic convulsion soon ensued. Death, apparently from asphyxia, occurred about two minutes after the injection. The heart continued to beat slowly for a minute or two after respiration ceased. Blood, drawn immediately after death, appeared to coagulate normally.

*Dose* (100 mg.) per kilo of body weight was 7 mg. *Post mortem.* Blood darker than usual. Right side of heart full of coagulum. Organs apparently normal.

*Experiment 29.* — Weight of the dog, 25 kilos. In morphin-atropin narcosis.<sup>1</sup> Physiological salt solution (0.8 per cent), containing 0.01 gm. of thorium chlorid per c. c. of solution, was injected at intervals slowly into a femoral vein. The following summary shows the frequency of the respiration and heart beat before, during, and after each injection :

	Time.	Respiration.	Heart beat.
2.36	P. M. . . . .	16	120
2.40	" . . . . .	20	108
2.45	" . . . . .	18	108
Injection :	3 c.c. (2.45-46:30)	18	102
2.50	P. M. . . . .	16	102
Injection :	5 c.c. (2.51-52:10)	16	102
2.55	P. M. . . . .	20	102
Injection :	5 c.c. (2.57-58)	22	102
3.01	P. M. . . . .	20	102
Injection :	7 c.c. (3.02-03:40)	16 (spasmodic)	162
3.06	. . . . .	20	112
Injection :	8 c.c. (3.08-09:10)	much faster	very rapid

Respiration stopped at 3.10, after a few gasps. The heart continued to beat a minute and a half longer.

*Total dose* (280 mg.) per kilo of body weight was 11 mg. *Post mortem.* Same as for Experiment 28.

<sup>1</sup> Morphin, 6 mg. per kilo; atropin, 0.6 mg. per kilo.

## SUMMARY OF RESULTS.

The results of our observations with thorium chlorid may be briefly summed up as follows :

Thorium chlorid readily dissolves in water. The solution is strongly acid in reaction, markedly astringent in effect, and has an acid, metallic taste. Aqueous solutions of thorium chlorid precipitate proteins and protoplasmic materials in general, blacken and precipitate blood, blanch and harden muscle, and harden and shrivel practically all tissues. Upon the unbroken skin it has no appreciable effect. Fairly concentrated aqueous solutions, when introduced into the stomach or rectum, harden and shrivel the gastro-intestinal parts involved ; when injected under the skin they induce local precipitation, hardening, and degeneration ; and, when passed into the blood, precipitate the proteins. The reflexes were abolished preceding death in their usual order.

Doses of more than 4 mg. per gram of body weight (4 gm. per kilo), whether introduced into frogs subcutaneously, *per os*, or *per rectum*, invariably resulted fatally. One of two frogs that received *subcutaneously* 4 mg. per gram of body weight survived, the other died at the end of thirty hours. One of two frogs that received such a dose *per os* died at the end of eight hours ; the second died, on the ninth day after dosage, possibly from influences independent of the treatment. Introduction *per os* caused irritation of the throat, increased mucous secretion, and ejection of gastric contents. Doses of 3 mg. per gram of body weight, introduced into frogs *per rectum*, were fatal in one to three hours. Symptoms appeared more quickly in frogs, after introduction *per rectum*, when all other conditions were equal, than they did after administration *per os* or subcutaneously.

In frogs, the following symptoms were usually elicited by the administration of lethal doses :

1. After *subcutaneous* injection : diminished activity, stupor, progressive weakness, lack of co-ordination in movements, paralysis of the limbs (the fore legs first), general prostration. Anhidrosis was occasionally observed. (Non-lethal doses appeared to cause merely moderate initial excitement.)

2. After administration *per os* : retching movements, marked gastro-intestinal irritation, stupor, weakness, lack of co-ordination in movements, paralysis of the limbs (the fore legs first), general prostration.

Specially large doses induced preliminary excitement and in one instance brought on convulsions. In some cases there were no apparent effects at first except retching movements. Anhidrosis was occasionally observed.

3. After introduction *per rectum*: weakness, paralysis of the limbs (*hind* legs first), progressive rigidity of the muscles, general prostration. Anhidrosis was observed in some cases, edema in others. The smaller doses failed to elicit immediate effects, the larger doses induced excited movements at once and twitching of the muscles.

Doses of 0.3 gm. per kilo in dogs, whether introduced subcutaneously or *per os*, failed to produce fatal results. The subcutaneous injection of 1.5 gm. per kilo in a mouse caused death in five hours (Exp. 22). An intravenous dose of 7 mg. per kilo in a dog caused almost instant death, but this animal may have been particularly susceptible to the effects of the thorium compound because of previous subjection (nine days before) to the influence of a large subcutaneous dose. Another dog (Exp. 29) withstood, a few minutes longer, a somewhat larger total dose of thorium chlorid injected directly into the circulation in quantities that did not exceed 70 mg., although very little of the compound was required intravenously to cause speedy death.

The symptoms usually observed in dogs were the following:

1. After *subcutaneous* injection (in non-lethal doses): local irritation and sloughing, restlessness, fever, thirst, loss of appetite, twitching of the muscles, sluggish movements, weakness.<sup>1</sup>

2. After administration *per os* (in doses that were not very toxic): vomiting, thirst, impaired appetite.

3. After *intravenous* introduction (in lethal doses): muscular twitching, general tremor, tetanic convulsions, asphyxia, death.

Reasons were given on page 458 for the discontinuance of the experiments before various other facts in related connections could be ascertained.

<sup>1</sup> Fatal effects on a mouse are given on page 470.



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