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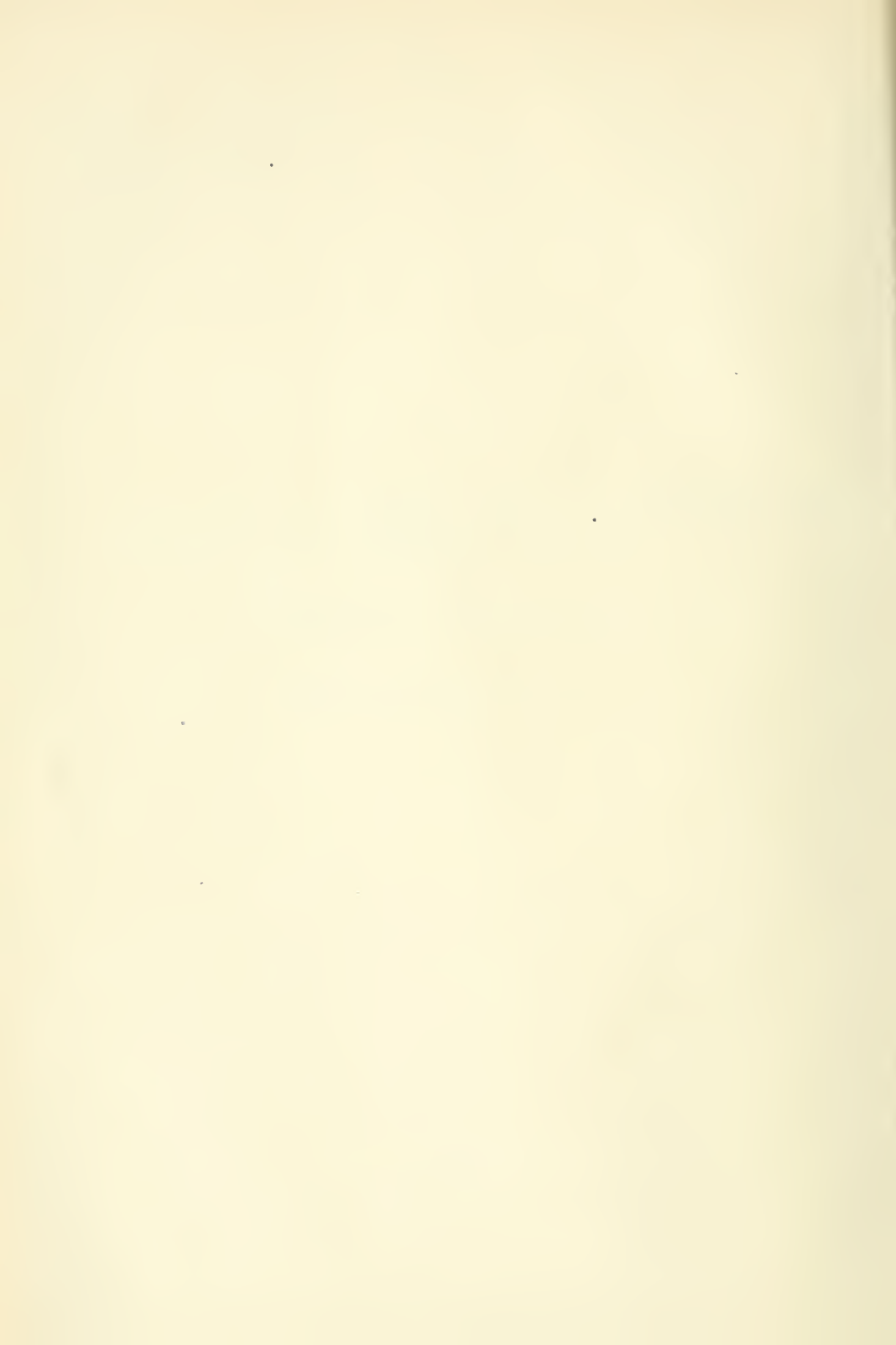
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TWENTY-FIRST ANNUAL MEETING.

BALTIMORE, DECEMBER 29, 30, and 31, 1908.



PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL
SOCIETY.

THE RELATION OF PULSE PRESSURE TO THE APPEARANCE OF
ALBUMIN IN A CASE OF ORTHOSTATIC ALBUMINURIA.

BY D. R. HOOKER, R. F. HEGEMAN, AND L. V. ZARTMAN.

IN a report before this Society in 1903¹ experiments were presented to demonstrate, among other things, a constant relationship between the pulse pressure and the appearance of albumin in a case of orthostatic albuminuria, as well as between the pulse pressure and the amount and composition of the urine.

These experiments are of importance in that they bear direct evidence in support of the theory that postural albuminuria results from vaso-motor instability as expressed by a decrease in pulse pressure. The observation of a second case is therefore of interest.

We were able to conduct in all eleven experiments during which we followed (a) the arterial blood pressure (maximum and minimum), (b) the pulse rate, (c) the amount of urine, and (d) the qualitative variations in the amount of albumin. Circulatory changes were induced by (a) alternately standing and lying, (b) changing the angle of the body with respect to the horizontal, (c) exerting pressure upon the legs with Crile's pneumatic suit, and (d) moderate exercise. Throughout these observations albumin appeared inversely as the magnitude of the pulse pressure with a single exception which occurred late in one experiment and which we believe may be accounted for without invalidating the contention that the appearance of albumin is dependent upon a relatively small pulse pressure.

¹ ERLANGER and HOOKER: This journal, 1904, x, p. xvi; The Johns Hopkins Hospital reports, 1904, xii, p. 145.

The amount of urine excreted was greater when the pulse pressure was relatively large. We could not, however, control our experimental conditions with sufficient accuracy to make this point decisive. Repeated observations failed to reveal casts in the urine.

I. CEREBRAL CIRCULATION IN SLEEP; II. METHODS AND RESULTS OF BLOOD-PRESSURE MEASUREMENTS.

By JOHN F. SHEPARD (by invitation).

THE first subject in this investigation is the same man who served for the work on waking reactions reported in the *American Journal of Psychology*, 1906, and the method of taking the curves is similar. The second subject was a student in the University who has a trephine on the right side of the forehead. The effects of movement were reduced to a negligible quantity by placing the subject's head in a swing.

The method of measuring blood pressure was a modification of the Erlanger method; but in addition a curve was taken from the lower arm by a soft rubber bulb connected to a piston recorder. Following is a summary of results: The volume of the brain and of peripheral parts (hand and foot) increases when the individual goes to sleep, and decreases when he awakes. There is sometimes a temporary fall of the brain volume preceding the more marked rise which shows itself as sleep becomes deeper. The size of arterial pulse from the brain increases with increase in volume, more markedly when the subject is sitting propped up. There is a prominent breathing wave in the plethysmographic records from both brain and periphery. The phasic relation is such that the fall in the circulation record very nearly corresponds to an inspiration, the rise to an expiration. Stimuli that disturb but do not awaken the subject cause a temporary increase in breathing in both chest and abdomen, a fall of volume of the brain and peripheral parts with comparative elimination of the breathing wave therein. When the subject is sleeping soundly and there are apparently no distinct stimuli acting, one often finds a more or less rhythmic repetition of such changes accompanying a break in breathing. The Traube-Hering wave is large during sleep, and the changes in brain and

periphery are always parallel or very nearly so. The wave is often much more marked in one than in the other. The general blood pressure is lower during sleep while the brain volume is highest, and rises when the brain volume falls with awakening. Fall in the brain volume accompanying a break in the breathing or an external stimulus is accompanied by rising pressure. In this particular subject the trough of the vaso-motor wave corresponds to highest pressure, but this is not so constant a condition.

Control of the brain circulation is not entirely a matter of control of general blood pressure; the supply of blood to the brain does not increase and decrease passively with rise and fall of pressure. Increase in volume of the brain, increase in size of arterial pulse from the brain and low blood pressure occur together. There is, apparently, constriction and expansion of the cerebral vessels themselves. Some changes in the other characters of the pulse indicate the same thing.

Sleep is not due to anæmia of the brain, and perhaps pressure has nothing directly to do with the matter. It may be that any considerable change in the size of the small vessels in the brain disturbs the connections of axones and dendrites sufficiently to bring on sleep when the neurones have already relatively lowered excitability and the subject is withdrawn from too strong stimuli.

THE RELATION OF INTENSITY OF STIMULUS TO RAPIDITY OF HABIT FORMATION.

BY ROBERT M. YERKES.

AN investigation of the value of different strengths of an electric stimulus (induced current from calibrated inductorium) in connection with the acquisition of a habit of visual discrimination by the dancing mouse indicates: (1) That the value of a stimulus which serves as a condition for habit formation depends upon the degree of difficulty of the discrimination demanded by the habit; (2) That in the case of very easy discrimination the rapidity of habit formation increases as the strength of the electric stimulus is increased from the threshold (the upper limit has not been definitely determined); (3) That in the case of moderately difficult discrimination a strength of stimulus between the maximal and

minimal stimuli is most favorable to the acquisition of a habit; (4) That in the case of very difficult discrimination a strength of stimulus much nearer to the threshold than the optimal stimuli in cases 2 and 3 is the most favorable condition for habit formation.

These several results suggest the following law of the relation of stimulus to modifiability of behavior: *As the difficulty of a habit increases, the value of the optimal stimulus (in case of the electrical stimulus, at least) approaches the threshold.* The easier a habit, the stronger its optimal stimulus; the more difficult a habit, the weaker its optimal stimulus.

MOTOR LOCALIZATION IN THE CEREBRAL CORTEX OF THE SHEEP.

BY JESSIE L. KING AND SUTHERLAND SIMPSON.

WE have experimented on seven specimens. The animals were anaesthetized and the cerebral cortex exposed in the usual way. For stimulation the unipolar method used by Grünbaum and Sherrington for motor localization in the chimpanzee was employed. The sheep were supported on a wooden frame with the limbs hanging vertically, but not touching the ground, so that the slightest response to stimulation could be detected.

The excitable area of the cortex occupies the posterior or caudal part of the superior frontal gyrus on the dorsal aspect extending over the border on to the mesial surface to a distance of three or four millimetres. It lies around a small sulcus in the gyrus which represents the upturned cephalic end of the splenial sulcus on the mesial aspect.

Faradic stimulation of the posterior part of this area leads to movements of the opposite hind-limb, while stimulation of the anterior part produces movements of the opposite fore-limb, but we find it difficult to define the anterior limit of the hind-limb area and the posterior limit of the fore-limb area, since they appear to some extent to overlap. So far we have not been able to find any cortical centres in the sheep controlling the movements of the eyes, neck, body, or tail.

The excitable motor cortex seems to be relatively much less extensive than it is in the rabbit, cat, and dog as determined in these

animals by Ferrier and other observers. It lies anterior to the cruciate fissure which separates the frontal from the parietal lobe, and is thus entirely confined to the frontal lobe. In this respect it corresponds to the pre-Rolandic position of the motor cortex in monkeys, apes, and man.

HEAT COAGULATION IN SMOOTH MUSCLE; A COMPARISON
OF THE EFFECTS OF HEAT ON SMOOTH AND STRIATED
MUSCLE.

BY EDWARD B. MEIGS.

THE striated muscle of the frog, when heated to 50° C., shortens to an extreme extent; whereas the smooth muscle of the same animal, when similarly heated, lengthens almost as markedly.

There is every reason to believe that the chemical and physical events, which take place in the two tissues at temperatures of 50° and lower, are similar. In both cases irritability is lost at about 40° ; irretrievably lost if the tissue be kept for five or ten minutes at 50° . In both cases a marked whitening and opacity occur at 50° , and the proteins in the extracts of the two tissues are precipitated at 50° , provided the reactions of the extracts are made neutral. In both cases very considerable amounts of lactic acid are formed at temperatures between 40° and 50° ; much more, it is true, in the striated than in the smooth muscle.

Finally, no considerable change in weight occurs in either tissue, if it be kept for thirty minutes at a temperature of 50° in 0.7 per cent sodium chloride solution.

The changes which occur in the two tissues at temperatures above 50° are of an entirely different character. The shortenings which occur at these temperatures are by no means characteristic for muscle. They occur more rapidly and to a greater extent in catgut, connective tissue, elastic tissue, nerve, etc. They are more marked in muscle, of which the proteins have been coagulated by two or three days' treatment with 70 per cent alcohol, than in fresh muscle. They are more or less reversible, and are always accompanied by a loss of fluid and consequently of weight.

The shortening of striated muscle and the lengthening of smooth muscle between the temperatures of 40° and 50° C. may be explained

in the following manner: The heating causes the production of lactic acid in both kinds of muscle. The presence of the acid causes the swelling of the sarcostyles in the one case and of the cells of the other at the expense of the interstitial fluids. The histological structure of the sarcostyle of striated muscle, on the one hand, and of the cell of the smooth muscle on the other, is of such a nature that swelling causes the former element to shorten and the latter to lengthen.

THE EFFECT OF CARBON DIOXIDE UPON THE PUPILS OF FROGS.¹

By J. AUER.

If frogs are placed in an atmosphere of almost pure CO₂ gas, the pupils begin to contract strongly within thirty seconds and within five to six minutes are almost maximally contracted. The same effect is produced when the excised bulbi are subjected to the gas, which shows that the point of action is, largely at least, peripheral, that is, upon the sphincter muscle of the iris. These experiments, however, do not exclude a possible central action of the gas which aids in the contraction of the iris when the bulbi are *in situ*.

If frogs' bulbi, under the influence of CO₂ gas, are treated with adrenalin, the mydriatic effect of this substance is exerted only slightly, and the pupils do not become large and round as do the controls.

If frogs, whose pupils have been fully dilated by adrenalin instillations or by injections of 0.1-0.2 c.c. into a lymph sac, are placed in an atmosphere of CO₂ gas, the pupils contract, but never to the same extent that is found in normal frogs; it seems therefore that CO₂ is unable to fully overcome the mydriatic effect of adrenalin when fully established, just as adrenalin is unable to exert its full effect when the iris is under the influence of CO₂ gas.

This behavior of the frogs' iris is interesting as it differs strikingly from the effect which asphyxia shows in the pupils of mammals, where dilatation is the chief phenomenon.

¹ Read by title.

THE INHIBITORY EFFECT OF LAPAROTOMY UPON SOME OF
THE FUNCTIONS OF THE SPLANCHNIC NERVES.

BY J. AUER.

DURING a series of experiments designed to test the influence of the extrinsic nerves of the stomach upon its motility, it was found that opening of the peritoneal cavity of those rabbits whose vagi alone had been resected beneath the diaphragm some days or months previously, no longer exercised any inhibitory effect upon the movements of the stomach; in these rabbits the movements of the stomach kept on when that viscus was fully exposed to view. Now, in normal rabbits opening of the abdomen produces stoppage of gastric movements, due to inhibitory reflexes which pass to the stomach through the splanchnic nerves, for as soon as the splanchnic nerves of these normal rabbits are cut the stomach shows strong and regular movements. Moreover, an examination of the rabbits, whose vagi had been resected beneath the diaphragm, showed that the ordinary stimuli, such as a whiff of ether or glacial acetic acid, a slight struggle, etc., still produced a prompt and temporary inhibition of gastric movements, which is easily observable through the intact belly wall of the rabbit. As this occurs in rabbits whose only connection with the central nervous system is the splanchnic nerve route, it is evident that the inhibitory impulses must have reached the stomach through them. Yet opening of the peritoneal cavity in these rabbits produces no stoppage. From these facts it seems clear that laparotomy in these animals must have abolished the inhibitory function of the splanchnic nerves, at least as far as stomach motility is concerned. This blocking of the inhibitory stimuli seems to occur in the spinal cord, for stimulation of the splanchnic nerves in some cases, not in all, caused a distinct reduction in the vigor of the gastric waves.

A full interpretation of the facts presented above cannot yet be given, especially as one control animal whose abdomen had merely been opened and immediately sutured showed, after twelve days, when the abdomen was again opened, a persistence of gastric movements which were apparently normal. Another control upon which the second laparotomy was performed nineteen days after the first one, showed, however, a normal picture of gastric inhibition. Still, the first control, alluded to above, indicates that opening of the peri-

toneal cavity alone may produce a blocking of the inhibitory impulses of the splanchnic nerves when the second laparotomy is performed within a certain time after the first one.

THE EXCRETION OF MAGNESIUM AND CALCIUM.

BY LAFAYETTE B. MENDEL AND STANLEY R. BENEDICT.

WHEN magnesium salts are introduced parenterally into animals, the elimination of magnesium takes place to a great extent through the kidneys within a relatively short period (forty-eight hours). Some of the magnesium thus injected may in part be retained in the organism for a period exceeding two weeks. It is not excreted in increased amounts with the *faeces*. Purgation is not observed after parenteral introduction of magnesium sulphate; if anything, the reverse effect may be noted. The increased urinary output of magnesium observed immediately after injection of its salts was regularly accompanied by an increase in the amount of calcium eliminated through the kidneys. There may be a decrease in the calcium excreted by the bowel. The elimination of magnesium through the kidneys after introduction of its salts appears to be more extensive in the rabbit than in the dog. The output of nitrogen and of chlorine was not appreciably affected by injections of magnesium salts.

When calcium salts are introduced into the circulation, calcium is abundantly excreted in the urine, particularly in the rabbit. There is simultaneously an increased output of magnesium by the kidneys.

Numerous other details will be published in a more elaborate report of the experiments.

ON THE RHYTHMICAL CONTRACTILITY OF THE ANAL MUSCULATURE OF THE CRAYFISH.

By F. R. MILLER.

I HAVE already recorded the fact that stimulation of any region of the ventral nerve cord of the Crayfish results in rhythmical movements of the anus. I have examined the anatomical structure of the parts involved and find that the muscular mechanism consists in the

first place of a dorsal and ventral series of muscular strands which are attached externally to the body wall, while internally, after undergoing considerable branching, they are inserted into the wall of the intestine. Secondly, there is an arch of muscle apparently a continuation backwards of the circular muscular coat of the intestine. In the absence of any definite anal sphincter one must conclude that the sole means of closing the orifice is the elasticity of the chitin. A similar mechanical device exists, according to Dahl, in the limbs of insects.

The physiological experiments conducted consisted in the first place of faradic stimulation of the nerve cord in the fourth or fifth abdominal segment, the movements of the anus being recorded by a long straw lever. The early periods of stimulation are characterized by the occurrence merely of a tonic contraction of the anus, but a transition speedily occurs from this condition to that of typical rhythmical contractility. Single break induction shocks are also capable of leading to rhythmical activity of the anus. Faradic stimulation applied directly to the intestine at the point where it emerges from the thorax leads to peristalsis of the intestine and also rhythmical movements of the anus. Dividing the intestine very close to the anal musculature usually prevents any rhythm of the anus on stimulating the nerve cord. I am therefore inclined to believe that the impulses generated on stimulating the nerve cord lead first of all to the development of peristaltic waves in the intestine and that these on arrival at the anus result in the activity of the latter.

ON THE INTERNAL SECRETIONS OF THE THYROID.

BY A. J. CARLSON AND A. WOELFEL.

THE only basis for the view that the internal secretion of the thyroid reaches the blood by way of the neck lymph appears to be histological — colloid being sometimes found in the tissue or lymph spaces.

(1) The main lymphatics for the thyroid leave the upper pole of the glands and join the main neck lymphatic trunk. In normal thyroids the lymphatics are relatively small, smaller than from the salivary glands of corresponding size. The flow of lymph from the normal gland is very slight, probably not exceeding 2-5 c.c. in twenty-four hours.

(2) All forms of glandular hyperplasia (goitre) in the dog are accompanied by an increase in the size of the gland lymphatics similar to that of the gland blood vessels. There is a corresponding increase in the quantity of lymph flowing from the gland. This condition obtains even after the gland has become hematomatous. In goitre of the size of the kidney the lymphatics are larger and the lymph flow is much greater than in the kidney. In large goitres the lymph production is probably from 50 to 150 c.c. in twenty-four hours. By massage of the gland more lymph can be collected from a large goitre in two hours than is yielded by a normal gland in five to seven days.

(3) Tests for thyroid secretions in the lymph. *A. Chemical.* All of our tests for iodine in the goitre lymph (quantities of 20 to 100 c.c.) have been negative. *B. Physiological.* (1) Intravenous injection of goitre lymph in a normal dog produces a rise of temperature ($2-3^{\circ}$ F.), irregularity of the heart beat, and usually tremor of the skeletal muscles. These phenomena disappear within ten to twenty hours. Intravenous injection of goitre lymph in dogs under general anaesthesia causes a gradual depression of the blood pressure accompanied by a rapid, feeble, and irregular heart beat. This is due partly to action on the vagi centres in the medulla. These phenomena suggest hyperthyroidism. (2) Hunt's acetonitrile method. The experiments have not yet yielded definite results. (3) The elimination of all the thyroid lymph in the fox and noting symptoms of thyroidectomy. Experiments in progress.

(4) While the colloid is a product of the secretory acini, it has probably no relation to the internal secretions of the gland that have physiological importance.

THE EFFECT OF CALCIUM INFUSIONS UPON THE IRRITABILITY OF THE HEART VAGUS.

BY J. AUER AND S. J. MELTZER.

In a previous paper we pointed out that an infusion of a 10 c.c. of $m/8$ calcium chloride solution would restore the irritability of the cardiac vagus which had been greatly reduced by an infusion of a magnesium salt. On the basis of this we made a series of experiments to determine whether calcium would increase the effectiveness

of the cardiac vagus in the normal animal. It was found that solutions of calcium salts, infused in *m/8* solution, produced, as one of the most striking results, a very strong reduction or even abolition of the cardiac vagus, while motor nerves such as vagus fibres for the œsophagus and the peripheral sciatic still produced on stimulation practically normal contractions.

RESUSCITATION BY THE DIRECT INJECTION OF ADRENALIN INTO THE HEART CAVITIES.¹

BY F. C. BUSCH AND T. H. MCKEE.

IN this series of experiments, in order to make sure that the heart had ceased to beat, the method was applied with an open thorax. Animals, dogs, and cats were apparently killed by ether or chloroform. The resuscitating agent was not used until other methods were exhausted, such as cardiac massage with artificial respiration. If resuscitation occurred by this means, the anesthetic was again administered until there was a second cessation of heart beat, or until the onset of fibrillation which could not be brought back to co-ordinate contractions by massage alone.

In most instances, after a variable time, sometimes only after repeated injections, but frequently almost immediately, coördinate heart contractions returned. In all cases, after a considerable period, respiration returned. In two instances (dogs) respiration returned while the heart was still fibrillating.

In a number of cases following the resumption of circulation after prolonged anemia, resuscitation of the cord, the bulb, and some parts of the brain occurred as shown by the reappearance of certain definite reflexes. The order of reflex return was as follows: respiratory, knee-jerk, pad-reflex, lip and lid, conjunctiva, and, lastly, reaction of pupils to light. In two cases an apparently complete return to consciousness returned. These animals were killed immediately. The respiratory centre, after its revival, was very sensitive to carbon dioxide. The first respirations, dyspnoëic in character, later became more regular and more nearly normal.

No attempt was made to protect the animals from lowered tem-

¹ Read by title.

perature, and the heart was handled rather roughly. In only one case did we find that cardiac massage induced intra-cardiac clotting.

We have still to apply the method with the closed thorax and to compare it with other methods of administering adrenalin.

TEMPERATURE SENSATIONS FOLLOWING NERVE DIVISION.¹

By SHEPHERD IVORY FRANZ.

WHEN nerves have been cut on areas of the skin which respond to stimuli of a protopathic nature (Head), a difference in temperature sensations are found. Head reported that in such areas hot and cold objects are sensed, but not warm and cool ones. Experiments on a case of nerve division show that, although the general statements are true, the sensations from hot and cold stimuli on the skin from which the epicritic supply is lacking are similar to those from warm and cool stimuli on the skin endowed with the epicritic sense. The areas responding to cold and cool and warm and hot differ, and there is not a sharp line of division between the protopathic and epicritic areas, as has been contended.

SENSIBILITY OF THE HAIRS FOLLOWING NERVE DIVISION.¹

By SHEPHERD IVORY FRANZ.

EXPERIMENTS carried out with a patient in whose arm the ulnar and median (probably also the medial antibrachial cutaneous) nerves were cut show that the distribution of sensibility is much like that described by Head and Sherren, there being well-marked areas in which all sensation is abolished and others in which only some sensations are lost. The mapping of the areas into those in which the epicritic, the protopathic, and the deep sensibilities are lost showed an interesting condition when the hairs were stimulated. In some areas in which, according to Head, the protopathic sensibility remained intact the hairs were found sensitive to light stimu-

¹ Read by title.

lation with cotton wool, but not to plucking. In these areas no sensation resulted, even when two or more hairs were simultaneously pulled out with their roots. These results indicate that in the hairs we have two forms of sensation that come from light brushing and from plucking respectively. This agrees with the findings of Tello, recently reported, of two kinds of sensory nerve endings in or near the hair bulbs, although Tello does not give any indication of the probable functions of these two kinds of nerve terminals.

A POSSIBLE HORMONE VASO-MOTOR MECHANISM.

BY A. J. CARLSON, A. WOELFEL, AND W. H. POWELL.

It occurred to us that hormones, or related bodies, may increase the blood flow through the organ by acting directly on the capillary wall in a way to produce dilation of the capillaries or to diminish the adhesion of the blood to the capillary walls, or possibly by diminishing the viscosity of the blood itself. Either factor would diminish capillary resistance and increase the blood flow through the organ, the general blood pressure remaining nearly the same.

It is well known that the increased blood flow through an active salivary gland more than suffices to meet the increased demand for oxygen. The same relation probably holds for all the glands having a watery external secretion. It would thus seem that in the glands of external secretion the blood flow is also correlated with the demands for water and salts, and we should look for a greater supply of the vaso-dilator metabolites in the organs of external secretion than in the other organs. Moreover, the concentration of these metabolites ought to diminish with the increasing fatigue of the glands.

We have tested the above hypothesis by comparing the relative depressor action of extracts of the glands of external secretion and other organs, and of resting and fatigued salivary glands. Equal weights of the fresh tissues were ground up in sand and equal quantities of physiological salt solution, filtered and injected intravenously, under light ether anæsthesia. The comparisons involve the following organs: salivary glands, stomach mucosa, intestinal mucosa, pancreas, liver, spleen, kidney, testes, thymus, thyroid lymph glands, lung, skeletal muscle.

Results. — (1) The greatest depression is produced by the extract of the pancreas, salivary glands, and the stomach and intestinal mucosa. Next in order comes the kidney, the lung, and the liver extract, while as prepared by us the extracts of the other organs tested gave practically no depression.

(2) The concentration of the depressor substances in the salivary glands decreases with the increasing fatigue of the glands.

(3) The depressor substances are present in the saliva.

(4) The depression is not due to action on the heart or on the vague centres in the medulla.

(5) We are probably dealing with a number of substances in these extracts, as boiling diminishes but usually does not completely abolish the depression.

THE INNERVATION OF THE CORONARY VESSELS.

BY CARL J. WIGGERS.

I. THE reaction of the coronary vessels to adrenalin furnishes presumptive evidence that they are supplied by sympathetic fibres. When the coronary vessels were perfused through cannulas inserted directly into their mouths, the addition of adrenalin caused a decreased outflow from the right auricle, if the heart was at a standstill and adrenalin caused no resumption of the beat. If such a heart was made to resume its beat, an increased flow was the rule. When tested on a beating heart, adrenalin invariably augmented the rate and strength of contractions and increased the outflow. In the last two cases the direct action of adrenalin on the vessels was obscured (1) by the inconstant capacity of the right auricle and ventricle which form an intermediary chamber between the coronary sinus and the registering apparatus; and (2) by the effect of the augmented and accelerated contraction on the flow through the coronaries.

When perfusing the coronary vessels of a stopped heart with a rhythmically interrupted stream of Locke's solution, the diastolic portion of the pressure curve recorded rose when amounts of adrenalin as small as 0.005 mg. were introduced.

II. Further evidence of the existence of nerve fibres to the coronaries was obtained by stimulating the vago-sympathetic nerve of

the dog. When this was done so that no change in the rate or strength of contraction was manifest either in the auricle or ventricle, and the pressure in the aorta and right auricle remained unaltered, a decreased flow from an incised superficial coronary vein was repeatedly obtained.

THE INFLUENCE OF ADRENALIN OVER INTERNAL HEMORRHAGES.

BY CARL J. WIGGERS.

I TAKE the position that the favored method of treating internal hemorrhages by further lowering the blood pressure is always unphysiological and if the pressure is already low may be dangerous.

It was found, in experiments in which the degree of hemorrhage from the vessels of the intestines and lungs was constantly recorded, that adrenalin would raise the arterial pressure while the effect on hemorrhage was as outlined below.

1. *On intestinal hemorrhages.* — Small intravenous injections (0.025 mg.) caused no increase or only a slight and temporary preliminary increase in hemorrhage, followed by a rapid and permanent decrease or total cessation. Larger doses caused an even more prompt cessation, but unfortunately also caused a greater preliminary increase of hemorrhage. The preliminary increase was due to the high pressure which could be largely avoided by the smaller doses. The action of these doses at the point of bleeding outlasted the general rise of pressure, thus diminishing the bleeding. This favored clot formation which alone was able to check the bleeding. The rise of pressure could be prolonged by the intramuscular or continuous intravenous introduction of adrenalin and the dose so adjusted that hemorrhage could be checked.

2. *On pulmonary hemorrhage.* — Therapeutic doses of adrenalin (0.025 to 0.05 mg.) even when they produced no rise of pulmonary arterial pressure caused only an increased flow from the large pulmonary arteries. Though the pressure in the left auricle fell, such doses only caused an increased flow from the peripheral end of a pulmonary vein. Hemorrhages from small vessels embedded in the lung tissue ceased very rapidly, so that no drug was needed.

A NOTE UPON THE FARADIZATION OF THE POSTCENTRAL CONVOLUTION OF THE HUMAN BRAIN IN CONSCIOUS PATIENTS.

By HARVEY CUSHING.

SHERRINGTON and Grünbaum's delimitation to a relative narrow precentral strip of what had previously been considered a widespread motor territory, left without definitely proven function the large portion of the original "motor area"—Munk's sensori-motor field—lying posterior to the central fissure.

The presumption that this is a sensory end-station has received support from certain histological studies and a number of carefully observed clinical cases.

Two patients afflicted with epileptic attacks, inaugurated in each instance by a sensory aura in the right hand, offered unusual opportunities for cortical stimulation while in a conscious state, during a "second-stage" operation.

In both of them the situation of the central fissure was determined by obtaining characteristic motor responses from the precentral gyrus, these motor responses being attended by no sensation other than that of the forced change of position which accompanies similar movements elicited by stimulation of a peripheral nerve.

On the other hand, in both of these patients stimulation of the postcentral convolution gave definite sensory impressions which were likened in one case to a sensation of numbness, and in the other to definite tactual impressions.

In both of the patients, furthermore, stimulation of the outlying convolutions gave no response whatsoever, either of a subjective sensation or of active movement.

FURTHER OBSERVATIONS ON THE MYENTERIC REFLEX.

By W. B. CANNON.

A YEAR ago evidence was given that the local reflex in the wall of the alimentary canal (the myenteric reflex) was reversed with increasing tonus. Observations on the cat's large intestine under warm normal salt solution prove that the production of a weak

tonus ring at the cæcum (by a pinch or better by applying $BaCl_2$) causes *peristaltic* waves to pass over the proximal colon. If the tonus ring is now made at the terminus of the peristaltic waves, antiperistaltic waves occur and sweep away the peristaltic waves. If the tonus ring is made midway in the proximal colon, the waves pass in either direction from the ring. The tonus ring is therefore the source of the waves, a fact noted by me in 1901¹ and by Elliott and Barclay-Smith² without, however, realizing the significance of that fact.

The peristaltic waves of the stomach, like those of the large intestine, are not abolished by injection of nicotine. They start from the tonically contracted cardiac end of the stomach. A tonus ring artificially produced near the pylorus reverses the gastric waves over the antrum. Frog stomachs filled with normal salt solution show peristaltic or antiperistaltic waves according to the end having a tonic constriction.

Loops of small intestine filled with Ringer's solution and placed in oxygenated Ringer's solution to which a small amount of strychnine sulphate has been added, exhibit, after a time, peristaltic waves closely following one another as in the stomach. A tonus ring produced midway in the loop causes the waves to pass in either direction from the ring.

IS THE PITUITARY GLAND ESSENTIAL TO THE MAINTENANCE OF LIFE?

BY LEWIS L. REFORD AND HARVEY CUSHING.

THE problem of the function of an organ can be attacked, roughly speaking, in two principal ways, — one by ascertaining the effects of injections of the material which it secretes, and the other by the study of symptoms which result from its total or partial extirpation.

With a somewhat improved operative method, conducted under the left temporal lobe in association with a large cranial opening over the opposite hemisphere to allow for the "principle of dislocation," we have carried out a series of twenty-five experiments to test the truth of the claim made by Paulesco that a successful extirpation made by a similar operative method is necessarily fatal.

¹ See this journal, 1902, vi, p. 265.

² Journal of physiology, 1904, xxxi, p. 280 *et passim*.

Out of these twenty-five operations twenty were surgically successful, with clean-cut extirpations and no complications which could have contributed to the result. After a period of consciousness and fairly normal activity during the following twenty-four hours the animals would, in the manner described by Paulesco, gradually become comatose, and with a peculiar symptom-complex similar to that produced by profound and fatal narcosis with drugs of the chloral group — a greatly lowered temperature, slowing of respiration and pulse, and a profoundly lethargic state — they would succumb.

Deviations from this typical course of events occurred only in cases in which extirpation had been not entirely a total one.

(In the Hunterian Laboratory an operative demonstration was given and a preliminary report made of the present status of the work which has followed the demonstration of the correctness of Paulesco's views in regard to total extirpation. Perfection of the operative method has enabled one of us, working this year with Drs. S. J. Crowe, John Homans, and G. J. Heuer, to remove either the anterior or posterior lobe of the gland. A series of animals were shown surviving after various partial extirpations and with hypophyseal transplantations, etc.)

THE EFFECT OF SUBMINIMAL ELECTRICAL STIMULATION OF THE VAGI UPON THE DEVELOPMENT OF CARDIAC RIGOR.

BY DON R. JOSEPH AND S. J. MELTZER.

At the last meeting we reported that effective stimulation of the peripheral vagi hastens the development of cardiac rigor. The explanation offered at that time for the result was that the repeated slowing and standstills of the heart caused by the vagus stimulation favored the development of premature asphyxia of the heart muscle, thereby hastening the development of rigor.

We have since studied the effects of subminimal stimulation of the vagi upon the onset of cardiac rigor. In each experiment two animals (dogs) were used. The vagi were cut in both animals, and in one they were stimulated alternately for one hour by a cur-

rent equal to either 100 or 200 mm. less than the least effective stimulus.

The averages for 15 experiments show a distinct retardation in the time between death and maximum rigor in the stimulated animals. There were some individual exceptions. Ten of the 15 experiments showed distinct retardation, 4 showed no change, and 1 a doubtful acceleration.

In 10 experiments in which the irritability was studied the loss of irritability in the ventricles (which runs parallel with the development of rigor) was even more strikingly affected than the rigor. For the left ventricle 7 animals showed a retardation of the loss of irritability, 2 showed no change, and 1 an acceleration. For the right ventricle, 9 showed retardation, 1 no change, and none showed acceleration.

In 3 pairs of dogs in which the effect of cutting the vagi was studied, the animals whose vagi were cut showed a distinct acceleration in the development of cardiac rigor — that is, the hearts influenced by vagus tonus went into rigor later than those deprived of that tonus.

THE MECHANICAL DESTRUCTION OF PEPSIN.

BY A. O. SHAKLEE AND S. J. MELTZER.

At various times since 1884 one of us (M.) studied the effects of shaking upon red blood corpuscles, bacteria, and arbacia eggs. Those experiments led to the general conclusion that shaking, by virtue of the mechanical factor, exerts a profound influence upon living cells. In the present series of experiments we intend to investigate the changes which shaking may produce in the action of ferments. We began with the study of pepsin, the determination of which is greatly facilitated by several recently described reactions.

Solutions of pepsin were shaken with air at room temperature and at a temperature of 33° C., for periods of different lengths, by means of shaking machines. Our results which we state here very briefly are unmistakable. Shaking under these conditions destroys pepsin. Even short periods of shaking greatly diminish its strength. If shaken long enough, it is completely destroyed. Shaking at 33° C. causes a more rapid destruction than at room temperature.

That the effect is not due to oxidation was proven by substituting for the air in the bottles hydrogen, carbon dioxide, or oxygen. There was no appreciable difference in the results.

That the effect is not due to heat was ascertained by fixing maximum thermometers in the bottles perpendicular to the direction of shaking. In no case did the shaking increase the temperature more than a fraction of a degree.

We have found further that the shaking which occurs in the animal body is sufficient to reduce materially the strength of pepsin. This was determined by introducing a small bottle containing some solution of pepsin into a dog's stomach through an esophageal fistula and permitting it to remain there for twenty-four hours or longer. A portion of the same pepsin solution was kept in the thermostat. The strength of the portion kept in the stomach was diminished as much as 40 per cent compared with that kept in the thermostat. Pepsin in a similar bottle kept in the peritoneal cavity of a rabbit for three days showed practically complete destruction.

THE REGULATION OF VENOUS PRESSURE AND ITS RELATION TO SHOCK.

BY YANDELL HENDERSON (WITH T. B. BARRINGER AND S. C. HARVEY).

WHEN the development of shock is followed by means of the volume curve of the heart, it is apparent that the fall of arterial pressure is caused by a diminution in the output of the heart. The fall is not due to abolition of the peripheral resistance in the arterial system. No inhibition, or fatigue, or failure of any sort occurs in the vaso-motor nervous system. On the contrary, this mechanism is intensely active in an effort to compensate the lessened blood stream. Nor is the heart itself weakened. When the pressure in the venous system is observed, it becomes evident that the apparent cardiac failure is the result of diminution of the pressure and volume of the venous stream to the right heart.

No special mechanism for the regulation of the tonus of the venous system is at present recognized. In experiments upon dogs, by Dr. T. B. Barringer and myself, we find that, after section of the vagi, stimulation of the splanchnic nerves sufficiently strong

to cause a considerable rise of arterial pressure produces a barely perceptible effect upon the pressure in the systemic veins. Injection of adrenalin, after vagus section, has likewise an insignificant influence upon venous pressure, unless the dosage is so large as to raise arterial pressure to the point at which the heart's action is interfered with. Experiments now in progress by Mr. S. C. Harvey and myself tend to show that neither section of the spinal cord just below the bulb nor direct stimulation of the cord has any marked direct effect upon venous pressure.

Nevertheless, there is a special mechanism controlling the tonus of the veins. When it is inhibited, venous pressure falls from a normal of 4 or 5 cm. of saline down to 1 or 2 cm. or even to the point at which the venous supply to the heart ceases and death occurs. When the mechanism is stimulated, venous pressure is raised to 12 or 15 cm. or even higher. This action has been demonstrated with and without section of the vagi, with and without curare, under natural respiration, artificial respiration, and the oxygen jet respiration of Vollhard. The inhibition occurs whenever the CO₂ content of the blood is diminished by excessive pulmonary ventilation (acapnia). Stimulation of the mechanism results whenever the body is forced to accumulate CO₂ (Hypercapnia).

The etiology of surgical shock is: pain, hyperpnœa, acapnia, venous stasis, cardiac failure, fall of arterial pressure, — except when the sequence is abbreviated by apnœa vera.

PROTEIN METABOLISM IN DEVELOPMENT.

By J. R. MURLIN.

EXPERIMENTS on pregnant dogs, lasting throughout the entire gestation period, were reported. In one of these a nitrogen balance was kept in weekly periods. It was found, in agreement with the work of Hagemann, Ver Ecke, and Jägerroos, that a minus nitrogen balance existed throughout the first four weeks and a progressively increasing plus balance throughout the last five weeks. Nitrogen and sulphur partitions were made on the urine on two days of each week in one case, and on one day of each week in the other. In a third case the dog was kept on a creatin-free diet during the first

and last weeks of gestation and the first week *post partum*. The creatinin output was constant, but creatin appeared in the urine two days before parturition and reached a maximum on the fifth day after parturition. This latter point probably marks the maximum of the involution process.

The theoretical significance of the minus nitrogen balance in the early part of the gestation was discussed.

TOTAL (OR ENERGY) METABOLISM IN DEVELOPMENT.

By J. R. MURLIN.

In two of the pregnancy experiments (on the same dog), reported under the previous title, the dog was kept in the respiration apparatus on the third day previous to parturition and (together with the puppies) on the first day after parturition. From the first pregnancy one puppy was born; from the second, five. The dog on each of these days, and on one day three weeks after the first parturition, was kept at the same temperature and had eaten the same diet. The results follow:

	Day, 1908.	Total N, gm.	Total C, gm.	Total, cal.	
3d Before	June 23	8.608	59.415	551.3	First pregnancy, one puppy born, weight 280 gm.
1st After	June 27	8.455	65.855	640.6	
	July 15	5.276	51.657	505.3	Sexual rest after lactation.
3d Before	Dec. 11	6.838	74.670	764.9	Second pregnancy, five puppies born, weight at birth 1560 gm.
1st After	Dec. 15	8.389	100.620	1058.8	

The metabolism due to the pregnant condition is found by subtracting the total metabolism on the day of sexual rest from that on the third day before parturition in each case. Thus:

$$551.3 - 505.3 = 46 \text{ Cal. and } 764.9 - 505.3 = 259.6 \text{ Cal.}$$

The extra metabolism due to the pregnant condition proves therefore to be almost exactly proportional to the weight of the puppies at birth; thus:

$$\frac{46 \text{ Cal.}}{280 \text{ gm.}} = \frac{259.6 \text{ Cal.}}{1560 \text{ gm.}}$$

The differences obtained by subtracting the total resting metabolism of the mother dog from the total metabolism of the mother and puppies on the day after parturition are not quite proportional to the weights, probably for the reason that the five puppy dogs helped to keep each other warm, thereby reducing the metabolism somewhat.

Respiration experiments were performed on the individual puppy dogs of the second litter immediately after birth and before they had nursed. The respiratory quotient was found to be almost exactly 1, indicating the combustion of carbohydrate (glycogen).

A METHOD OF STUDYING THE PHYSIOLOGY OF MAMMALIAN HEART TISSUE.

BY JOSEPH ERLANGER.

A CAT is bled to death, its heart quickly excised and perfused with Locke's solution. After it has begun to beat, strips are made of the auricles. These are quickly connected with levers and with the terminals of an induction coil, when they are immersed in Locke's solution through which oxygen is bubbled.

The results obtained differ with the part of the auricles of which the strips are composed and are not quite constant in the case of strips taken presumably from the same part. Parts containing some of the sinus region may beat spontaneously; however the beats soon cease or become unrecognizably small. The appendicular parts have never contracted spontaneously. Tetanic stimulation of the strips momentarily suspended in air after all motion has ceased usually elicit from sinus strips a single initial contraction, which may be followed by a few low, irregular contractions. When now immersed the strip usually (sometimes only after several trials) gives a fine series of beats of some minutes' duration. Such series can be re-elicited at will, sometimes for as long as twenty-four hours. Only once have such beats been obtained from strips composed exclusively of right auricular appendage. Strips composed of the left auricular appendage alone have never contracted except during stimulation. Stimulation of a strip while beating usually elicits again a single initial contraction or the beats become smaller and

less frequent, occasionally more frequent and irregular. Upon re-immersion a finer series of beats usually develops. Atropin, short of doses that annul rhythmicity, has not checked the phenomenon. The sinus region is always the most rhythmical, but its contractions are low. The converse is true of the appendicular regions. When the strips are composed of the two parts, the former at first alone beats; the movements of the lever are slight. As the beats of the sinus are improved by stimulation, the contractions spread into the appendicular part (often after a preliminary partial block), when the movements of the lever become surprisingly ample (329).

ON THE INTERVAL BETWEEN THE MINIMAL AND MAXIMAL
RISE IN BLOOD PRESSURE FOLLOWING STIMULA-
TION OF THE SCIATIC NERVE.

BY W. T. PORTER AND R. RICHARDSON.

THE percentile rise in blood pressure following stimulation of the sciatic nerve varies from "accidental errors." When the number of excitations with any given strength of stimulus is practically sufficient, the deviations above the mean value will balance those below it, and the true mean will be revealed. By this method of accidental errors we have studied the reflex rise of blood pressure with stimuli increasing from the minimal to the maximal value. The curve obtained is similar to that given by the extirpated auricle of the tortoise heart when the latter is also stimulated with induction currents of increasing intensity. In the first instance, the muscle is part of a reflex arc connected with at least three neurons; in the second there is no connection with the vasomotor nervous system; yet the mean contraction values rise with the same curve from minimum to maximum. So far as our present information goes, this result seems to justify the statement that *the vasomotor cells have no "constant" influence upon the reflex rise of blood pressure.* Further, these results suggest that the vasomotor neurons take no part in the reflex rise, either "constant" or "accidental." It is possible that they are concerned only in the maintenance of arterial tonus.

ON THE INTERVAL BETWEEN THE MINIMAL AND MAXIMAL
FALL IN BLOOD PRESSURE FOLLOWING STIMULATION
OF THE DEPRESSOR NERVE.

BY W. T. PORTER AND F. H. PRATT.

THE curve obtained by applying the method of accidental errors to the fall in blood pressure upon stimulation of the depressor nerve, with induction currents increasing from minimum to maximum intensity, is similar to that obtained by stimulation of the sciatic nerve, so far as can be determined by the data now in hand. Should this result be confirmed by the continued examination of this question now in progress, it will follow that *the vasomotor cells have no "constant" influence upon the reflex fall of blood pressure*, and it will be probable that pressor fibres and depressor fibres have essentially the same relations to the vasomotor neurons.

MAXIMUM VASOMOTOR REFLEXES OBTAINED BY STIMULATING PORTIONS OF THE SCIATIC NERVE.

BY W. T. PORTER AND R. RICHARDSON.

FOLLOWING a suggestion of Dr. Clark's that perhaps one half the sciatic nerve would give a rise in blood pressure equal to that obtained by stimulating the undivided nerve, the sciatic nerve was split longitudinally near its exit from the pelvis and the several portions stimulated with maximum induction currents. The maximum rise in blood pressure was obtained with one quarter of the nerve trunk and all portions larger than this. There was no escape of stimulating current. Efforts to determine whether the vasomotor fibres ran in one portion of the nerve rather than another were not definitely successful.

USES OF THE ALTERNATING CURRENT IN THE
PHYSIOLOGICAL LABORATORY.

BY E. P. LYON AND E. M. WILLIAMS.

1. WE use the alternations at the central power plant instead of a tuning-fork for all experiments requiring the measurement of

fractional parts of a second, such as velocity of the impulse, reaction time, etc. The electric signal will give two vibrations for each complete cycle of the current. With the usual 60-cycle dynamo each double vibration of the electric signal, therefore, represents $1/120$ second. This is a little unhandy, but the student readily reduces his results to decimal form. The accuracy attained will depend on the constancy of velocity maintained at the power plant. Under good conditions the variations should not exceed 2 per cent, which is about as good as can be expected from the ordinary tuning-fork. The alternating current is easier to use than the electric tuning-fork and far easier than the simple hand fork. For student use, especially, the alternating current is recommended instead of the tuning-fork. The record of time is so easily made that he is not distracted from the most essential features of the experiment.

2. The alternations of the current may be used to induce a tetanizing current without the use of the automatic make and break device, thus saving the latter from being sparked out. The induced current resulting from the alternations is not so strong as when batteries are used, because the make and break are not so sudden. However, using the coil in series with a 32 C. P. lamp on the 110 volt circuit, one gets a tetanizing current sufficient for nearly all purposes and perfectly uniform in intensity after being started.

3. By using the automatic make and break the current may be used with the inductorium in place of a battery. It may also be used for the electric pendulum, metronomes, etc. In fact it serves all purposes except where a single make or break shock is desired or for such experiments as electrotonus, when a direct current must be used.

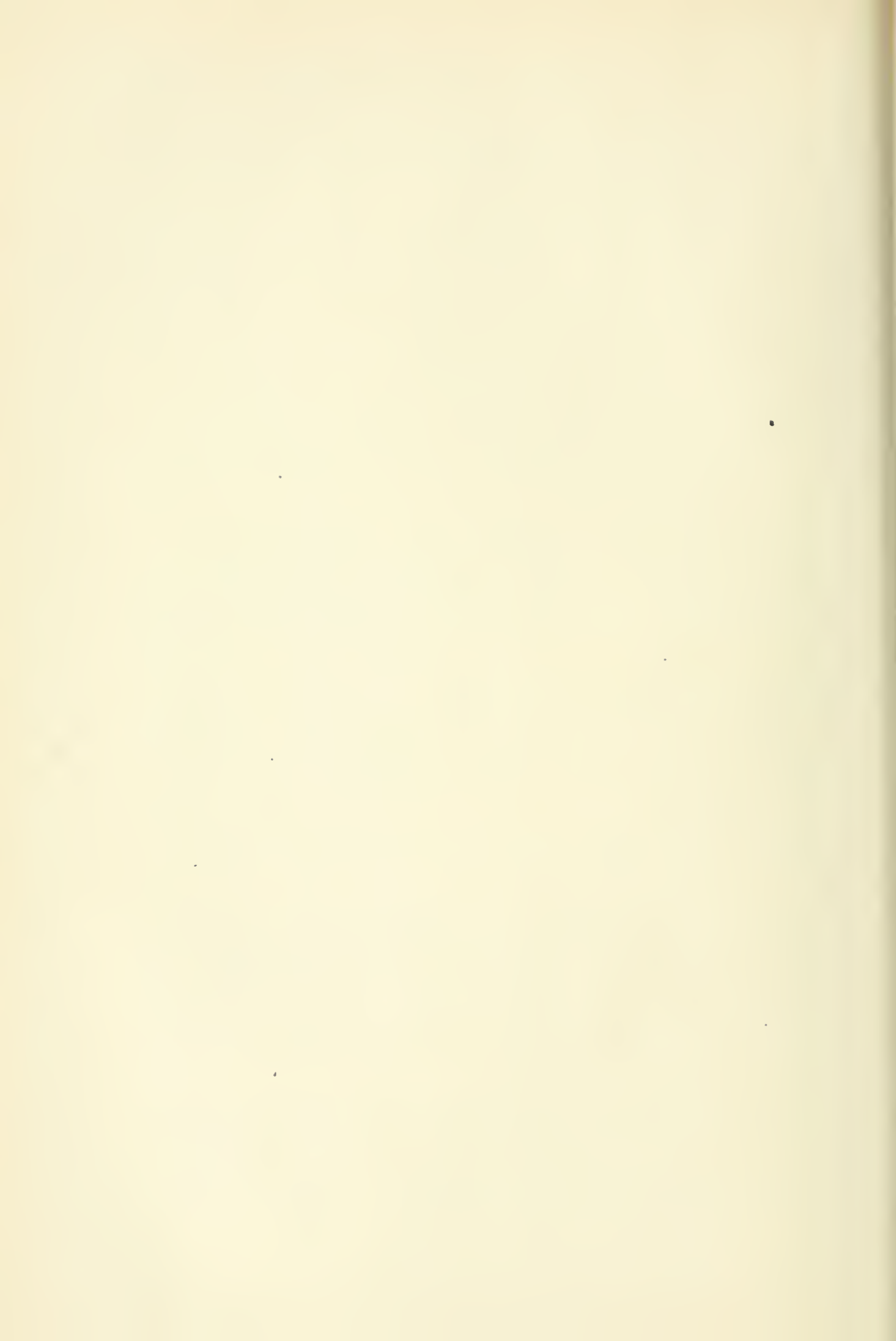
The only apparatus needed for any of these purposes is a suitable lamp resistance, a small shunt resistance to cut down the voltage of the current used, and a short-circuiting key. The latter is quite essential on account of the slowness with which the carbon filament of the lamp warms up when the current is started at the lamp.

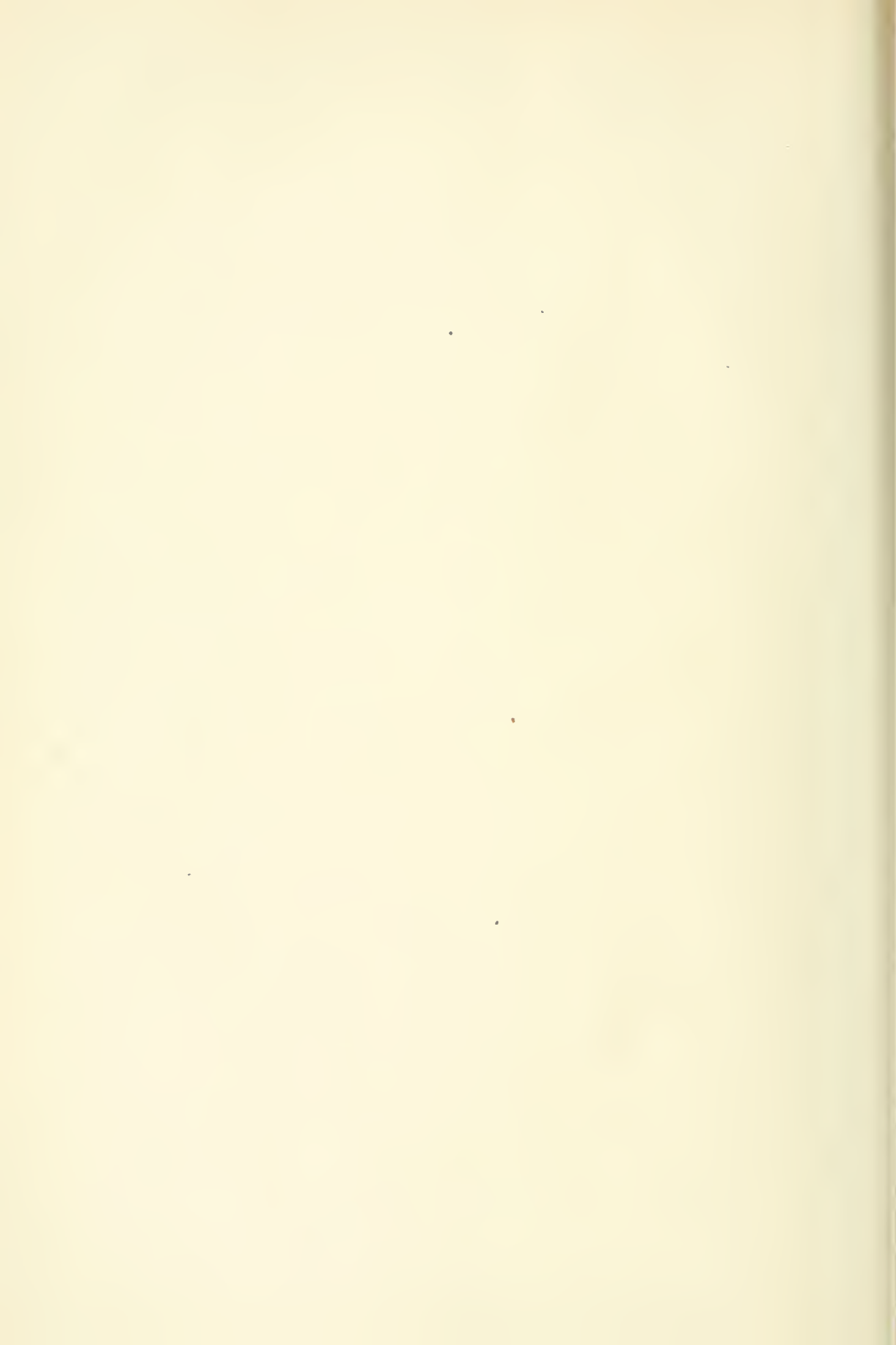
The following communications were read by title:

- FATIGUE OF MUSCLE STIMULATED DIRECTLY AND INDIRECTLY. BY F. S. LEE and S. EVERINGHAM.
- THE PRODUCTION, BY HYDROGEN PEROXIDE, OF RHYTHMICAL CONTRACTIONS IN THE MARGINLESS BELL OF GONIONEMUS. BY O. P. TERRY.
- THE ABSORPTION, EXCRETION, AND DESTRUCTION OF STROPHANTHIN IN THE ANIMAL ORGANISM. BY R. A. HATCHER.
- FURTHER OBSERVATIONS UPON SURGICAL SHOCK. BY G. W. CRILE.
- RHEOTROPISM IN FISHES AFTER BLINDING ONE EYE. BY E. P. LYON.
- THE INFLUENCE OF THE LEFT SPLANCHNICUS MAJOR UPON THE VASCULARITY OF THE NORMAL RIGHT AND THE DENERVED LEFT KIDNEY. BY R. BURTON-OPITZ and D. R. LUCAS.
- ON THE VASOMOTOR NERVES OF THE SPLEEN. BY R. BURTON-OPITZ.
- THE DESTRUCTION OF BODY PROTEIN IN FEVER. BY P. A. SHAFFER.
- ON THE ANTAGONISTIC ACTION OF AMMONIUM AND CALCIUM SALTS. — A CONTRIBUTION TO THE KNOWLEDGE OF ACIDOSIS. BY C. VOEGLIN and J. KING.

The following demonstrations were made:

- AN INSTRUMENT FOR THE DETERMINATION OF THE VENOUS PRESSURE IN MAN. BY D. R. HOOKER and J. A. E. EYSTER.
- A METHOD OF STUDYING THE PHYSIOLOGY OF MAMMALIAN HEART TISSUE. BY J. ERLANGER.
- A DIAGRAM OF THE NORMAL BEHAVIOR OF THE HEART AT ALL RATES OF BEATS. BY Y. HENDERSON.
- DEMONSTRATION OF A NEW METHOD OF DETERMINING THE RATE OF BLOOD FLOW THROUGH AN ORGAN. BY T. G. BRODIE (by invitation).
- SOME RECENT VARIATIONS IN THE ANALYSIS OF THE GASES IN SMALL VOLUMES OF BLOOD BY THE CHEMICAL METHOD. BY T. G. BRODIE (by invitation).
- USES OF THE ALTERNATING CURRENT IN THE PHYSIOLOGICAL LABORATORY. BY E. P. LYON.
- THE INTRACENTRAL PORTION OF THE SEVENTH NERVE ROOT DEMONSTRATED BY INDIRECT WALLERIAN DEGENERATION. BY S. SIMPSON (by invitation).
- DEMONSTRATION OF THE THREAD GALVANOMETER AND SOME OF ITS APPLICATIONS IN PHYSIOLOGY. BY C. D. SNYDER.





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THE EXCRETION OF KREATININ AND KREATIN IN HEALTH AND DISEASE.

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IT is the purpose of this paper to present results on the excretion of kreatinin and of kreatin by normal and by pathological subjects, in the hope of throwing some further light upon the physiological significance of these two substances.

Previous to 1904 the Neubauer method¹ of precipitation with zinc chloride was used for the determination of kreatinin; while for the determination of kreatin the substance was isolated as such and weighed. In neither case are the results reliable from a quantitative standpoint.

A complete review in this paper of the observations made by the use of these methods would serve no purpose, since the literature of this subject has been fully treated by recent writers.² The more important of these observations may, however, be again stated very briefly.

¹ NEUBAUER: *Annalen der Chemie und Pharmacie*, 1861, cxix, p. 33; SALKOWSKI: *Zeitschrift für physiologische Chemie*, 1886, x, p. 113; SALKOWSKI and TANIGUTI: *Ibid.*, 1890, xiv, p. 471.

² VON NOORDEN's *Handbuch der Pathologie des Stoffwechsels*: HOOGENHUYZE and VERPLOEGH: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 415; MELLANBY: *Journal of physiology*, 1908, xxxvi, p. 447.

Acid human urine contains kreatinin; and little or no kreatin; an alkaline urine contains kreatin instead of kreatinin (Voit³). The amount of kreatinin excreted by an adult was found to vary between 0.4 gm. and 1.5 gm. (Voit,³ Munk,⁴ Neubauer,⁵ Hofmann⁶), and was believed to depend upon the amount of protein as well as upon the amount of kreatin (Gruber,⁷ Hofmann,⁶ Meissner,⁸ Mallet⁹) in the food. Regarding other factors, Hofmann⁶ found that the amount of kreatinin excreted increases with growth, and declines in old age; that the amount excreted by women is somewhat lower than that of men; that body height has no influence, and that there is some relationship between body weight and the amount of kreatinin excreted, but apparently he did not consider the last-mentioned point of great importance, since he did not give the weights of any of his subjects.

In view of the close chemical relationship between kreatin and kreatinin and of the supposed ease with which one is converted into the other, it is very generally believed, but without any definite proof, that the kreatinin of the urine is derived from the kreatin of the muscles.

Conflicting statements are to be found concerning the influence of muscular work. Moitessier,¹⁰ Grocco,¹¹ and Gregor¹² found an increased excretion of kreatinin following muscular work, while Hofmann⁶ and Meissner⁸ reached the opposite conclusion.¹³

With pathological individuals and using the Neubauer method, Hofmann,⁶ Schottin,¹⁴ and Munk,⁴ found an increased excretion in

³ VOIT: *Zeitschrift für Biologie*, 1868, iv, p. 77.

⁴ MUNK: *Deutsche Klinik*, 1862, No. 30, p. 299.

⁵ NEUBAUER: *Annalen der Chemie und Pharmacie*, 1862, cxx, p. 27.

⁶ HOFMANN: *VIRCHOW'S Archiv für pathologische Anatomie*, 1869, xlviii, p. 358.

⁷ GRUBER: *Zeitschrift für Biologie*, 1901, xlii, p. 416.

⁸ MEISSNER: *Zeitschrift für rationelle Medicin*, 1865-68, xxiv, xxvi, and xxxi.

⁹ MALLET: *Bulletin No. 66*, 1899, U. S. Office of Experiment Stations.

¹⁰ MOITESSIER: *Thesis*, 1891. Cit. by Hoogenhuyze and Verploegh.

¹¹ GROCCO: *Cit. MALY'S Jahresbericht*, 1886, xvi, p. 199.

¹² GREGOR: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 98.

¹³ I need not refer here to the recent experiments on this point. By the use of the FOLIN method it has been shown that neither an increased (HOOGENHUYZE and VERPLOEGH) nor a decreased (SHAFFER) muscular activity has any effect upon the amount of kreatinin excreted.

¹⁴ SCHOTTIN: *Cit. by KRAUS in VON NOORDEN'S Handbuch der Pathologie des Stoffwechsels*, 1906, i, p. 137.

fevers. Low results were found in chronic under-nutrition (Hofmann⁶); in convalescence (Munk⁴); in chlorosis (Hofmann⁶); in pernicious anæmia, in myelogenous leucæmia, and in lymphatic leucæmia (Stejskall and Erben¹⁵); in pseudo leucæmia (Moraczewski¹⁶); and in muscular atrophy (Weiss, Jakubowitsch, Langer¹⁷). A high excretion of kreatinin was noted in diabetes (Senator, Gathgens, and others¹⁸), probably because of the kreatin and kreatinin in the meat eaten.

But on account of the inaccuracy of the analytical method used and of the great variations found in the amounts of kreatinin excreted by normal persons, these pathological results have lacked any great significance, and must now be accepted with caution.

With the advent of Folin's quick and relatively accurate method¹⁹ for the determination of kreatinin and kreatin, interest concerning these substances was greatly revived; in consequence, our knowledge concerning them has been materially advanced, and in several important instances the conclusions of earlier investigators as stated in the foregoing pages have already been disproved.

Folin first showed that the amount of kreatinin excreted in the urine by a normal individual is, contrary to Hofmann's conclusion, quite independent of the amount of protein in the food, or of total nitrogen in the urine; the amount of kreatinin excreted from day to day is practically constant for each individual (presumably under the same conditions of health and muscular activity).²⁰

This constancy of kreatinin excretion has been fully confirmed by Hoogenhuyze and Verploegh,²¹ Klercker,²² Closson,²³ and the

¹⁵ STEJSKALL and ERBEN: *Zeitschrift für klinische Medicin*, 1900, xxxix and xl.

¹⁶ MORACZEWSKI: *VIRCHOW'S Archiv für pathologische Anatomie*, 1898, cli, p. 22.

¹⁷ WEISS: *Wiener klinische Wochenschrift*, 1877, p. 701; JAKUBOWITSCH: *Neurologisches Centralblatt*, 1884, p. 279; LANGER: *Deutsche Archiv für klinische Medicin*, 1883, xxxii, p. 395.

¹⁸ Cit. by VON NOORDEN: *Handbuch der Pathologie des Stoffwechsels*, 1907, ii, p. 90.

¹⁹ FOLIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223.

²⁰ FOLIN admitted that the amount excreted might be dependent upon the amount of muscular work (*This journal*, 1905, xiii, p. 86).

²¹ HOOGENHUYZE and VERPLOEGH: *Zeitschrift für physiologische Chemie*, 1905, xlvi, p. 415.

²² KLERCKER: *Biochemische Zeitschrift*, 1907, iii, p. 45.

²³ CLOSSON: *This journal*, 1906, xvi, p. 252.

writer.²⁴ If the subject is receiving sufficient food, the kreatinin excretion is the same with 16 gm. as it is with 4 gm. total nitrogen in the urine. Folin²⁵ and Klercker²² further showed that, contrary to the previously accepted views, the excretion of kreatinin is wholly unaffected by the ingestion of kreatin; and this result has been confirmed by Wolf and the writer.²⁶

Since kreatinin, unlike any other known product of normal metabolism excepting perhaps uric acid, is independent of the total amount of protein katabolized, it appears to be of the greatest interest to learn the full significance of kreatinin, and to determine further the factors which may influence the amount of this substance excreted in the urine.

THE NORMAL EXCRETION OF KREATININ.

While the kreatinin excretion is practically constant from day to day for each healthy individual, different persons excrete different amounts; and Folin pointed out that "the chief factor determining the amount of kreatinin eliminated appears to be the weight of the person."²⁷ He further realized that the amount of adipose tissue must be considered, because he noted that the fatter the subject the less kreatinin is excreted for each kilo of body weight. Folin considered these points rather briefly, but the conclusion from his results is that the amount of kreatinin excreted depends primarily upon the mass of active protoplasmic tissues.

Since we have no method of accurately measuring the amount of adipose tissue in various individuals, the best that we can do is to express the amount of kreatinin excreted as milligrams kreatinin, or, as seems to me preferable, as milligrams kreatinin-nitrogen per kilo of body weight. This ratio, milligrams kreatinin-nitrogen per kilo of body weight, I have called the "kreatinin coefficient,"²⁸ and this term will be used in the following pages.

²⁴ SHAFER: This journal, 1908, xxii, p. 445.

²⁵ FOLIN: HAMMARSTEN'S Festschrift, 1906, iii, p. 1.

²⁶ WOLF and SHAFER: Journal of biological chemistry, 1908, iv, p. 439.

²⁷ FOLIN: This journal, 1905, xiii, p. 85.

²⁸ "The effect of muscular activity on kreatinin excretion; with preliminary observations on the excretion of kreatinin in health and disease." Proceedings of the American Physiological Society, New York, Dec. 1906. In this paper the kreatinin coefficients were expressed as milligrams kreatinin, but I have since

Table I contains averages of kreatinin values determined by Folin's method, in the urine of supposedly normal persons; most of these results have been taken from the literature, only the last ten being my own. Almost all of the figures are averages from several days and in some cases represent much longer periods. These figures contain evidence in favor of the factors, body weight and amount of adipose tissue, suggested by Folin, as influencing the kreatinin excretion. But these are certainly not the only factors of influence, and because of the complexity of the subject it is difficult to show the effect of any one factor alone.

These figures, covering 37 supposedly normal cases, show an average of 8.1 with a maximum of 11.7 and a minimum of 5.4. These we may for the present accept as the normal limits for kreatinin excretion,²⁹ although I believe that a closer analysis and further experience will show that kreatinin coefficients below 7 are normal only for elderly, inactive, poorly developed, or excessively fat subjects, and, strictly speaking, none of these conditions is normal.

HOURLY EXCRETION OF KREATININ. .

In view of the remarkable uniformity in the excretion of kreatinin from day to day, it seemed desirable to learn the extent of variation during various periods in the twenty-four hours. A large number of determinations in the urine of seven subjects showed just as great uniformity in the hourly excretion of kreatinin as is found in the daily excretion. A portion of these results is given in Table II.

The time of each period was exactly noted, and an effort was made to empty the bladder as completely as possible each time in order to get quite all of the urine secreted in the time stated. No meat products were contained in the diets except where noted in the table.

concluded that it will be more consistent, and in the end less confusing, to express the kreatinin coefficient, like results of other nitrogenous substances, in terms of nitrogen.

²⁹ The kreatinin coefficients of normal dogs are practically the same as those given above for man. WOLF and OSTERBERG (*Biochemische Zeitschrift*, 1907, v, 304) found an average of 8.2 for one dog (fourteen days) and 7.0 for another (thirteen days). Results of my own from four dogs lie between the above figures. The amount of kreatinin excreted from day to day is as constant for dogs as for men (OSTERBERG and WOLF, *loc. cit.*, and unpublished experiments of the writer).

It is by no means an easy matter without some practice to empty the bladder completely, especially at frequent intervals, and it is not unlikely that at least some of the slight variations found are to be explained by this difficulty. Where this is so, a low result will of course precede or follow a high result.

The lack of effect of diuresis on the excretion of kreatinin is shown in some of these figures.³⁹ With subject M. S. the volume of urine varied from 24 c.c. to 934 c.c. per hour, the latter after taking 2 gm. diuretin, but the amount of kreatinin excreted in each period was practically the same (0.059 and 0.054 gm.). The same thing is shown in the results from P. A. S. Between 1 and 2 P.M. on one day, for instance, 432 c.c. of urine was secreted, containing 68 mg. of kreatinin; the next hour only 66 c.c. of urine was secreted, but this contained 70 mg. kreatinin.

A great increase or decrease in the amount of protein ingested at a single meal, resulting in marked change in the amount of total nitrogen excreted per hour, is also without effect upon the hourly excretion of kreatinin. (See the results from M. S.)

These results appear to show that the regularity of excretion of kreatinin is to be explained by a regularity of formation, and not merely by a regular secretion by the kidneys.

There are slight variations during the twenty-four hours, but the results are, on the whole, remarkably regular, and justify the belief that kreatinin is formed during a process in the body which varies very little in intensity from hour to hour.

The papers by Hoogenhuyze and Verploegh and by Klercker contain data on the hourly excretion of kreatinin; but their results do not show any such regularity. The greater variations found by these investigators are possibly due to some inaccuracy in the length of periods, etc.; in any event, my results show that there is a far greater regularity in the hourly excretion than their observations indicate.

NORMAL EXCRETION OF KREATIN.

As mentioned earlier in this paper, Voit believed that an alkaline urine contains kreatin instead of the kreatinin which he found when

³⁹ When less than about 8 mg. of kreatinin was contained in 25 c.c. urine, the latter was made slightly more acid with HCl, and evaporated to a smaller volume before the determination. Except on rare occasions and for very small quantities, kreatin was not present in these urines.

the urine was acid. This is not correct. Normal fresh urine, whether acid or alkaline, contains kreatinin, and if the normal subject has not taken kreatin in his food during the preceding few days, his urine will not contain kreatin, whatever its reaction. There is no normal excretion of endogenous kreatin.³¹

EXCRETION OF KREATININ BY PATHOLOGICAL SUBJECTS.

During the past three years we have been carrying on in this laboratory metabolism experiments upon various hospital patients, many of whom were in bed. From these subjects we have found, very frequently, much lowered kreatinin coefficients, notwithstanding the fact that many of the patients were more or less emaciated, and therefore, because of the little adipose tissue, we might perhaps have expected high kreatinin coefficients. A number of times it has been further observed that when patients improved, got out of bed, and walked around, the kreatinin coefficient was raised to some extent. When I referred to some older results³² from patients at the McLean Hospital for the Insane, many of whom were not in bed and had no definite disturbance of their vegetative organs, but who were not taking much physical exercise, I found again many instances of a much lowered kreatinin coefficient.

These findings seemed at first to point clearly in one direction,—that the lowered kreatinin excretion was the result of the small amount of muscular energy expended by such individuals. I therefore proceeded to determine the effect of muscular activity, particularly of a greatly diminished activity, on kreatinin excretion, and, incidentally, upon the general protein metabolism.

These experiments, which are described in a separate paper,³³ led to the conclusion that the amount of muscular activity is in itself wholly without effect upon the amount of kreatinin excreted. We may, therefore, leave out of consideration the factor of muscular work. Some other explanation must be found for the very low kreatinin excretion noted in abnormal subjects.

We have made, in this laboratory, observations on the kreatinin

³¹ FOLIN has shown that kreatin, when ingested, is largely retained in the body unless the food contains a large amount of protein (HAMMARSTEN'S *Festschrift* iii). See also KLERCKER, *loc. cit.*

³² FOLIN: *American journal of insanity*, 1904, xli, p. 299.

³³ SHAFFER: *This journal*, 1908, xxii, p. 445.

excretion of more than two hundred different persons, representing a variety of conditions. Folin's method has been used exclusively. The lowest kreatinin coefficient (2.4) was found in a subject of lymphatic leucæmia, who was extremely ill and died about a week later. Coefficients of 3 or 4 are comparatively common in patients who are confined in bed on account of weakness, whatever may be the disease. From these low values the coefficients vary up to the normal figures, 8-11. In subjects of acute fevers, in the early stages, the excretion of kreatinin is quite normal or high, but with the disappearance of the fever the kreatinin falls to below the normal.³⁴ The publication of all our results is unnecessary; a few will be found at the close of this paper, while some others will be given in forthcoming papers from this laboratory.

The facts which I wish to emphasize in this paper are that *a low excretion of kreatinin is found in a remarkably large number of pathological subjects, representing a variety of conditions, and that the excretion of an abnormally small amount of this substance is by no means peculiar to any one disease.*

What is the significance of low kreatinin excretion? According to Folin,³⁵ the kreatinin excreted, on a kreatinin-free food, is an index of and wholly derived from endogenous or tissue katabolism. Folin has not defined his "tissue katabolism," but if we understand that term to cover all of the processes taking place in the cells of the body in which the body protein is broken down, the *total* endogenous katabolism, we shall be compelled to modify this idea of the origin of kreatinin.

At a meeting of the American Physiological Society (New York, December, 1906), after comparing normal results, as given in a previous chapter of this paper, with low results from a number of pathological cases, I suggested that the kreatinin of the urine is derived from, and an index of, not the total tissue or endogenous katabolism, but of one process of this tissue katabolism; and I then pointed out that on this latter process appears to depend the muscular, or general cellular, efficiency.

³⁴ LAMBERT and WOLF: Proceedings of the American Society of Biological Chemists, 1907, i, p. 28; FOLIN: unpublished results, personal communication; SHAFER: unpublished results; LEATHES: Journal of physiology, 1907, xxxv, p. 275.

³⁵ FOLIN: This journal, 1905, xiii, p. 84.

In May, 1907,³⁶ I presented further low kreatinin coefficients from subjects of exophthalmic goitre, whose total endogenous metabolism was above the normal, as shown by rapid loss of weight and emaciation; and the conclusion was again drawn, "that kreatinin is not a product of total tissue catabolism, but is a product of certain *normal* cell processes, which in many diseased conditions may be extremely sluggish in their intensity, even though, as in exophthalmic goitre, the total tissue catabolism may be much increased. The low kreatinin coefficients in all marked cases of exophthalmic goitre — subjects of which disease are especially prone to muscular weakness — are also accepted in support of the author's hypothesis that kreatinin is an index of muscular tonus, or of muscular and perhaps of general cellular efficiency."

Whether kreatinin arises in this katabolic process in all of the tissues of the body, or whether it is alone formed in the muscles, cannot be decided without further experiments;³⁷ but for the muscular tissues, at any rate, facts support the belief that the amount of kreatinin excreted is an index of their efficiency, — not the amount of work which the muscles are doing at the time, but the amount of work which they are capable of doing.

In October, 1907, a paper appeared by Spriggs,³⁸ who, using Folin's method, came to essentially the same conclusion. Spriggs found the kreatinin excretion very low (2.2 and 4 mg. kreatinin nitrogen per kilo body weight) in two cases of muscular dystrophy (decrease of muscular bulk); very low (1.9 mg. kreatinin-nitrogen per kg.) in a case of amyotonia congenita; slightly low (5.6 mg.) in a case of myasthenia gravis; normal in one case of locomotor ataxy (7.1); and slightly high (?) in two cases of tetanus (7.8 and 9.3).

Spriggs concluded from these cases that "kreatinin is connected with the nutritional metabolism of the muscle fibre, and is not a

³⁶ SHAFFER: Proceedings of the American Society of Biological Chemists, 1907, i, p. 22.

³⁷ The experiments of GOTTLIEB and STANGASSINGER (*Zeitschrift für physiologische Chemie*, lii and lv) appear to indicate that the formation of kreatin and kreatinin takes place in the glandular organs as well as in the muscles. This phase of the subject, the site of formation of kreatinin, and its possible relation in the body to kreatin will be treated of in a future paper. Experiments on these points by Dr. R. A. HATCHER and the writer were begun over a year ago and are still in progress.

³⁸ SPRIGGS: The quarterly journal of medicine (Oxford), 1907, i, p. 63.

substance formed in the act of contraction. If we liken the muscles to a machine, kreatinin as a waste product would stand in relation to the structure of the machine, and not to the fuel which the machine uses." This statement is quite in accord with my own views, as presented to the American Physiological Society and to the American Society of Biological Chemists:

If this idea is correct, it merely means that in a muscle in a high state of nutrition and development certain processes which cannot be at present fully defined, but which lead to the formation of kreatinin as a waste product, are proceeding at a greater speed than in a muscle organically weak or diseased.

The actual efficiency of a muscle depends upon a number of factors, one of which is the nerve impulse sent to that muscle; but my view in this connection, and as I understand it the view held by Spriggs, considers the muscle only as an individual machine, and as being quite independent of nervous stimulation. If the motor nerve to one of the skeletal muscles is cut, that muscle is actually unable to work, but on account of the lack of a stimulus, and not because of any inefficiency of the muscle fibres. Potentially the muscle is as good as before. This applies of course only to the immediate effects; the nervous mechanism being destroyed or diseased, the muscle becomes atrophied from disuse, its nutrition is interfered with, and then, as a secondary effect, the efficiency of the muscle as a machine is decreased. I believe it is only this secondary effect which under these conditions would lead to a decreased excretion of kreatinin.

I should not expect temporary anæsthesia, for instance, to cause any marked decrease in kreatinin excretion, although it would for the time destroy the actual muscular efficiency; a long illness or old age, on the other hand, which leaves the person muscularly weak should, and does, cause a corresponding decrease in the amount of kreatinin excreted.

Some reservation must be made for the effect of fever, which probably increases, though in my experience not greatly, the excretion of kreatinin; and it cannot be supposed that fever increases temporarily the efficiency of the muscles. The greater excretion in fever may be ascribed to a pathological increase of the kreatinin forming process, which is perhaps due solely to the higher temperature or to the action of bacterial toxins, and which is coincident with the increased destruction of body protein. For non-febrile

individuals my results indicate that the amount of kreatinin excreted bears a direct relation to the potential efficiency of the muscles, and is a reliable index of the muscular development of an individual.

A number of the variations in the kreatinin coefficients of normal persons (Table I) may be explained by a consideration of the varying muscular efficiency of the subjects. Of those of the individuals known to me, the ones with the better muscular development and capable of the greater amount of muscular work have the higher kreatinin coefficients, and *vice versa*.

The idea outlined above has already received some consideration at the hands of other workers. Benedict and Meyers³⁹ report determinations of kreatinin in the urine of twenty-six women, all of whom were patients in a hospital for the insane. Most of these cases, it seems to me, fully bear out the factors above outlined as influencing the amount of kreatinin excreted and its relation to muscular efficiency. I shall cite only the high and low extremes in these cases.

Case I was a female, 85 years old, senile dementia, and weighed 39 kg. "Subject in bed, old, feeble, withered, and very inactive." Kreatinin coefficient, 2.0.⁴⁰

Case XVI. Female, age 92 years, weight 63 kg. "Subject rather decrepit, partly paralyzed, fat and flabby. Spends most of time in bed." Kreatinin coefficient, 2.0.

This case was doubtless muscularly stronger than Case I, since she was out of bed a part of the time. With relatively the same amount of adipose, she should have had a higher coefficient than Case I; but she was also "fat and flabby," and in consequence had relatively less muscular tissue than Case I.

Case XXIII. A nurse, 25 years old, weighing 52 kg., just convalescing from typhoid fever, and was considerably under normal body weight." Kreatinin coefficient, 3.1.

In convalescence from typhoid there is usually a marked muscular weakness.

Some of these cases had coefficients near the normal.

³⁹ BENEDICT and MEYERS: This journal, 1907, xviii, p. 377.

⁴⁰ BENEDICT and MEYERS express the kreatinin coefficients in mg. kreatinin; for comparison with my results I have converted their figures into mg. kreatinin-nitrogen.

Case XIII. Manic depressive insanity, depressed form, "in a fair physical condition and extremely active." Kreatinin coefficient, 5.6.

Case XXII. Dementia præcox, age 45 years. "Subject rather inert as a rule," in bed because of erysipelas. Kreatinin coefficient, 5.9.

Benedict and Meyers conclude that "the kreatinin excretion of women is, in general, much lower than that of men." This is doubtless true, and is to be explained by the fact that most women are poorer developed muscularly than men; the kreatinin-forming process is less active, and they have at the same time a lower muscular efficiency.

I have had the opportunity of determining the kreatinin coefficients of only a few strong and hearty women, but these and my results from pathological subjects indicate that if the factors of muscular development or efficiency and the amount of adipose are nearly the same, there is no difference between the kreatinin coefficients of men and women. Sex, *per se*, has, I believe, no influence.

The results of Amberg and Morrill⁴¹ are in agreement with my hypothesis. These investigators find from very young infants kreatinin coefficients between 1.46 and 2.6 (mg. kreatinin-nitrogen). These figures are what should be expected from the smaller bulk of muscle tissue, and the low muscular tonus of infants only a few days old.

In experiments upon a fasting dog, poisoned with phosphorus, Lusk found "a gradual fall in the amount [of kreatinin] eliminated — independent of the tone and strength of the muscle," but states in his summary that "the kreatinin output is scarcely affected."⁴²

A calculation from his results shows that the kreatinin coefficient of the dog on the third day of the fast was 7.0, which fell to 6.3 on the sixth day, and to 5.5 on the seventh day. A dog's muscles are certainly less efficient on the seventh day of a fast than on the third day. The subsequent rise of the kreatinin excretion after the injection of the phosphorus is doubtless due to the toxic action of the latter, and, I think, speaks neither for nor against my hypothesis. Such a toxic increase is quite analogous to that seen during fever. On the other hand, it should not be concluded, because the dog was severely ill (from the poisoning with phosphorus), and was unable to stand, that his muscles, considered as individual machines, lost

⁴¹ AMBERG and MORRILL: *Journal of biological chemistry*, 1907, iii, p. 311.

⁴² LUSK: *This journal*, 1907, xix, p. 464.

in potential efficiency to a corresponding degree. It seems to me more probable that the muscular weakness of this dog was due more to a depression of the nervous mechanism, and not to any great extent to a decreased efficiency of the muscles themselves.

Mention must also be made of the recent admirable paper by Mellanby⁴³ on "Creatin and Kreatinin." This author presents a large number of valuable facts concerning the kreatin content of muscles from many different animals, and concludes that "in the formation of kreatinin muscle plays a small part," and that "the liver is intimately connected with the production of kreatin and the excretion of kreatinin." He introduces distinctly a new point of view in believing that the liver forms kreatinin from other substances, that this kreatinin is in part converted into kreatin and stored in the muscles until the amount of kreatin in the muscles reaches the "saturation point," after which the excess of kreatinin, continually being formed by the liver, is excreted in the urine.

According to Mellanby's idea one would expect a low kreatinin excretion from subjects of diseases interfering with the normal function of the liver; and he presents such data. But subjects of cirrhosis and other pathological conditions of the liver are far from being the only instances of low kreatin excretion, and in view of the large number of subjects having low kreatinin-coefficients, and in whom there is no reason for suspecting any disturbance or diminution of liver function, it does not appear to me probable that there can be such a relation between the activity of the liver and the formation and excretion of kreatinin as he suggests. The relationship between body weight and the amount of kreatinin excreted may also be mentioned as an argument against the correctness of his idea.

KREATIN.

As stated earlier, kreatin is not present in normal urines unless kreatin is taken in the food. This substance is, however, excreted in various pathological conditions even when the food is free from kreatin. It is logical to suppose, as do Benedict⁴⁴ and Mellanby, that the kreatin excreted has its source directly in the kreatin of the muscles; and this I believe to be true in view of the fact that, according to my observations, kreatin is invariably excreted where

⁴³ E. MELLANBY: *Journal of physiology*, 1908, xxxvi, p. 447.

⁴⁴ BENEDICT: *This journal*, 1907, xviii, p. 406.

there is a rapid loss of muscle protein. It is excreted in acute fevers, in the acute stages of exophthalmic goitre, and in tumor cachexia, in all of which conditions emaciation is taking place, with probably a breaking down of muscle tissue.

The largest excretion of kreatin which I have so far encountered is in women during the first week *post partum*, when I have found as much as 1.50 gm. (as kreatin) in twenty-four hours; it is during this stage that the resolution of the muscular wall of the uterus is proceeding most rapidly. It may also be excreted even when the body is increasing in weight, as I have observed in a case of exophthalmic goitre who was improving rapidly. But it is conceivable that this may be explained by the persistence in the muscles of a pathological katabolism, otherwise masked by the regenerative processes going on at the same time.

The following are given as instances of kreatinin excretion and of kreatin excretion in pathological individuals. The figures are in nearly all cases averages of a considerable number of days. The diets were always free from kreatin and kreatinin.

Kreatinin-nitrogen is abbreviated to K_1-N , kreatin-nitrogen to K_2-N , an kreatinin-coefficient to $K_1-Coef.$

Subject	Age	Weight	K_1-N	$K_1-Coef.$	K_2-N
I. A. N. Exophthalmic goitre. Female. Large frame, but had lost about 7 kg. some months earlier. Able to move about the house, but with effort. A little later was much weaker than during the experiment.	35	59 kg.	0.22 gm.	3.7	0.03
II. A. M. H. Exophthalmic goitre. Female. Slow onset, marked symptoms. In bed, not emaciated. Improved, able to be out of doors. After greater improvement. Much stronger.	38	49 "	0.163 "	3.3	0.12
III. Perpall. Exophthalmic goitre. Female. Extremely ill, emaciated to last degree. Died several weeks later.	0.217 "	4.4	0.03
IV. Redlin. Exophthalmic goitre. Female. Extremely ill. Lost much weight dur- ing last year.	0.273 "	5.5	0.17
V. Mrs. J. Exophthalmic goitre. Female. Rapid onset, acute case. Very weak and losing weight.	..	35	0.11 "	3.2	0.11
VI. Duffy. Exophthalmic goitre. Female. Severe case. Extremely emaciated and not able to sit up.	18	41 "	0.13 "	3.2	0.12
	39	50 "	0.14 "	2.8	0.18
	..	45 "	0.166 "	2.6	0.11

Subject	Age	Weight	K ₁ -N	K ₁ -Coef.	K ₂ -N
Three months later after very great improvement patient was much stronger, walking out of doors and doing housework	61	kg.	0.28 gm.	4.6	0.08

Fifteen other cases of exophthalmic goitre gave kreatinin coefficients from 3.0 to 6.0. One of the characteristics of this disease is the muscular weakness which F. Müller by direct measurement has shown to be very great in severe cases.

Subject	Age	Weight	K ₁ -N	K ₁ -Coef.	K ₂ -N	Glucose
VII. C. B. D. Permanent biliary fistula. (This journal, xvii, p. 362.) Female. Rather weak, but walking about the house	61	55 kg.	0.27 gm.	4.9
Greater lassitude and weakness.			0.22 "	4.0
Walked two miles each day. Much stronger			0.33 "	6.0
VIII. Ward. Chronic nephritis. Male	52	80 "	0.36 "	4.5
Had been quite muscular and a hard worker, but had been in bed nearly three months.						
IX. Malkus. Chronic nephritis. Male	65	"	0.25 "	3.8
Alcoholic. In bed.						
X. Kennedy. Chronic nephritis. Male	55	kg.	0.21 gm.	3.8
XI. Boggs. Flat foot, obesity. Male	67	147.5	0.79 "	5.35
Very inactive, but able to walk about.						
XII. Lymphatic leucæmia. Male	63		0.152 "	2.4
Extremely ill.						
XIII. Mrs. A. G. Diabetes. Female. Fair condition, nutrition good, but muscles flabby and adipose excessive. Walking about. Much meat in diet.	55	77 "	0.36 "	4.65	0.25	0.5% to 2.7%
XIV. Miss E. W. W. Diabetes	50	91 "	0.43 "	4.7	0.19	3.5% to 5%
Nutrition fair, but muscles are flabby and adipose excessive. Able to walk about. Much meat in diet.						
XV. Miss A. S. Chronic nephritis. A large, athletic, and very muscular woman, but with much adipose. In bed for therapeutic reasons.	24	99.6	0.73 "	7.3	0.18	..

Subject	Age	Weight	K ₁ -N	K ₁ -Coef.	K ₂ -N	
XVI. Mrs. A. B. Normal pregnancy. Only fair muscular development; slender and active. During course of observation patient took much exercise (chiefly walking) out of doors, and materially increased in strength. Normal labor Oct. 18. Third day <i>post partum</i>	30	52 to 55	0.33	"	6.35	0.045 July 31
			0.345	"		0.052 Aug. 28
			0.362	"		0.033 Sept. 11
			0.365	"	6.75	" 30
			0.355	"		Oct. 5
			0.342	"	6.2	" 10
			0.36	"	0.225 " 21	
Subject			K ₁ -N	K ₁ -Coef.	K ₂ -N.	
XVII. Mrs. A. H. Normal labor Mar. 21 On third day <i>post partum</i> urine contained " fifth " " " " " "					0.15	
					0.23	
XVIII. Mrs. Smith. Normal labor. Urine third day <i>post partum</i> contained					0.36	
XIX. Johnson. Normal labor. Urine of third (?) day <i>post partum</i>					0.26	
XX. Dudd. Normal labor. Urine second (?) day <i>post partum</i>					0.56	
XXI. Rupin. Typhoid fever. Male. 12th day of disease, temp. 102.4° to 104.8° F. 43rd " " " " normal, convalescent The patient had lost but little in weight and was in comparatively good physical condition.			0.54	10.0	..	
			0.41	7.7	..	
XXII. Paponis. Typhoid fever. Male. 14th day of disease temp. 102° to 103.4° F. 57th " " " " 100° to 103° F. Great emaciation.			0.54	9.0	0.67	
			0.28	5.5	0.00	
XXIII. Sparks. Typhoid fever. Female. July 25 temp. high Aug. 1 temp. normal Aug. 14 relapse Aug. 27 temp. normal			0.31	5.5 (?)	0.115	
			0.123	..	0.058	
			0.334	..	0.112	
			0.19	..	0.037	

CONCLUSIONS.

I. The amount of kreatinin excreted by strictly normal individuals is between 7 and 11 mg. kreatinin-nitrogen per kilo body weight. The amount excreted by any one individual is constant not only from day to day but from hour to hour; and the amount is independent of the volume of urine as well as of the total nitrogen.

II. The kreatinin excretion of pathological subjects is usually low, varying from the normal to 2 mg. kreatinin-nitrogen per kilo body weight in twenty-four hours. The amount of kreatinin-nitrogen per kilo — the kreatinin coefficient — shows a direct parallelism with the muscular development or strength, or "muscular efficiency," of the individual.

III. Kreatinin is not an index of the total endogenous protein katabolism. Subjects of exophthalmic goitre and others in whom the total endogenous katabolism is probably much increased may excrete very little kreatinin.

IV. Kreatinin is derived from, and its amount, expressed in milligrams per kilo body weight, is an index of, some special process of normal metabolism taking place largely, if not wholly, in the muscles. And upon the intensity of this process appears to depend the muscular efficiency of the individual.

V. The kreatinin excretion is slightly increased in acute fevers, and in this condition does not run parallel to the muscular efficiency of the individual.

VI. Kreatin is not a normal product of endogenous metabolism, and is not present in normal urines, unless the individual has taken kreatin with the food.

VII. Kreatin may be excreted by subjects of acute fevers, in the acute stages of exophthalmic goitre, in other conditions in which there is a rapid loss of muscle protein, and by women during the *post partum* resolution of the uterus.

VIII. The source of endogenous kreatin is probably the kreatin of the muscle tissues, and its appearance in urine probably indicates that muscle protein is being absorbed.

TABLE I.

KREATININ EXCRETION AND KREATININ COEFFICIENTS—NORMAL. (KREATININ COEFFICIENT = MG. KREATININ-NITROGEN PER KG. BODY WEIGHT.)

Investigator.	Subject.	Muscular development.	Remarks.	Body weight.	Kreatinin-nitrogen.	Kreatinin coefficient.
				kg.	gm.	
Folin	J. H. B.	} Young and active male nurses in Hospital . . .	57.4	0.584	10.2
"	E. H. S.		66.5	0.543	8.2
"	R. L. J.		70.4	0.584	8.3
"	G. E. C.		70.0	0.658	9.4
"	M. H.		56.5	0.506	9.0
"	S. R. B.		58.0	0.587	10.1
"	O. F.	Good	Little adipose	65.2	0.587	9.0
"	"	"	Little adipose	64.1	0.554	8.6
"	"	"	Little adipose	68.3	0.584	8.5
"	H. B. H.	Good	Fat	86.0	0.584	6.8
"	"	"	Fat	86.0	0.561	6.5
"	"	"	Fat	91.0	0.673	7.4
"	E. S. A.	Fair	Lean	55.9	0.410	7.3
"	"	"	Lean	55.9	0.420	7.5
"	"	"	Lean	54.2	0.443	8.2
"	A. H.	Good	Moderately fat	70.1	0.513	7.3
"	"	"	Moderately fat	70.5	0.513	7.3
"	Dr. H.	6 ft. tall, bony but not muscular	69.5	0.502	7.2
"	Mr. B.	5 ft., 11 in. tall	75.0	0.576	7.7
"	Mr. Bu.	5 ft., 7 in. tall	64.5	0.495	7.7
"	Dr. K.	Very good	5 ft., 8 in. tall	69.5	0.685	9.9
"	Mr. W.	Fair	Lean	62.0	0.547	8.3
"	Mr. T.	Fair	5 ft., 8 in. tall	70.0	0.569	8.1
Hoogenhuyze and Verploegh	Student	71.0	0.830	11.7

TABLE I (Continued).

KREATININ EXCRETION AND KREATININ COEFFICIENTS—NORMAL. (KREATININ COEFFICIENT = MG. KREATININ-NITROGEN PER KG. BODY WEIGHT.)

Investigator.	Subject.	Muscular develop- ment.	Remarks.	Body Weight.		Kreatinin nitrogen.	Kreatinin Coefficient.
				kg.	gm.		
Hoogenhuyze and Verploegh	Student	80.0	0.804	10.0	
	"	57.0	0.629	11.0	
	"	79.0	0.822	10.4	
	"	63.0	0.632	10.0	
Closson	G. M. B.	Age 33, frail in physique .	61.5	0.431	7.0	
	"	Age 32, not corpulent . .	70.0	0.439	6.3	
	"	Slender	57.2	0.410	7.2	
	"	Age 47, slender	57.0	0.331	5.8	
	"	Age 47, slender	58.0	0.312	5.4	
Three days excluded from average.	"	Age 47, slender	59.0	0.323	5.5	
	F. P. U.	Good	Age 26, not fat	65.0	0.394	6.1	
Osterberg and Wolf Klercker	O. E. C.	Age 24	62.5	0.495	7.6	
	E. O.	Good	Age 40, not corpulent . .	70.0	0.465	6.65	
	K. O. K.	187 cm. tall, well nourished, not fat	87.7	0.638	7.85	
Shaffer	O. T.	Very good	Very little adipose	68.0	0.603	8.9	
	"	"	Very little adipose	69.0	0.591	8.6	
	"	Good	Very little adipose	58.0	0.472	8.15	
	"	Good	Moderately fat	75.0	0.550	7.3	
	"	Fair	Slender	65.0	0.584	9.0	
	"	"	Slender	65.0	0.577	8.9	
	"	Fair	Slender	65.8	0.528	8.0	
	"	Good	Not fat	67.0	0.572	8.55	
	"	Good	Not fat	67.5	0.558	8.3	
"	Mrs. S. T.	Good	Good	52.0	0.40	7.7	

TABLE II.
HOURLY EXCRETION OF KREATININ.

Subject.	Time.	No. of hours.	Urine per hour.	Tot. N. per hour		Kreatinin per hour.	Remarks.
				c.c.	gm.		
M. S.	8 -10 A.M.	2.0	39	0.39	0.066	Net weight 62.5 kg.	
"	10 -12 M.	2.0	30	0.36	0.062		
"	12 - 2 P.M.	2.0	27	0.32	0.057		
"	2 - 4 P.M.	2.0	25	0.35	0.065		
"	7 - 9.15 A.M.	2.25	50	0.64	0.068		
"	9.15-11.30 A.M.	2.25	79	0.73	0.066		
"	11.30- 1.30 P.M.	2.0	63	0.62	0.061		
"	1.30- 3.30 P.M.	2.0	67	0.62	0.062		
"	3.30- 5 P.M.	1.5	46	0.56	0.059		
"	5 - 7 P.M.	2.0	72	0.56	0.064		
"	7 - 9.30 A.M.	2.5	54	0.45	0.063		
"	9.30-11.30 A.M.	2.0	67	0.51	0.055		
"	11.30- 2 P.M.	2.5	45	0.46	0.061		
"	2 - 4 P.M.	2.0	54	0.56	0.063		
"	4 - 6 P.M.	2.0	58	0.57	0.062		
"	9.15-10.50 P.M.	1.58	45	0.51	0.065		
"	10.50- 3.40 A.M.	4.83	31	0.46	0.064		
"	3.40- 5.30 A.M.	1.83	33	0.46	0.062		} Sleeping except for time to urinate at 3.40.
"	5.30- 8 A.M.	2.5	40	0.49	0.052		
"	8 -11.30 A.M.	3.5	31	0.44	0.065		
"	11.30- 2.30 P.M.	3.0	46	0.60	0.066		
"	2.30- 4.30 P.M.	2.0	49	0.61	0.061		
"	4.30-11.10 P.M.	6.67	39	0.55	0.062		
"	11.10- 8.20 A.M.	9.17	24	0.41	0.059		} Sleeping.
"	8.20-11.30 A.M.	3.17	25	0.44	0.065		
"	11.30-12.45 P.M.	1.25	220	0.74	0.068		
"	12.45- 2.45 P.M.	2.0	60	0.69	0.061		
"	2.45- 4.15 P.M.	1.5	202	0.95	0.063	} Took 2 gm. diuretin about 4 P.M., and continued drinking much water during the afternoon.	
"	4.15- 5 P.M.	0.75	930	1.11	0.063		
"	5 - 5.30 P.M.	0.5	934	1.19	0.054		
"	5.30- 6 P.M.	1.0	757	0.77	0.058		
"	6.30-12 N'T.	5.5	111	0.76	0.053		
"	12 - 9 A.M.	9.0	55	0.50	0.056	} Sleeping.	
"	9 -11.15 A.M.	2.25	60	0.45	0.062		
"	11.15-12.45 P.M.	1.5	32	0.42	0.056		
"	12.45- 4.30 P.M.	3.75	57	0.52	0.068		
"	4.30- 6 P.M.	1.5	124	0.53	0.064		
Average kreatinin					0.0625 gm.		
					(or 1.0 mg. per kg. per hour.)		

TABLE II (Continued).

Time.	No. of hours.	Urine per hour. c.c.	Kreatinin per hour. gm.	Time.	No. of hours.	Urine per hour.	Kreatinin per hour. gm.
M. S. (one month earlier than the above). ¹				S. P. B.			
2.15- 3.15 P.M.	1.0	50	0.067	10 -11 A.M.	1.0	34	0.074 ¹¹
3.15- 4.15 P.M.	1.0	..	0.067	11 -12 M.	1.0	31	0.067
10.12-11.12 A.M.	1.0	38	0.071	12 - 1 P.M.	1.0	28	0.061
11.12-12.12 P.M.	1.0	29	0.067	1 - 2 P.M.	1.0	32	0.069
12.12- 1.12 P.M.	1.0	30	0.064	R. A. H.			
1.12- 3.12 P.M.	2.0	42	0.070	10- 11 A.M.	1.0	34	0.075 ¹²
3.12- 4.12 P.M.	1.0	53	0.071	11 - 3 P.M.	4.0	39	0.072
Average kreatinin 0.068 gm. (or 1.09 mg. per kg. per hour).				Average kreatinin 0.0735 gm. (or 1.3 mg. per kg. per hour).			
P. A. S. ²				O. T.			
Whole day	24.0	49	0.065	11 -1 P.M.	2.0	64	0.074 ¹³
7 -12 M.	5.0	34	0.068 ³	1 -3 P.M.	2.0	65	0.081
12 - 2 P.M.	2.0	96	0.068	Average kreatinin 0.0778 gm. (or 1.19 mg. per kg. per hour).			
3 - 8 P.M.	5.0	33	0.068	J. T.			
8 -11 P.M.	3.0	60	0.061	11 - 1 P.M.	2.0	70	0.049 ¹⁴
11 -12 N'T	1.0	45	0.066	1 - 3 P.M.	2.0	38	0.052
12 - 7 A.M.	7.0	36	0.060 ⁴	Whole day	24.0	66	0.059 ¹⁵
7 - 8 A.M.	1.0	28	0.063	Average kreatinin 0.0533 gm. (or 0.89 mg. per kg. per hour).			
8 -11 A.M.	3.0	87	0.070 ⁵	J. T. (eight months later, when in better physical condition).			
11 - 5 P.M.	6.0	103	0.071	1.30- 4.30 P.M.	3.0	33	0.068
P. A. S. (one month later).				I. G.			
7 - 9 A.M.	2.0	43	0.076	2 - 3 P.M.	1.0	38	0.069 ¹⁶
9 -12 M.	3.0	100	0.077	9 -11 A.M.	2.0	37	0.062
12 - 2 P.M.	2.0	231	0.074 ⁶	11 - 1.30 P.M.	2.5	34	0.070
2 - 4 P.M.	2.0	85	0.072	Average kreatinin 0.0686 gm. (or 1.13 mg. per kg. per hour).			
4 - 6.30 P.M.	Lost	J. T. (eight months later, when in better physical condition).			
6.30- 9 P.M.	2.5	77	0.066	I. G.			
9 -10 P.M.	1.0	40	0.069	I. G.			
10 - 7 A.M.	9.0	26	0.061 ⁷	I. G.			
7 -10 A.M.	3.0	73	0.067	I. G.			
10 -12 M.	2.0	57	0.062	I. G.			
12 - 1 P.M.	1.0	230	0.074 ⁸	I. G.			
1 - 2 P.M.	1.0	432	0.068	I. G.			
2 - 3 P.M.	1.0	66	0.070	I. G.			
3 - 5 P.M.	2.0	46	0.068	I. G.			
5 - 9 P.M.	4.0	41	0.073 ⁹	I. G.			
9 - 7 A.M.	10.0	40	0.070 ¹⁰	I. G.			
7 -11 A.M.	4.0	55	0.071	I. G.			
11 - 5 P.M.	6.0	62	0.072	I. G.			

TABLE II (Continued).

Time.	No. of hours.	Urine per hour. c.c.	Kreatinin per hour. gm.	Time.	No. of hours.	Urine per hour. c.c.	Kreatinin per hour. gm.
I. G. (one month later).				10 - 6 A.M.	8.0	26	0.063 ¹⁸
				9 - 10 A.M.	1.0	31	0.067
				Average kreatinin 0.0669 gm. (or 1.07 mg. per kg. per hour).			
11 - 1 P.M.	2.0	42	0.069	10.15-11.15 A.M.	1.0	40	0.084 ¹⁹
1 - 5 P.M.	4.0	34	0.073	11.15-12.15 P.M.	1.0	44	0.174
5 - 11 P.M.	6.0	35	0.070	12.15- 2.15 P.M.	2.0	44	0.138
11 - 7 A.M.	8.0	25	0.063 ¹⁷	2.15- 5.15 P.M.	3.0	51	0.101
7 - 10 A.M.	3.0	24	0.070	5.15-12.06 A.M.	6.85	35	0.084
10 - 1 P.M.	3.0	37	0.067	12.06- 7.20 A.M.	7.23	29	0.0725
1 - 2 P.M.	1.0	50	0.069	Total 21.03 1.9400			
2 - 4 P.M.	2.0	43	0.062				
4 - 6 P.M.	2.0	28	0.064				
6 - 10 P.M.	4.0	28	0.065				
1.94 less 1.41 (21.08 × 0.0669) = 0.53 gm. = 76% of kreatinin ingested was excreted in 21 hours.							
<p>¹ Weight same as above. ² Net weight 60.5 kg., some meat eaten. ³ A very little meat eaten at 8 A.M. ⁴ Sleeping. ⁵ Meat eaten at 8 A.M. ⁶ Drank cocoa at noon. ⁷ Sleeping. ⁸ Drank three cups of strong cocoa at 12.45. ⁹ Stewed chicken eaten at dinner. ¹⁰ Sleeping from 10 to 7. ¹¹ Net weight 56.5 kg. ¹² Net weight 65.3 kg. ¹³ Net weight 60 kg. ¹⁴ Ate some meat. ¹⁵ Net weight 62.5 kg. ¹⁶ Sleeping. ¹⁷ Sleeping. ¹⁸ Sleeping. ¹⁹ Received 0.70 gm. pure kreatinin in 250 c.c. water at 10.40 A.M.</p>							

AN IMPROVED OUTFLOW-RECORDING APPARATUS.

By CARL J. WIGGERS.

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

MANY forms of apparatus, simple and complex, have been suggested by means of which to study changes in the rate of flow from organs. Each has some claim of advantage as regards simplicity, accuracy, or adaptability to a particular need. During the course of numerous experiments, varied in character, which required outflow measurements, it was usually found that the very point that made the apparatus of special value in one class of experiments rendered its employment valueless in others. It thus became a matter of convenience to devise one form of apparatus which should serve a variety of needs. In order to do this, the apparatus must satisfy the following requirements:

1. *The outflow must be recorded continually.* — All methods by which the amount of fluid which is passed from an organ in definite time intervals is measured by a graduate or recorded by some apparatus, lack this requirement. I have previously taken occasion to discuss the fallacy of such procedures, but, as the method is so commonly employed, the criterion may be repeated in the form of a table. Column I of Table I shows the amount of fluid collected from the veins of a perfused kidney during ten-second intervals. Distinct outflow changes are evident. Column II illustrates how perfectly all traces of these changes would be covered by estimating the flow for thirty-second periods. Such errors can only be obviated by the use of an apparatus recording the outflow continuously, for then the most temporary changes become evident. On the other hand, if it becomes desirable to express results in terms of flow per units of time, these units can be so chosen as not to conceal outflow changes.

2. *Single drops or a continuous stream must be registered interchangeably.* — For this reason all forms of apparatus which record, either by a tambour system or electric contact, the drops as they leave

the vessel, are limited in their use and entirely unsuited for many experiments.

3. *The magnification of the rate of flow must be adjustable in different experiments, in proportion as these changes are large or small.* — Changes in flow may be so small that, if not properly mag-

TABLE I.

I. Flow per 10 seconds.	II. Flow per 30 seconds.
c.c.	c.c.
4	
4	12
4	
3	
2	12
7	
6	
3	12
3	
3	
4	11
4	

nified, they escape observation, or they may be so great that, if too much magnified, the delicacy of the apparatus prevents the production of a useful record.

4. *The drip-collecting surface must be wide or funnel-shaped and without impeding parts above it that would prevent a free entrance of the drip from vessels in which no outflow cannulas have been inserted.* — Most forms of apparatus acceptable in other respects do not comply with this requirement, nor can they be readily adapted to it.

5. *All kinds of fluid, watery, viscid, and coagulable, must be recorded.* — Ordinary forms of apparatus usually make no provision for this.

In 1907 I reported an improved form of apparatus to record continually the drip from many sources, whether flowing in drops or in a continuous stream.¹ It possessed, however, no arrangement for adjusting the magnification and was not adapted to viscid or coagu-

¹ WIGGERS: This journal, 1907. xx, p. 207.

lating solutions. For these reasons the form of apparatus described below was devised.

The principle for the construction of this apparatus was suggested by Professor Lombard's balance for estimating continually changes in human body weight.² As the writer was fortunate enough to witness the development of the various parts of this balance, the idea gradually suggested itself to register the outflow by some form of continuous weighing.

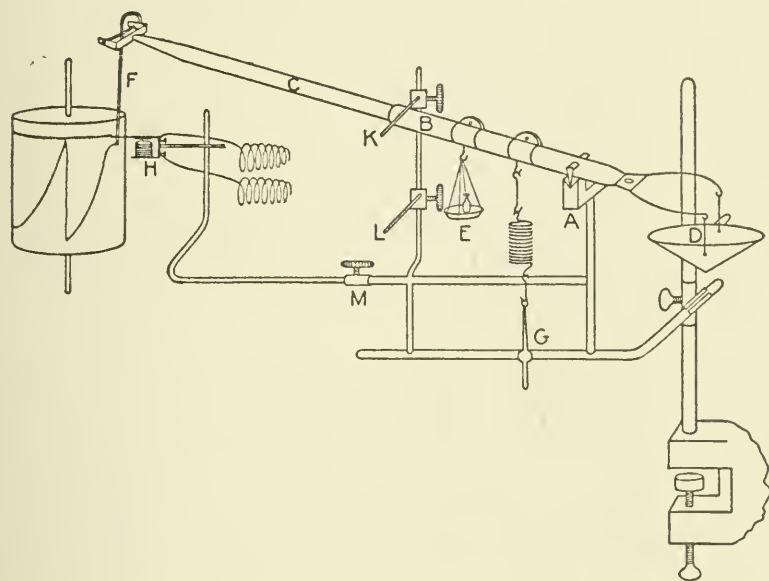


FIGURE 1.

Fig. 1 shows the apparatus diagrammatically. It is essentially a balance arrangement supported on a horizontal L-shaped rod clamped to an upright. The beam of the balance consists, on one side of the fulcrum (A), of an aluminum tube (B) into which a similar tube (C) slides, and, on the other side, of a forked projection suspending the drip-receiving pan (D). By sliding the smaller aluminum tube (C) in and out of the larger tube (B) the excursion of the celluloid pointer (F) is suitably magnified. The drip-receiving pan, made of light metal, is wide and shallow, the actual width being 6 1-2 inches and the depth 2 inches. It is cone-shaped so that,

² LOMBARD: Journal of the American Medical Association, 1906, xlvii, p. 1790.

as fluid accumulates, the centre of gravity practically does not shift in relation to the axis. The pan is counterbalanced by appropriate weights placed on a small scale pan (*E*), the number depending on the magnification. This small scale pan (*E*) is attached to the aluminum beam so that it and the centre of the drip-receiving pan are equidistant from the fulcrum.

The beam and knife edge serve as a lever; so, in order to allow sufficient excursion, the knife edge was made as narrow and its rest as

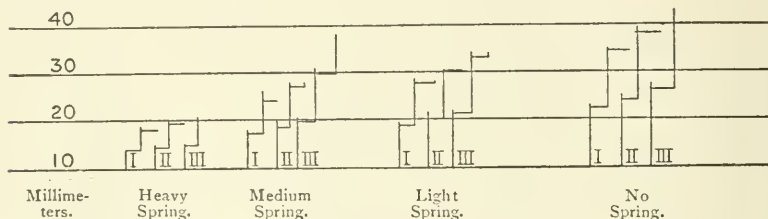


FIGURE 2.—Vertical lines show in millimetres the actual distances moved by pointer after addition of 1 gm. to drip-receiving pan. Horizontal lines represent distances of 10 mm. each. Roman numerals refer to magnification used with each spring, as follows: I = 3.2, II = 3.6, III = 4.2.

wide as was mechanically feasible. The weighing is in reality accomplished by a spiral spring of phosphor bronze or music wire, attached near to the axis in order to minimize changes in the direction of its pull as the lever moves up and down. Any tendency for friction or inertia to hinder the production of an accurate record is adequately guarded against by the evenly repeated jars of a second's signal (*H*) attached to the upright supporting the knife edge. Two movable stops (*K*, *L*) limit the excursion of the celluloid pointer to the width of the paper.³

Calibration.—With the celluloid pointer touching the bottom of the paper, one-gram weights were added to the centre of the drip pan, and the vertical distances moved by the pointer in consequence were recorded on a drum. It was thus found that the distances moved at the top and bottom of the paper were greater than toward the centre. This was due to the fact that as the aluminum lever moved from a horizontal to an oblique position it subscribed an arc, while the celluloid pointer accommodated itself to the vertical surface of the drum and so slightly changed the length of the lever. As

³ To avoid corrosion of the knife edge or its rest, a metal shield has recently been so arranged above them as to avoid accidental contact with the outflowing liquid.

this difference by adjustment could be reduced to fractions of a millimetre, and as it was found by calibration that from 4 to 16 mm. equalled 1 c.c. of water, the error could be disregarded for the class of experiments for which this apparatus was designed. Fig. 2 shows the nature of the results obtained by calibration at the middle of the drum. It is there shown how, by adjusting the magnification and substituting heavier or lighter springs, almost any magnification of the outflow may be obtained.

During an experiment the part whence the fluid is derived must be suspended above the drip-receiving pan and the apparatus allowed to write on an evenly moving drum. As long as the weight of the fluid entering continues the same, it writes an oblique line, which becomes more inclined to the horizontal, however, if the amount entering becomes less, and more to the vertical if it becomes greater. Subsequent to an experiment the actual vertical rise can be deciphered at any point for any length of time, and converted into grams of fluid by reference to a calibration table, *or* to cubic centimetres by additional multiplication by the specific gravity of the fluid received.

By means of this apparatus the flow of fluid from perfused organs, the flow of lymph, the amount of hemorrhage from veins and arteries, the secretion of urine, saliva, bile, gastro-enteric secretions, etc., can be studied without the insertion of any outflow cannulas.

In conclusion, I wish to acknowledge the extent to which Professor Lombard's kindness in lending his valuable experience in balance construction has aided in the successful completion of this apparatus.

CONCERNING THE ACTION OF CURARA AND PHYSOSTIGMINE UPON NERVE ENDINGS OR MUSCLES.

BY CHARLES WALLIS EDMUNDS AND GEORGE B. ROTH.¹

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

INTRODUCTION.

FOR the last fifty years one of the first facts learned by students of physiology and pharmacology has been that curara caused a paralysis of the endings of the motor nerves. This fact, first demonstrated by Claude Bernard and by Kölliker, has been confirmed by later workers, until it has been accepted as one of the fundamental truths in the study of drug action, resting not only upon a physiological basis but also upon histological evidence, as will be pointed out later. Notwithstanding this widely accepted view, some facts have been advanced which seem to indicate a direct action on the muscle cell itself instead of upon the nerve ending.

In 1883 Heidenhain² found that if one half of the tongue is paralyzed by cutting a hypoglossal nerve, and varying periods of time are allowed for the peripheral end to degenerate, and then nicotine is injected into the circulation, the drug will strengthen fibrillary contractions which may be present in the paralyzed muscle, and will also produce a distinct tetanic contraction of that part of the tongue. These effects are prevented by previous injections of curara, and as the nervous tissue had degenerated, both nicotine and curara must have acted upon the muscle cell directly.

Much more recently Langley³ has advanced the same view, here again offering as evidence the relation of curara to some effects produced by nicotine. His reason may be briefly summarized as follows: Nicotine produces in certain muscles of the fowl a tonic

¹ Fellow in Pharmacology, Parke, Davis & Company.

² HEIDENHAIN: *Archiv für Anatomie und Physiologie*, 1883, Suppl., p. 133.

³ LANGLEY: *Journal of physiology*, 1906, xxxiii, p. 374.

contraction which is removed by curara and which may later be reinstated by new injections of nicotine; the two drugs therefore are antagonistic to one another and act upon the same structure. This relation still persisting after degenerative section of the motor nerves, both drugs must act upon some constituent of the muscle cell itself, which Langley calls the "receptive substance."

The existence of some structure intermediate between the histologically demonstrable nerve ending and that constituent of the cell upon which it depends for its characteristic action has been postulated by some writers to explain the results obtained by the use of several drugs which retain their action upon certain peripheral structures after degenerative section of the nerves going to the structures. If, now, on such a denervated tissue a drug will still retain its activity, it must act directly upon the cells of that tissue. But similar tissues in different parts of the body may be affected in different degrees by the same drug. It is necessary, therefore, in order to explain these results, to suppose that such similar tissues possess chemical differences which may reside either in the substance characteristic of the cells of the individual tissues or in some other constituent of the cells, and this structure has been named the "receptive substance."

It is natural that different writers, in advancing such views, should have used different names for the same structure, and confusion has naturally arisen. Brodie and Dixon⁴ speak of the nerve ending "as the connecting link between nerve fibre and muscle fibre and which . . . does not necessarily . . . degenerate when the nerve fibre which terminates in it degenerates." There is grave objection to this view, because the term "nerve ending" has already a well-established place in histological and physiological literature. It is a structure demonstrable by histological methods, being the terminal arborization of the nerve, probably a prolongation of the axis cylinder, and which, as Huber⁵ has shown, undergoes degeneration when the nerve trunk is cut. Also from the pharmacological standpoint an action on nerve ending has always been considered as one which remains after simple section of the nerve trunk, but not after degenerative section, as pointed out by Langley and Anderson.⁶

For these reasons the definition given by Brodie and Dixon should be rejected.

⁴ BRODIE and DIXON: *Journal of physiology*, 1904, xxx, p. 494.

⁵ HUBER: *This journal*, 1900, iii, p. 341.

⁶ LANGLEY and ANDERSON: *Journal of physiology*, 1905, xxxiii, p. 437.

Elliott⁷ calls such an intermediate structure the "myoneural junction," the same being part of the muscle in as far as its trophic centre lies in the muscular nucleoplasm.

Anderson⁸ suggests that the term "myoneure" be used to designate that part of the myoneural junction which persists after degenerative section of the nerve trunk.

Langley,⁹ as explained above, considers that in cells there are two constituents: (1) a substance concerned with carrying out the chief functions of the cells, as secretion, contraction, etc.; and (2) a receptive substance capable of setting the chief substance in action and which does not degenerate when the nerve is cut. The views of Elliott and Langley are essentially the same in that both assume the existence of an intermediate substance. Elliott, however, localizes the substance as being in the immediate neighborhood of the nerve ending, and considers it as having been developed as a result of the union of the cell with a nerve fibre.¹⁰

NICOTINE AND CURARA ON NORMAL MUSCLE.

In some experiments carried out upon the relation of nicotine and curara upon the normal and degenerated muscles of fowls we were struck by the slight, and in very many cases the utter, lack of effect produced by curara against nicotine in the degenerated muscle. This fact has been explained by Langley as being due to the increased irritability of the degenerated muscle, and as this action upon the denervated muscle practically determines the point of curara action, we have studied the question in all stages of muscle degeneration.

We adopted the method of Langley, using the muscles of the fowl's leg in both normal and denervated conditions. To avoid as far as possible individual variations shown by different fowls to the two drugs, all the work was done on Plymouth Rocks of about the same age (six months), all obtained from the same barnyard and weighing from 1400 gm. to 1650 gm. each. In almost every case they were cockerels.

⁷ ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 436.

⁸ ANDERSON: *Journal of physiology*, 1905, xxxiii, p. 437.

⁹ LANGLEY: *Journal of physiology*, 1905, xxxiii, p. 399.

¹⁰ The nomenclature introduced by Langley will be used in this paper, as being the clearest and most satisfactory in the present state of our knowledge.

They were anesthetized by means of paraldehyde, 2 c.c. per kg. given by the rectum, in most cases this amount insuring a very satisfactory anesthesia, but in a few instances a second small injection had to be given. When anesthesia was complete, a cannula was inserted in the trachea and a venous cannula in the jugular vein. The sciatic nerve was exposed and cut either before or at the time of the experiment. If it was desired to study the action on a degenerated muscle, the bird was given ether, the nerve exposed and cut and the wound sutured, a collodion dressing applied, and the bird kept alive for the desired length of time.

Perhaps the simplest way to expose the nerve is by cutting through the skin and muscles (gluteus and biceps flexor cruris) high up in the thigh. The nerve is easily found lying beside the artery, from which it is carefully separated, raised, and cut. At the time of the experiment, after the nerve has been prepared, the tendo Achilles is dissected free for a distance of 4 to 5 cm. up to the beginning of muscular tissue proper of the gastrocnemius, so that the greatest amount of freedom is obtained without interfering with the blood supply to the muscle. An incision is now made through the skin and muscles for a distance of 4 or 5 cm. over the femur, and the tissues stripped from the bone a sufficient distance to allow a screw clamp to be inserted and fastened to the bone. By means of this clamp, which is fastened to a standard, and a second clamp which is fastened to the ankle joint, the legs of the bird are completely immobilized. The tendons which have been separated from the surrounding structures are now fastened by threads to writing levers which record the curves on a kymograph in the usual manner. Except as will be noted, the weights used were 22 gm. on each lever. In all cases artificial respiration was started before the beginning of the experiment.

The drugs used were curara (Merck), which was made up in the strength of 1 per cent in physiological salt solution, and nicotine chloride ($\frac{1}{2}$ per cent) made by neutralizing nicotine with $n/20$ hydrochloric acid.

The symptoms produced in the fowl by the injection of, say, 1 mg. of nicotine have been described by Langley, so that it is hardly necessary to discuss them at length. They consist essentially of a slow tonic contraction of certain muscles, a contraction which may be best studied in the gastrocnemius. These begin to contract within a few seconds after the injection, and the maximum is reached in varying lengths of time, — in some thirty seconds, while in others the lever may continue to rise for two minutes. The contraction may then remain at its maximum for a few minutes, or a gradual relaxation may begin at once and last for ten to thirty minutes or

even more, but in no case does the lever return to the original level, there being always a contraction remainder. If a sufficient dose of curara be administered while the contraction or relaxation is progressing, the nicotine action may be overcome within a few seconds, as is shown in Fig. 1; but, as will also be seen by this curve, the contraction is not completely removed, the same remainder (contracture) is still present. In only one instance in all the

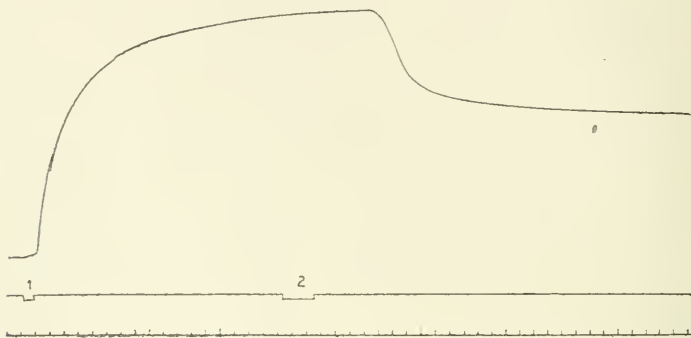


FIGURE 1.—About one third the original size. Tracing from normal gastrocnemius of fowl. Effect of injection of 2 mg. nicotine chloride (1) followed by 12 mg. curara (2). Time in ten-second intervals.

curves we have taken has the lever returned to its original level under the influence of curara. No explanation can be given for this one exception beyond a possible alteration in susceptibility. If, now, after the curara has been given, a sufficient dose of nicotine is administered again, the contraction will return in much the same manner as at first, and it in turn may be removed, as before, by a sufficient quantity of curara, the two drugs being antagonistic.

NICOTINE AND CURARA ON DEGENERATED MUSCLES.

Langley investigated the relation in five fowls in which the sciatic nerves had been cut, six, eight, twenty-seven, thirty-eight, and forty days each respectively. He found that "curara still exercised its antagonistic effect, but the antagonism was distinctly less than normal."¹¹

This action of the two drugs on denervated muscle we have examined in quite a large series of experiments in which the nerves have been cut for from two to fifty-three days. In those fowls in

¹¹ *Op. cit.*, p. 395.

which the nerves were allowed to degenerate only from two to five days we have carried out two or three experiments for each period in order to confirm the results obtained in the first, as it is on these days that the most important changes were found. In all cases tracings were taken from both the normal and operated legs to enable a comparison to be made, and also, with the same object in view, the initial injection of nicotine was 1 mg. and that of curara 15 mg. A study of the curves obtained in the different stages of degeneration showed the following:

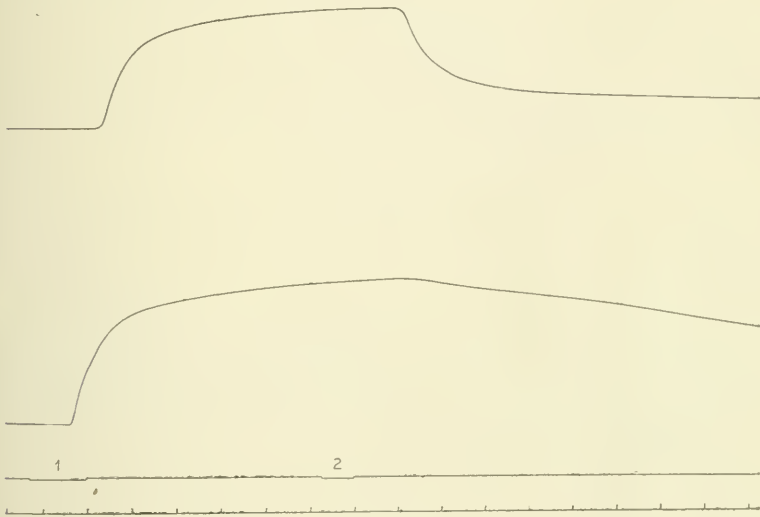


FIGURE 2. — About two fifths the original size. The upper curve from the normal and the lower from the degenerated gastrocnemius, on the third day. Lower curve shows the slight effect of curara (2) on the nicotine (1) contraction where the nerve ending has partially degenerated.

Two-day degeneration. — Stimulation of the cut end of the sciatic by the faradic current showed it to be still active, and curves obtained from the two legs were practically identical.

Three-day degeneration. — In one fowl the sciatic was still slightly active, and here repeated injections of the drugs showed a very distinct effect from curara in the curve of the degenerate muscle, but the relaxation was not as marked as in the normal leg.

In two more fowls stimulation of the sciatic elicited no response. In both of these nicotine caused a good contraction, and in one the curara action was distinct (Fig. 2) but slight, while in the other no curara action could be made out.

After the third day electrical stimulation of the cut nerve brought out no response.

Four-day degeneration. — In both fowls curara had no distinct effect whatever upon the contraction due to nicotine.

Five-day degeneration. — Repeated injections of the two drugs, given at first in the usual doses and later in larger amounts, showed that the nicotine curve from the operated leg just after injecting the

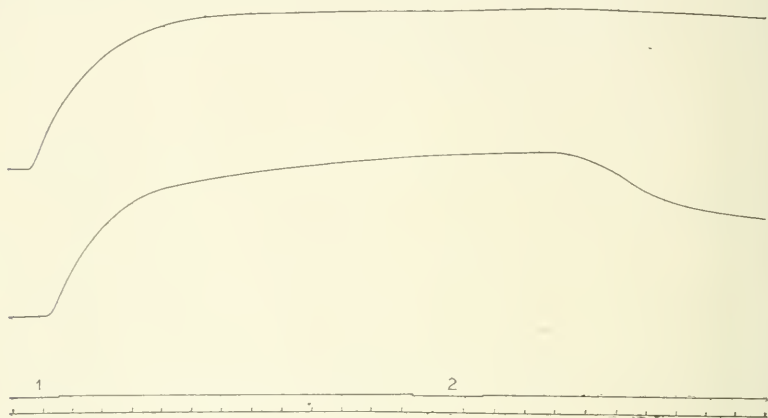


FIGURE 3. — About one fourth the original size. The lower curve is from a normal muscle. The upper curve is from a muscle in which the nerve has been cut five days. At (1) nicotine was injected, at (2) curara. Note slight alteration in direction of upper curve when curara was injected.

curara suffered a slight alteration in direction, producing a very slight angle in the curve, as will be seen in Fig. 3.

Seven-day degeneration. — This curve was rather unique in that it showed a double rise, the second rise being preceded by a distinct fall. The first injection of curara met the curve of the degenerate leg on its second rise, and far from causing a fall it merely delayed the upward progress of the curve for a brief space. On a second nicotine curve curara produced the same suggestion of an angle in the curve as mentioned under the fifth day, but there was no distinct fall.

Curves taken on the eleventh, fourteenth, twenty-first, thirty-third, and fortieth days all show essentially the same action, namely, that after about the fifth day curara has no effect on the course of the curve from the degenerate leg except in some to produce the angle referred to. In all our experiments there was only one excep-

tion to this statement, and that was in a fowl in which the nerve had been cut twelve days and in which curara produced a very definite fall in the curve due to nicotine.

In a curve of the fortieth day successive injections of 1, 3, 3, 5 mg. of nicotine produced contractions which were absolutely unchanged by 15, 50, 50, 50 mg. of curara respectively.

In support of our experiments those of Langley himself may be brought forward as showing that curara has little effect on degenerated muscles except to produce the angle in the curve, the explanation of which will be taken up later.

He says¹² that 15 mg. curara caused in the nicotine curve a "slight increase in the rate of relaxation." Later injections of nicotine produced rises in the lever, and curara was given, but in neither case does he mention the curara action, although the nicotine effects are noted. The conclusion may be drawn that there were no effects.

In the other protocol given (Experiment III.) all the effects of the nicotine are given, but the curara effects on the degenerated muscle in all three injections are again omitted, justifying the conclusion again that there were none. Also, in the only curve he gives of curara on the degenerated muscle the first injection of 10 mg. apparently caused a slight pause in the upward movement of the lever, while the second injection of 20 mg. produced only the suggestion of an angle in the curve described by us; so that, as far as can be judged by the protocols and curve, Langley's results agree exactly with our own.

Langley explains this lack of action of curara by increased sensitiveness of the muscle to nicotine. That the muscle which is partially degenerated is more irritable to certain forms of stimuli is well known and has been proved by Elliott, Anderson, Lewandowsky, and others. We have also found the same to be true in this case: such a muscle will always contract first to the nicotine and to smaller doses than the normal muscle, and, as will be described later, physostigmine and barium also produce contractions earlier in a denervated than in a normal muscle. An increased irritability to nicotine then is present, and it is possible that this may explain the lack of curara action, even large doses failing to overcome the nicotine action in several experiments in which we gave much larger doses than would be necessary for the normal muscle.

¹² LANGLEY: *Journal of physiology*, 1906, xxxiii, p. 396, Experiment I.

In all our experiments with one exception the failure of curara to affect the nicotine curve began about the third day of degeneration; that is, the onset of the exaggerated susceptibility to nicotine coincided with the degeneration of the nerve ending which was shown by Huber¹³ to exhibit marked alterations in the ending at about the end of two days, at which time the muscle also began to fail to respond to nerve stimulation. These changes consisted in their earliest stages of round or oval thickenings which appeared on the arborizations of the motor endings; later the endings failed to take the differential stain by the methylene blue method, so that further changes could not be made out. This coincidence in time between the failure of the curara effect and the degeneration of the nerve endings certainly suggests a relation between curara and the nerve ends, and it is not at all necessary from this point of view that the failure to respond to the two forms of stimuli should appear at exactly the same time.

In addition there is direct histological evidence that curara acts upon nerve ends.

Miura¹⁴ showed that if frogs were paralyzed with curara and then kept alive for five or six weeks, an examination of the muscles showed atrophy of the nerve endings, the end plate stained badly, and the granular substance was reduced. The cells of the cord, the nerve trunks, and the muscle substance proper were not altered.

M. Cavalié¹⁵ found in rabbits and in the torpedo that under curara the muscle fibres are intact as to their nuclei; that the nerve trunk is not altered save at its termination in the motor plaque; the nuclei of arborization are less numerous and altered in outline and stain badly. The primary nerve branches are more irregular than normal, and the secondary terminal nerve branches do not stain.

Also Herzen and Odier¹⁶ showed that under the action of curara the terminal digitations of the nerve fibres are no longer regular, but present a series of swellings. The axis cylinder in place of staining a uniform violet is covered by fine granulations indicating structural changes; these being most marked near the terminal arborization.

These histological examinations would certainly be additional proof that the nervous tissue is affected by curara, especially when taken in connection with the physiological facts given above, but at

¹³ HUBER: This journal, 1900, iii, p. 341.

¹⁴ MIURA: VIRCHOW'S Archiv für pathologische Anatomie, 1886, cv, p. 129.

¹⁵ CAVALIÉ: Société de Biologie, 1903, lv, p. 615.

¹⁶ HERZEN and ODIER: Archives internationales de physiologie, 1904, i, p. 364.

the same time too great weight must not be laid upon such evidence on account of the great difficulties and recognized uncertainties connected with the histological study of nerve endings.

But if we believe the curara action to be merely one of nerve ends, how shall we explain the changes in the form of the curve obtained in some cases from the denervated muscle under its action? What evidence is there of a direct effect of curara on the muscle structure itself? It must be admitted that this evidence is very conflicting.

Cl. Bernard, Kölliker, v. Bezold, and Rossbach and Anrep found that curara had no effect upon the muscle substance proper. Boehm found the same to hold true for curarin. On the other hand, the majority of workers have found that the muscle curve is considerably altered by the injection of curara. Among those holding this view are Valentine, Vulpian, Buchheim and Loos, Mendelssohn, and Rossbach and Rosenthal. Also Rossbach and Clostermeyer, together with Couty and Lacerda, Bochefontaine, Nikolski and Dogiel, and finally Gréhant and Quinquand, all describe changes in the reaction of muscular tissue due to an injurious action of curara.

From the mass of conflicting evidence there would seem to be little doubt that curara has a direct action on muscle tissue apart from its paralyzing effect upon the "nerve endings." As to just what the effect consists in it is harder to say. In small doses several observers have ascribed to the drug an action increasing the irritability of the muscle. The great difference, however, in the findings lies in the effects of larger doses. Here some workers, notably Rossbach, found the duration of the contraction was much shorter than normal, while opposed to this view is that of Bochefontaine and others who compare the curara muscle curve to a veratrine curve. The cause of this wide divergence in views may possibly be due to impurities in the curara itself, these differing depending upon the source and mode of preparation of the individual specimen used. This idea would receive support from the work of Boehm, who using pure curarin found no muscular effect even from very large doses.

Some of the writers quoted above used Merck's curara, which was also employed by us in our work. We therefore carried out a series of experiments upon frogs, rabbits, and on a fowl to ascertain whether our specimen had any action upon muscle, and if so whether it shortened or prolonged the curve.

The animals were anaesthetized and prepared as for our earlier experiments. Direct stimulation of the muscle by the tetanizing current was carried out by needle electrodes inserted directly into the muscle and connected with a secondary coil. The strength of the current was the same before and after the curara injection, and the sciatic nerve was cut in all cases. The amount of curara injected into the jugular vein was 20 mg., and the muscle was stimulated immediately after the curara injection and later at intervals of three minutes with practically identical results. These consist essentially of a diminished strength of contraction and almost total disappearance of contracture, the duration of the curve being much shortened, the shortening taking place mainly in the descending limb of the curve. This would give the curve obtained by a very short stimulus more of a pointed appearance. The results then agree exactly with those of Rossbach and others, and applying these findings to the curara action on the nicotine curve in denervated muscle we think there would be sufficient explanation of the change in the direction of the curve. The lessened irritability of the muscle to a uniform stimulation would naturally cause the curve to decline somewhat, giving it the slight angle to which attention has been called. Another fact in favor of this view is that the change produced by curara is most marked in the early injections of the drug, and, as we have found, may entirely disappear later, which it certainly should not do if it was due to a true antagonistic action of the curara.

The evidence presented thus far may be summarized briefly as follows: the denervated muscle responds with greater promptness and to smaller doses of nicotine than the normal muscle, but to curara it loses its response at practically the same time as its nerve ending degenerates, after which time the only curara effect obtainable is a slight alteration in the nicotine curve, which is probably due to a direct action of curara on the muscle structure proper.

But how is the exception in the twelfth-day muscle to be explained? Clearly it cannot be explained in the above manner, but could be easily accounted for on Langley's theory of increased irritability. In this muscle for some reason the susceptibility to nicotine action was not as high as on the others, and therefore curara was able to throw nicotine out of its combination.

It was necessary, therefore, in order to prove or disprove the hypothesis of "increased susceptibility" to find some other substance

which would bring about a contraction similar to that of nicotine but which would not possess such a strong affinity for the muscle cell as nicotine. Under such conditions it might be possible to throw it out of combination by means of curara.

Physostigmine was tried for this purpose on account of certain peculiar muscular symptoms it produces, some of which resemble those from nicotine, and also because of the great similarity in action existing between the two drugs, as brought out by Rothberger, who showed¹⁷ that both nicotine and physostigmine are antagonistic to curara, so that curarized animals may have their voluntary movements restored by the careful injection of either drug. Physostigmine was used in the form of the salicylate¹⁸ dissolved in salt solution. All the other experimental arrangements were the same as used with nicotine excepting that 2 gm. weights were used on the levers. In all our later experiments a preliminary injection of atropine was given to paralyze the vagi, and also to prevent the muscle twitchings, which at times were very marked when this was omitted.

PHYSOSTIGMINE ON THE NORMAL MUSCLE OF FOWLS.

When the drug in doses of from 2 mg. to 5 mg. is injected in the normal bird, no change is observed for about ten, twelve, or even fifteen minutes, when a contraction begins. The curve differs in the individual birds, but generally the course is about as follows: there is a gradually increasing tone, shown by a very slight rise in the lever, beginning, as stated, on an average of ten minutes after the drug is given; this is followed after about one or two minutes by a more rapid contraction, lasting about ten minutes, and then relaxation begins, but is very slow and in some cases may have progressed very little in thirty minutes. The curve seems to be essentially like that produced by nicotine, but with the difference that it is much slower in every part of its course, — in its appearance, rise, and descent. We have never seen the muscle relax completely, there being always the contracture remaining. Also the curve rarely rises as high as it does under nicotine.

If atropine has not been given previous to the physostigmine, the

¹⁷ ROTHBERGER: *Archiv für physiologie*, 1901, lxxxvii, p. 145; *Ibid.*, 1902, xcii, p. 408.

¹⁸ Melt. point, 179°.

curve drawn by the levers will show irregularities produced by the muscle twitching so characteristic of physostigmine action; these twitches, as is well known, are either prevented or removed by atropine.

If atropine is injected during the course of the tonic contraction, it has very little effect; small doses of from 1 to 5 mg. seemed to hasten the contraction in case it was given while the contraction was coming on, while still larger doses of 10 to 20 mg. in many cases given during the height of contraction had no effect, while others produced a very slight degree of relaxation. The general impression made was that atropine had practically no effect upon this tonic contraction.

Curara.— This drug injected in doses of 5 to 15 mg. produced at once a relaxation of the physostigmine contraction exactly like that produced in a nicotine contraction, but as with nicotine it never brought about complete relaxation; a condition of contracture always remained. Efforts to reproduce the contraction by further injections of physostigmine were successful in proportion to the dose of curara given. In one bird, after 15 mg. of curara, physostigmine given in divided doses up to 115 mg. did not cause any distinct contraction, but only a stage of increased tone. In another bird 100 mg. of physostigmine in divided doses only caused increased tone after a previous injection of 10 mg. curara. In a third bird, after 5 mg. of curara had removed the primary physostigmine contraction, 60 mg. of physostigmine caused an increase in tone in the normal leg, while a further injection of 10 mg. produced the typical physostigmine contraction, thus demonstrating the direct antagonism between the two drugs.

PHYSOSTIGMINE ON DENERVATED MUSCLES.

Experiments were carried out on a large series of fowls in which the sciatics had been cut and allowed to degenerate for varying periods of time ranging from twenty-four hours to fifty-three days. The description of one will essentially apply to all. The most noticeable difference from the normal muscle consisted in the increased sensitiveness of the denervated muscle, which is very plainly seen even twenty-four hours after the nerve has been cut. The degenerated muscle always responds first, and in some cases the contraction appears within one minute after the physostigmine has

been injected. The contraction progresses with varying degrees of rapidity, in one or two cases the ascending limb of the curve rising nearly as abruptly as with nicotine, while in others it is much less so. The contraction remains at the maximum for a few minutes, and then partial relaxation may set in, frequently to be replaced later by a second rise giving the double form of curve which was described under nicotine. Subsequent physostigmine injections were frequently followed at once by a slight relaxation followed by increased contraction. We have obtained a response of this kind as late as fifty-three days after the nerve was cut. However, after the thirty-fifth day the contraction is not as marked as it was previous to that time, because the muscle has undergone such extensive degenerative changes that it does not respond as well to physostigmine. Very marked reactions were obtained in the third or fourth week, as will be seen by Figs. 4 and 5, and at this time there can be no doubt that the nervous tissue has degenerated.

CURARA VERSUS PHYSOSTIGMINE ON DENERVATED MUSCLES.

Curara antagonizes physostigmine during all the stages of degeneration. This is seen very plainly during the first week or two of degeneration, when even 5 mg. of curara removes the physostigmine contraction (except the condition of contracture). When the muscle is very irritable and sensitive to the physostigmine, as it is during the third and fourth week, it is necessary to give larger doses of curara, and, as seen in Fig. 4, on a twentieth day muscle which was unusually sensitive 10 mg. of curara only altered the direction of the curve slightly. On less sensitive muscles the curara action is plainly to be seen.

It is very easy to show the antagonism existing between the two drugs on the denervated muscles. Even after comparatively large doses of curara (15 mg.) further injection of physostigmine in doses of 10 to 15 mg. will almost immediately reinstate the physostigmine contraction, which may again be removed by curara.

DISCUSSION.

Point of curara action.—Physostigmine proves itself a much more favorable drug to investigate the action of curara than does

nicotine, because the muscle is much less sensitive to its action, as is shown by the slow onset of the contraction which it produces. This (what may be called) lessened affinity existing between the cell and the physostigmine allows the latter to be thrown out of its combination more easily by curara, and enables the action of the latter to be seen plainly even on degenerated muscles, which are

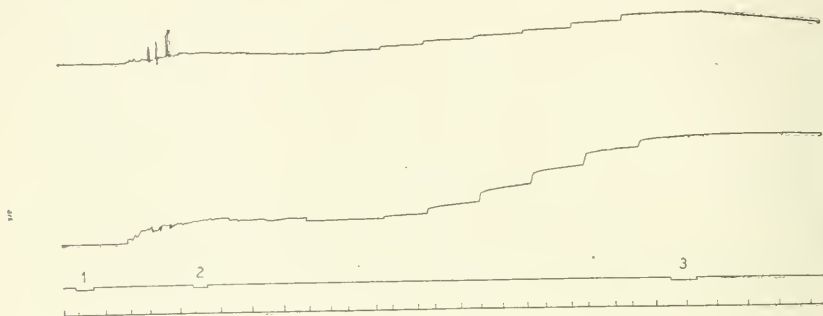


FIGURE 4. — Two sevenths the original size. The upper curve is from a normal, and the lower from a "twenty-day" degenerated muscle (at (1) 5 mg. physostigmine were injected; at (2) 2 mg. atropine; at (3) 10 mg. curara). Early part of tracing shows convulsive movements produced by physostigmine. Atropine had not been injected before the point marked (2). Drum stopped at intervals.

naturally much more sensitive to stimulation. By the use of these drugs it was possible to prove the correctness of the point raised by Heidenhain and Langley, that curara acts on some non-degenerating constituent of the muscle cell, and also of Langley's explanation of the failure to show the curara relaxation in degenerated muscle as being due to the increased sensitiveness of denervated muscle to nicotine.

Point of physostigmine action. — Physostigmine acts directly upon the muscle cell itself, as it causes contraction seven or eight weeks after the nerve has been cut and the nerve ending has degenerated. The muscular action of physostigmine has such an important bearing upon the whole question that it will be discussed more fully. That the drug acts upon muscle is not new, as Harnack and Witkowski¹⁹ say that the drug stimulates muscle and not nerve, but they suggest that it may stimulate some nervous structure lying peripheral to that paralyzed by curara, but that between a muscle and nerve action the probability is in favor of the former. Schmie-

¹⁹ HARNACK and WITKOWSKI: *Archiv für experimentelle Pathologie und Pharmakologie*, 1876, v, p. 401.

deberg²⁰ says physostigmine probably acts on all contractile tissues, the nervous tissue not being affected directly.

Rothberger²¹ describes the fascicular contractions produced by physostigmine as appearing in curarized animals, but as being stopped by atropine and not reinstated by additional physostigmine.

On the other hand, there is much evidence that physostigmine

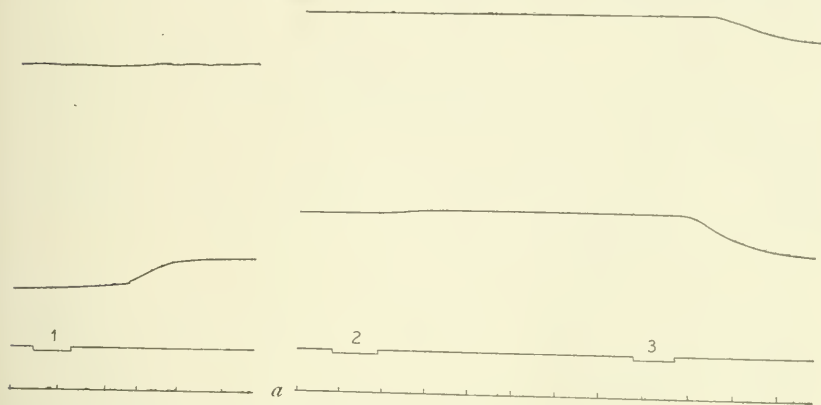


FIGURE 5. — The effect of physostigmine in normal (upper curve) and in "thirty-one day" degenerated muscle (lower curve) (1) 5 mg. physostigmine; (2) 10 mg. atropine; (3) 15 mg. curare. Five-minute interval at *a*.

has an action on nerve endings. Schweder²² ascribed the contractions as being due to an action on the intramuscular nerve endings. Magnus²³ showed that if the nerve going to a muscle is cut and allowed to degenerate, the drug will no longer call forth the muscle twitching it produces in the normal condition, but it might be questioned whether the lack of action is not due to degenerative changes in the muscle rather than to the loss of nerve endings.

If we accept Magnus's experiment as demonstrating the action on nerve ends, we have also our own experiments proving a direct muscular effect, which might be said to confirm the views of Harnack and Witkowski and others mentioned. But, as far as we know, the effects we get are altogether different from any heretofore described. They differ entirely from those given by Rothberger in that they are not stopped by atropine. So we think they really shed no light

²⁰ SCHMIEDEBERG: Grundriss der Pharmakologie, 1906, p. 179.

²¹ ROTHBERGER: Archiv für die gesammte Physiologie, 1901, lxxxvii, p. 138.

²² SCHWEDER: Inaugural Dissertation, Dorpat, 1889, p. 137.

²³ MAGNUS: Zentralblatt für Physiologie, 1907, xxi, p. 497.

upon the debated question of the point of physostigmine action, for the reason that this tonic contraction in the fowl's muscle seems to be an exceptional property of certain muscles, and the action of drugs thereon can hardly be taken as indicating their point of action under what might be termed "normal" conditions. If we cannot do this with physostigmine, neither can we with curara. Under the conditions of these experiments curara has a direct muscle effect, but this need not exclude it from a nerve end action when under "other conditions." There is some evidence of such a nerve end action in the histological changes mentioned earlier, and in addition we have the fact, already pointed out in our work, of the marked change in the curara effect against nicotine, which occurs at practically the same time as the nerve end fails to respond to electrical stimulation, but this fact would not carry any great weight, as the change may be explained by increased sensitiveness.

It is possible that we have in the past tried to localize the point of drug action too closely, confining it to nerve endings or to some muscle cell constituent, whereas either of these may be more or less complex in structure, one drug only acting (that is, entering into chemical combination with it) upon one component, while another drug is capable of spreading its action more widely.

The latter might help to explain the relationship between physostigmine on the one side and curara and atropine on the other.

Antagonism between physostigmine and curara.— The antagonism existing between these two drugs was pointed out by Pal,²⁴ who found that curarized animals regained their movements under the influence of physostigmine. This question was taken up by Rothberger,²⁵ who showed that the antagonism was a peripheral one, and located the action as being on the nerve endings. Schmieberg²⁶ claims that the antagonism is not real but only apparent.

However, our results show a real antagonism, as the two poisons act upon the same organ in an antagonistic sense,²⁷ and moreover the point of action is upon the muscle cell.

²⁴ PAL: *Centralblatt für Physiologie*, 1900-01, xiv, p. 255.

²⁵ ROTHBERGER: *Archiv für Physiologie*, 1901, lxxxvii, p. 119.

²⁶ SCHMIEBERG: *Grundriss der Pharmakologie*, 1906, p. 179.

²⁷ ROSSBACH: *Verhandlung der physikalische medicinischen Gesellschaft in Würzburg*, 1874, vii, p. 29.

CONCLUSION.

The action of nicotine, and more especially physostigmine, against curara on the muscles of the fowl proves that the latter drug acts under these conditions upon the muscle substance proper, but the question is raised whether these results render unnecessary the view that it exercises an action upon the nerve ends under other more normal conditions.

Also on the fowl physostigmine produces a tonic contraction by direct action on the muscle cell, and this contraction differs from previously described muscle effects produced by physostigmine in that it is not affected by atropine.

A direct antagonism between physostigmine and curara is shown.

ACTION OF BARIUM CHLORIDE ON THE FOWL'S MUSCLE.

BY CHARLES WALLIS EDMUNDS AND GEORGE B. ROTH.¹

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

IN the course of our experiments upon the action of nicotine, physostigmine, and curara upon the fowl's muscle to determine whether the action of the latter was upon the nerve ends or upon the muscle, we examined the effect of barium chloride as being a drug whose characteristic action is believed to be upon the contractile substance of the muscle cell, producing a tonic contraction of all muscle tissue due to the toxic action of the drug.

We studied the question upon the normal and upon the denervated gastrocnemii of fowls, the plan of the experiment being exactly the same as we have described in our other paper.² We will first describe effects upon the normal muscle of the injection of from 10 to 15 mg. of barium chloride. If injected at the beginning of an experiment, its action is merely to produce an increase in tonus, this developing gradually during the first five minutes after the administration of the drug. If, now, following this primary injection, a drug such as nicotine is given, the latter will of course call forth its usual tonic contraction, which passes off in the usual manner in twenty or thirty minutes. If at this time the injection of barium is repeated in the same-sized dose as before, it will at once produce a tonic contraction which reaches maximum in about five minutes. The muscle may remain thus contracted for from ten to thirty minutes, or it may begin at once to relax very slowly, but we have never seen complete relaxation take place. Curara does not prevent this contraction nor does it appear to influence its course in any way.

Barium on denervated muscles. — Primary injections of barium in fowls whose sciatic nerves have been cut for periods varying from two to fifty-three days produce contractions which are similar in character to those described above as being given by the normal

¹ Fellow in Pharmacology, Parke, Davis & Company.

² This journal, 1908, xxiii, p. 30.

muscles upon secondary injections of barium (Fig. 1). Curara had no effect upon the course of the contraction. In the earlier stages of degeneration the operated leg usually contracts more than the normal leg, but after about the fifth week the contraction of the normal leg was usually the greater, due, no doubt, to the degenerative changes the denervated muscles had undergone.

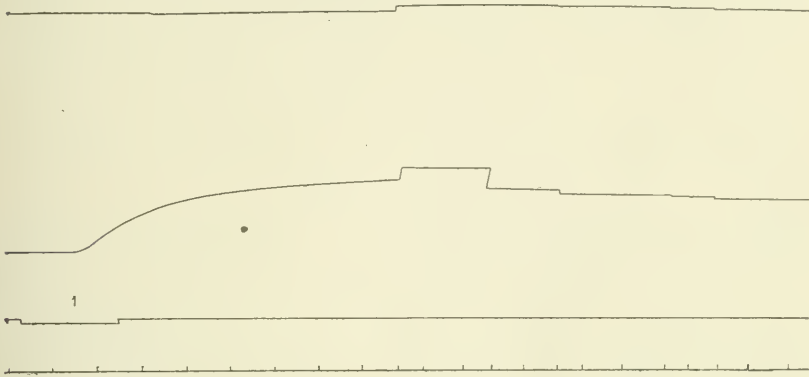


FIGURE 1. — About two fifths the original size. The upper curve is from a normal, the lower from a denervated muscle (sixteenth day). At (1) 10 mg. of barium chloride were injected. Drum stopped at intervals.

The great difference, then, between the results obtained in the normal and in the denervated leg is in the effects of the primary injection of barium; in the former it produces only an increase in tonus, while in the latter a strong contraction results. During the process of degeneration the muscle must undergo some change rendering it more susceptible to the drug's action. This same sensitizing action against barium can apparently be exercised by drugs causing contraction of the muscle, these substances apparently leaving some constituent of the muscle cell in a condition which renders it more irritable to the action of barium, so that to subsequent injections the normal muscle responds by a contraction.

The failure of curara to affect this contraction would point to a more peripheral action of barium as compared with that of curara and its antagonists, nicotine and physostigmine. This would agree, then, with the generally accepted view of the action of barium as being possibly upon the contractile substance of the muscle cell, while the other drugs mentioned would act upon some other constituent of the cell which might be the "receptive substance."

THE ADJUSTMENT OF PARAMECIUM TO DISTILLED WATER AND ITS BEARING ON THE PROBLEM OF THE NECESSARY INORGANIC SALT CONTENT.

By J. FRANK DANIEL.

[From the Laboratory of Experimental Zoölogy, Johns Hopkins University.]

I. INTRODUCTION.

THE question whether organisms can or cannot live in a medium of pure water is one upon which much experimental work has been done, and on which investigators are in no wise agreed; one class holding that distilled water *per se* is toxic, the other that the toxicity recorded is due to impurities contained in the water. Both of the above opinions take into account the water only, when in fact two factors, both fundamentally important, are involved. While it is both difficult and essential to obtain water free from contamination, this alone is not sufficient for a correct solution of the problem; the physiological condition and power of adjustment in the organism itself must likewise be taken into account.

This paper is the outgrowth of a series of experiments carried on for the double purpose of obtaining a pure standard medium in which to test organisms and of observing in how far adjustments to this can be made. The medium used was pure distilled water; the organism studied was a unicellular animal, paramecium.

In this place I wish to thank Professor H. S. Jennings, under whose direction this work has been carried on, for his interest and kindly criticism, and to express my appreciation to Dr. B. B. Turner, of the Johns Hopkins Chemical Laboratory, who made all of the conductivity measurements herein given and offered valuable suggestions on the preparation of pure water.

II. LITERATURE.

A brief review of the literature showing the development of the subject is well worth consideration at this point.

Probably no single paper has been so important in this field as has that by Carl V. Nägeli ('93).¹ Step by step this investigator traced the toxic effects of water, usually finding the source extraneous. Thus he observed that tap water standing in contact with pipes and brass faucets was poisonous to *Spirogyra*. After running for a time, however, the same water produced little, if any, ill effects. Water allowed to stand in glass or platinum was found to leave on the surfaces of these vessels its poisonous effects, which could be removed only by the use of such substances as nitric or sulphuric acid. By re-distilling in glass, and rejecting the first part of the distillate, water was obtained with sufficient purity to maintain life.

Locke ('95)² in repeating the experiments of Ringer and Phear ('95)³ confirmed Nägeli's results; he showed that both the tadpole and the annelid *Tubifex rivulorum*, which they had found were killed by distilled water, would live in water re-distilled from glass.

Re-distilling in glass was a simple solution to the problem, and one that seemed for a time to be satisfactory. Some years later, however, a series of investigators, Bullof (:04),⁴ Lyon (:04),⁵ and Peters (:04),⁶ working upon both single and multiple celled animals, raised the objection that simple re-distillation in glass was in itself inadequate to obtain water physiologically pure. Bullof found that water re-distilled in either glass or quartz is toxic to the fresh-water *Gammarus*. Lyon, experimenting on sea urchin eggs, added that a volatile toxic something may pass over in simple distillation. This may be held back by adding to the retort such reagents as sulphuric acid, or sulphuric acid and potassium bichromate. Peters made it clear that the term "distilled water" is very indefinite. He applied the measurement of electrical conductivity, the test for chemical purity, to physiological experiment. In water showing a very low conductivity he found that *Stentor ceruleus* began disintegration

¹ NÄGELI, CARL VON: *Neue Denkschrift Schweizer. Gesellschaft für Naturforschung* (Nouvelle Mémoires de la Société Helvétique des Sciences Naturelles, 1893, xxxiii), Abth. I, second contribution, pp. 1-52.

² LOCKE, F. S.: *Journal of physiology*, 1895, xviii, pp. 319-331.

³ RINGER, S., and PHEAR, A. G., *Journal of physiology*, 1895, xvii, pp. 423-432, and *Proceedings of the Physiological Society, Journal of physiology*, 1895, xvii, pp. xxiii-xxvii.

⁴ BULLOT, G.: *University of California publications, Physiology*, 1904, i, pp. 199-217.

⁵ LYON, E. P.: *Biological bulletin*, 1904, vi, pp. 198-202.

⁶ PETERS, A. W.: *Proceedings of the American Academy of Arts and Sciences*, 1904, xxxix, No. 20, pp. 441-516

in from three fourths of an hour to an hour's time (Peters, :04, p. 476).

The work just summarized has then added to our knowledge the following important points as to methods: (1) Simple distillation in itself is insufficient for obtaining physiologically pure water. (2) A volatile toxic substance may pass over with the distillate, but this may be held back by appropriate reagents. (3) "Distilled water" is an indefinite term that may be made definite by the measurement of its electrical conductivity.

An important contribution has been made recently by Livingston (:07).⁷ His results confirm those of Nägeli as to the presence and source of contamination in water. Livingston's paper adds most, however, in offering a method for the removal of such volatile substances as Lyon described.

Nägeli pointed out the advantages of adding such insoluble solids as graphite, or coal-dust, in rendering water of better physiological quality; his interpretation of their action was that the large surface of the solid thus exposed took up the toxic substances. Following this and the work of Breazeale (:06),⁸ Livingston has shaken distilled water of different kinds with such various solids as carbon-black, ferric hydrate, aluminum hydrate, and quartz-flour. Distilled water, thus treated, is held to have removed from it both the volatile and non-volatile poisons; thus often making it a better medium for growth than water of a low conductivity, distilled in contact with reagents.

But the question immediately suggests itself, May not the so-called "insoluble" substance be to a considerable extent soluble and may it not add something that aids growth? Livingston makes it probable, however, that the beneficial effect is due to a taking up of harmful substances from the water. Thus he shows that thoroughly washed carbon-black, ferric hydrate, and aluminum hydrate, though chemically different, all have a similar effect on wheat seedlings, grown in distilled water. This would not be expected if these solids gave up their different chemical substances to the water. Further he adds that water thus treated shows practically no increase in its electrical conductivity. From this it would appear that the benefit results from a removal rather than from an addition of substances.

⁷ LIVINGSTON, B. E.: Further studies on the properties of unproductive soil. Bulletin No. 36, 1907, Bureau of Soils, Washington, D. C.

⁸ BREAZEALE, J. F.: Botanical gazette, 1906, xli, pp. 54-63.

As stated above, re-distilling in glass usually gives a good quality of water; if, now, carbon-black is able to remove the volatile toxic substance which may pass over even in the most careful re-distilling, then, by combining the advantages of both of these methods we should be able to obtain water of a low conductivity and at the same time of physiological value.

To a preparation of such water we shall now turn.

III. PREPARATION OF DISTILLED WATER.

Two main difficulties have presented themselves in the use of carbon-black: (1) If it is shaken with water of a low conductivity (e. g., 1.6×10^{-6}), it often remains suspended colloid-like for days. Livingston has shown, however, that water of a low conductivity re-distilled in contact with reagents and shaken down with solids has little, if any, advantage for physiological purposes over the same treatment of ordinary distilled water. Since carbon-black settled readily in ordinary distilled water, I have obviated this first difficulty by shaking the carbon-black with once distilled water, of a good quality. (2) Carbon-black introduces into the water a trace of oil which is in evidence even after the H_2O has been shaken down two or three times. Filtering "treated" water to remove the oil and the particles of the solid increases its electrical conductivity. In order to correct for both the need of filtering and the possible ill effects from the oil, I have re-distilled the water *after* having shaken it with the insoluble solid.

By thus removing the volatile toxic substance with carbon-black, then re-distilling in glass to hold back the non-volatile substances, the method for the production of a non-toxic water has been reduced to a single source of error — that of the re-distilling in glass. This may be made negligible by using Jena glass which has been thoroughly steamed.

Method. — Once distilled water from the laboratory supply was put into a three-litre, straight-necked bottle (bottle No. 1), to which sufficient carbon-black had been added to cover the bottom for a depth of three or four inches. This water was thoroughly shaken down, and after the solid had settled to the bottom and the film of oil had risen to the top, sufficient water was added to carry the film over the mouth of the vessel. One third or one half of the water was then thrown out, more added and shaken as before. In

a day or more of this treatment practically all traces of oil were removed. The water was then siphoned into bottle No. 2 (4 litres capacity), containing carbon-black which had been repeatedly washed in distilled water. This was treated in essentially the same way as bottle No. 1. After this double treatment with the solid the water was ready for re-distillation in glass.

The still used consisted of a four-litre retort and a continuous Jena condensing tube so bent as to prevent spray from passing from the retort to the tube. Connection between the two was made of cork. The upper part of the retort was so covered with asbestos as to prevent condensation by cooling. Both retort and condensing tube after having been thoroughly cleansed with chromic acid were steamed for days to remove the traces of alkalinity from the surfaces in contact with the water. "Treated" water was then transferred by means of siphon from bottle No. 2 to the retort, and distillation was effected without reagents. To prevent traces of foreign substances, volatile or non-volatile, from passing over, the first fourth of the distillate, which experiment has shown most likely to carry volatile substances, was rejected; and the last part, containing the non-volatile, was not distilled. The remaining second and third fourths were allowed to drop four or five inches from the end of the condensing tube to the collecting bottle in order to insure the necessary aeration.

By this method water of a low electrical conductivity (*c. g.*, 3 to 2×10^{-6} reciprocal ohms), as well as of a good physiological quality, was readily obtained in contact with the air.

IV. THE ORGANISMS.

A. Observation. — Having a water upon which we may depend, a study of the animal itself may now be undertaken. That different organisms do not react in exactly the same way to a given stimulus has been emphasized by various observers. In fact, it has been made clear that the same organism at different times may show marked changes in behavior (Miss Towle,⁹ p. 223).

For reasons that will be evident the present study, considering the single organism *Paramecium*, is further limited to the single species *P. caudatum*.

Four strains of this species, which I shall designate as types A,

⁹ TOWLE, ELIZABETH W : This journal, 1905, xii. p. 223.

B, C, and D, have been collected under essentially different conditions of environment, and it is these strains that I have studied in detail.

Type A was a mixed culture containing both *P. caudatum* and *P. aurelia*. These cells had been taken from various cultures at different times, and added to a medium of tap water containing a small amount of hay. They were prosperous, generally free-swimming and of a hardy stock.

Type B.— This strain comes from a medium rich in decayed matter, made by adding to tap water a small bit of hay and some old leaves; it was probably as near an approach to a natural culture medium as could well be maintained under laboratory conditions. In this the animals collected loosely near the surface film on the sides of the vessel. As will be seen later, this type was also strong in resistance.

Type C.— These choice cells grew in a stentor culture of tap water, chara, and a little hay. While many of them were free-swimming, a large number often collected on the side of the jar an inch or two below the surface. This strain was large and exceptionally fine for experimental study.

Type D.— A laboratory culture kept for over seven months in a strong hay infusion. These animals gathered in a fantastically irregular line along the sides and at varying depths from the surface film. Although vigorous, these cells were of a small size, owing, doubtless, to their being kept under abnormal conditions. Power of resistance low.

I take it that the four strains herein described are typical of the different types that have been studied by various observers.

B. Experimental.— To each of four preparation dishes, containing 30 c.c. of known "treated" water, a single drop of paramecia of the above types was added; a drop of Type A to dish 1, a drop of B to 2, etc. This gave opportunity for observing to what extent different strains from different environments show a similarity in behavior and like powers of resistance.

Type A.— Paramecia from Type A when put into the pure medium moved rapidly forward. After a short time, however, the organisms quieted down and showed no evidences of injury. At the end of five days a number of these animals were collected in a small amount of water (5 drops) by means of a capillary pipette, and were again transferred to a second 30 c.c. of "treated" water.

After remaining in this for three days a few of them (six) were transferred a third time in a similar way to a like amount of "treated" water. Three of these were seen three days later and one at the end of the fifth day (thirteen days after the first transfer). These then were able to withstand three transfers in pure water and lived for days without food.

Doubtless experimenters who have worked upon paramecia of this sort would not hesitate to say that distilled water is not in itself destructive of cell life.

Type B differed from the foregoing in several respects. When first subjected to the pure medium, the animals showed greater activity. Backing, and later swelling, was a typical sequence. After remaining an hour and a half the majority gave signs of injury, many moving sluggishly along the bottom, others distorting. By the end of two hours movement had generally ceased. Of this group, however, three were alive and swimming at a normal rate for several days.

Type C. — These large cells, unlike either types A or B, showed extreme sensitiveness to the new medium. Backing was followed by an early swelling; the animals, which at first darted here and there in trying to escape stimulation, soon settled to a slow movement along the bottom. At the end of an hour distortion was marked, the body bursting usually in the middle region and flattening out. A few of this type moved an hour and ten minutes, after which careful observation with the low power showed none in which ciliary movement had not ceased.

Type D. — The cells of Type D acted essentially as did those of Type C, but manifested a still lower resistance. In these death generally came about at the end of thirty minutes; in none of the many cases observed have any of them lived longer than fifty-five minutes. These animals, as explained above, grew under abnormal conditions, and experiment upon them in five different ways gave the same result, death and disintegration within a very short time.

It will be noted that both this and the foregoing Type C died quickly in the same distilled water in which A lived for days. An investigator working upon either of Types C or D would doubtless have been as firmly convinced that distilled water *per se* kills as would the observer on Type A have been of the opposite opinion.

From the results thus far observed, it might be maintained that both are possibly right — that in the latter case the water was

destructive and in the former it was not. Since the same water was used throughout my experiments, the question is: Was not the difference due to the differing physiological conditions of the organisms themselves rather than to toxic influences of the water?

If, for example, we have two strains of paramecia — one living in a dense hay infusion, the other in a pure natural culture — is it not probable that those of the purer medium, if transferred to distilled water, will be better able than will those of the infusion to make the change necessary, since that change is actually not so great?

In other words, may it not be that the greatness of the change is the deciding factor? If this be true, injury would then be due, not to a fundamental incompatibility of distilled water to life, but to the fact that these organisms are now adjusted to other conditions.

In opposition to this view, many investigators have maintained that the inability of organisms to live in distilled water is owing to a fundamental cause, that is, to a lack of a necessary salt content in the water. Our next task must be to determine which of these views is correct.

V. THE PROBLEM OF THE INORGANIC SALT CONTENT.

A. Discussion. — Investigators who hold to the theory that the destructive effects of distilled water are due to a fundamental lack of the salts necessary to sustain life, would of course maintain that life in distilled water would be impossible, both to Paramecia that had lived in nearly pure water and to those that had lived in a dense hay infusion. For the reason why distilled water kills is that in a medium of lower salt content than that of the body protoplasm of the animal a loss of body salts results. If the medium is practically free from inorganic salts (as is distilled water), loss of the body salts continues and death follows. The fact that paramecia were present in both the dense hay infusion and the "purer" medium is sufficient, according to this theory, to show that both contained a sufficient salt supply; a transfer to pure water from either, being fundamentally the same, would therefore cause death.

But if distilled water necessarily kills because of its purity, that is, its lack of inorganic salts, why is it that Type A in the above observations lived for days in a medium of distilled water? To

explain such a case the answer might be made (Peters, :08, p. 109) that distilled water to which organisms have been added does not remain distilled water, as can be shown by its increase in electrical conductivity. This increase in the conductivity of the water is held to mean an increase in the salt content due to salts abstracted from the body of the organism (Peters, :04, p. 513). In case considerable numbers of organisms are added they may be able to give up sufficient salts to raise the content to a life-sustaining plane, provided the animals are kept in the same original quantity of the distilled water (Peters, :08, pp. 117, 119).

But is it safe to assume that because an increase is shown in conductivity this is necessarily due to an increase in the salt content? Carbon dioxide is known to affect conductivity measurements powerfully. The investigations of Jennings ('97)¹⁰ and Barratt (:05)¹¹ have shown that paramecia produce carbon dioxide. This must, then, be a factor to the increase in conductivity. As to the influence of numbers on the salt content, it must be recalled that as many were present in either C or D which died as in A that survived. Furthermore, the third transfer of Type A was made with only a few, and a few could not be assumed to raise the content of 30 c.c. of pure water sufficiently to save life.

The survival of the individuals of Type A (and a few of B) when the others died, is therefore not explicable upon this theory, and we must therefore seek an explanation other than that conditioned on the medium alone.

Peters¹² (:08) has shown, by an ingenious use of the centrifuge, that when animals (paramecium and stentor) are "kept" in pure water by constantly changing this medium at short intervals death ensues. By this method a medium of great purity can be obtained. Thus in his first experiment (Table I, p. 112), by centrifuging and adding 14 c.c. at a time, a volume of 196 c.c. of pure water was added to 1 c.c. of culture which was increasing in purity at every centrifuge; while in Table III 420 c.c. was added to 1 c.c. (30 centrifugings 14 c.c. at a time).

The same dilution of a small amount of culture fluid with a large quantity of pure water, that Peters accomplished by the use of the centrifuge, we have brought about in the simpler experiments de-

¹⁰ JENNINGS, H. S.: *Journal of physiology*, 1897, xxi, pp. 258-322.

¹¹ BARRATT, J. O. W.: *Zeitschrift für allgemeine Physiologie*, 1905, v, pp. 66-72.

¹² PETERS, A. W.: *This journal*, 1908, xxi, pp. 105-125.

scribed above. It may be remarked further that in his experiments and my own death occurred in so nearly the same way, both in time and manner, as to make extremely doubtful the necessity for maintaining the water "in its condition of original purity" (Peters, :08, p. 107).

Thus, to compare some of Peters' results with those which I have described above, we find in his first experiment (Table I) after the twelfth centrifuging, the following: "All dead. Most forms disintegrated." Time, 50 minutes (10.15 to 11.05). In Table III, after the twenty-eighth centrifuging: "Very few alive." Time, 2 hours, 7 minutes (9.35 to 11.42). Those of Table I thus acted much like Type C of my experiments (see p. 54); those of Table III like Type B, while Types D and A of my observations gave results lying at the two extremes, — D dying earlier, A living for days in pure water.

Another point in Peters' experiments is of much importance. Animals from normal culture media with an electrical conductivity of from 616 to 643×10^{-6} were suddenly subjected to a medium of pure water having a conductivity as low as 2.3 to 3×10^{-6} . A change of this sort is evidently extreme, and the question immediately reinforces itself: May not death have been due to this rapidity (or greatness) of the change rather than to the purity of the water?

Both this question and that of the importance of a low salt content can be put to direct experimental test. We shall turn first to the latter problem, — that of the importance of a low inorganic salt content.

B. Method for Experimentation. — If we consider a single drop x from a dense culture of paramecia, it will evidently contain the salt content necessary for life, otherwise the animals would not be found in it. If, now, from the same dense culture a few cubic centimetres be taken and added to a considerable amount of pure water, and if after a day a few cubic centimetres of this containing paramecia be transferred again to a considerable amount of pure water, it is clear that the medium in which the animals are will rapidly become purer and purer as the transfers continue. After having been transferred a number of times, a drop y of this medium will contain a much lower salt content than will that of drop x . If now drop x and y each be added to an equal amount of pure water, since x has taken into the pure medium more inorganic salts and has many more animals to raise the salt content, we would expect the organisms in

it to survive longer than those of drop *y*. If not, the experiment would indicate that a low salt content is not a fundamental factor in determining life or death.

Before proceeding to the experiments it will be necessary to obtain a method by which the organisms for drop *y* can be gradually transferred into a pure medium, approximating that of distilled water.

In this method of transfers adjustment on the part of the animal would be expected. This would make the element of time important. Further, a change to purer and purer water might be accompanied by osmotic effects. Whether these be of importance the method should show.

To correct for the element of time a day or two has been given to each transfer. If osmotic effects be a considerable factor, they would probably occur in the first or greater changes, and so these transfers should be made gradually. With this in mind 18 c.c. of a normal culture was added to an equal amount of pure water; this I endeavored to bring slowly down to a small amount of culture (1 drop), plus a large amount of pure water (30 c.c.). Transfers through 18 c.c. of culture + 30 c.c. pure water; 12 c.c. + 24 c.c.; and 6 c.c. + 30 c.c. were made with safety, but an attempt to add 1 c.c. (of the above 6 c.c. + 30 c.c.) to 30 c.c. of pure water, though tried many times, invariably resulted in the death of the animals.

By repeated trials it was found that as small an amount as 6 c.c. of a normal culture could be put directly into the 30 c.c. without ill effects; 1 c.c. of this dilution, however, transferred to 30 c.c. of pure water caused death, as in the above experiments. From this it was evident that the difficulty lay not in the higher changes but in those in which the amount of the already highly diluted fluid was reduced from 6 c.c. to 1 c.c. These changes it is evident are so slight as to render osmosis a doubtful factor.

By running through a series 6, 3, and 1 c.c. diluted culture + 30 c.c. pure water, about 8 to 10 per cent of the paramecia (in 1 c.c. + 30 c.c.) were found to live. This medium was sufficiently low in salt content to satisfy the conditions of the proposed experiment, to compare *x* and *y* in equal amounts of pure water, and thus to show the influence of a low salt content. With drop *y* containing only a few paramecia from the above pure medium, and drop *x* containing many from the original culture, the following experiments were performed.

C. Experimental. — The experiments will be expressed in brief

by means of symbols: part (a) being the control animals; part (b) the animals subjected gradually to purer and purer water. The original culture from which the animals came will be given in capitals as C or D; and the dilutions in figures 1 to 4. Following each division (a) or (b) is the "Result," giving material for comparison of animals from the natural culture with those of the purer medium.

Experiment 1 (a). C → 1

C = the original culture (from which both drops *x* and *y* came).

May 5. 1 = a drop *x* of culture C suddenly put into 30 c.c. pure water ($k = 2.4 \times 10^{-6}$).

That is, a drop of the original culture C was put suddenly into 30 c.c. of pure water ($k = 2.4 \times 10^{-6}$), giving a test for the natural or control animals.

Result: Animals slowing in movement in from thirty to forty-five minutes; some distorting at fifty minutes, and all dead at one hour.

(b). C → 1 → 2 → 3 → 4

C = the original culture as above.

May 5. 1 = 6 c.c. of culture C + 30 c.c. of pure water.

" 6. 2 = 3 " " dilution 1 + " " " " "

" 8. 3 = 1 " " " 2 + " " " " "

" 9. 4¹³ = drop *y* of " 3 + " " " " " ($k = 3.2 \times 10^{-6}$).

That is, drop *y* (originally from C) which had been brought gradually into pure water to give test for acclimatized animals was put into 30 c.c. of pure water ($k = 3.2 \times 10^{-6}$).

Result: Six alive at the end of one hour, ten minutes; three swimming at two hours, and the last one moving slowly at five hours.

In the above no benefit resulted to *x*, although it carried a much greater salt content than did *y*. But this experiment is inserted only as the first trial after the development of the method of transfers; the results in later cases were much more striking.

The following one, which is typical of the perfected method, gives characteristic results.

Experiment 2 (a). C → 1.

C = the original culture.

May 14. 1 = a drop *x* of culture C suddenly added to 30 c.c. pure water ($k = 3 \times 10^{-6}$). *

¹³ Twelve paramecia in the drop.

Result: Animals on the bottom and swelling in thirty minutes; dead at fifty minutes.

(b). $C \longrightarrow 1 \longrightarrow 2 \longrightarrow 3 \longrightarrow 4$.

C = the original culture.

May 14. 1 = 6 c.c. of culture C + 30 c.c. pure water.

" 15. 2 = 3 " " dilution 1 + " " " "

" 16. 3 = 1 " " " 2 + " " " "

" 17. 4 = drop y of " 3 + " " " " ($k = 4.5 \times 10^{-6}$).

Result: Seven alive and swimming twenty-four hours later; four alive at the end of six days; two of these slow and wobbly and two that swim at a normal rate.

In every point does Experiment 2 mark the inadequacy of a low inorganic salt content as an explanation for the results obtained. First, drop x took into the pure medium a much larger quantity of salts than did y ; and secondly, it had about 200 paramecia to raise the content still further, while y had only 8. But in spite of both these facts the organisms of x died early; those of y lived for days.

Not only do these experiments prove the inadequacy of the theory that death is due to a low salt content, but they show further that in a medium practically free from all inorganic salts paramecia can live provided time is given for adjustment.

To the experiments just described, the objection may possibly be made, that since the animals of drop y in both Experiments 1 and 2 were tested in water of a slightly higher electrical conductivity than were those of x , this put the latter (those of x) at a disadvantage. Let us repeat the experiment in the following form, giving x the advantage of (1) a higher normal salt content, (2) a greater number of animals in the drop, and (3) a test in water of a higher electrical conductivity.

Experiment 3 (a). $C \longrightarrow 1$.

C = the original culture.

May 22. 1 = a drop x of culture C put suddenly into 30 c.c. of pure water ($k = 4.4 \times 10^{-6}$).

Result: Some on the bottom and swelling in thirty minutes; slowly moving forty minutes; at one hour no movement could be detected by the unaided eye, but by the use of the microscope two were seen to "jerk," up to one hour twenty-five minutes.

(b). $C \longrightarrow 1 \longrightarrow 2 \longrightarrow 3 \longrightarrow 4$.

C = the original culture.

May 22. $1 = 6$ c.c. of culture $C + 30$ c.c. pure water.

" 23. $2 = 3$ " " dilution $1 +$ " " " "

" 25. $3 = 1$ " " " $2 +$ " " " "

" 26. $4^{14} =$ drop y of " $3 +$ " " " " ($k = 2.7 \times 10^{-6}$).

Result: On the following day these were moving actively; in good condition on the second day; sixth day four seen — one very "wabby"; two alive on the eighth day; and one lived up to the tenth day.

Drop x , with every advantage, when subjected suddenly to the pure medium died. That it was not the purity of the water that killed is made evident from the fact that drop y transferred gradually from the same culture into water even purer lived for days. That the rapidity of the change was the destructive factor is demonstrated by the opposite proposition; drop y transferred by a series of steps properly graduated lived for days in a purer water than that which killed x , subjected suddenly.

The results of these experiments show that adjustment is a factor so important as to demand consideration in work of this nature. Without giving it proper weight we cannot hope to arrive at an explanation adequate to account for the variations observed.

To the foregoing results it is necessary only to add that Types A and B were easily acclimated to pure water. The same method was used for B as for C. Two differences in behavior, however, were noted. In the transfer from 2 to 3 (when 1 c.c. of 2 was added to 30 c.c. of pure water) many more of B than of C lived. In one case with B where a drop of 3 had been added to 30 c.c. of pure water there was division in the distilled water. Only one paramecium was originally introduced, but this by the end of the fifth day had increased to five.

In none of the many trials that I have made with Type D have I been able to acclimatize it by the series 6, 3, 1 c.c. + 30 of pure water. I have recently found, however, that if instead of 6 c.c. only 3 c.c. of the original culture be added directly to 30 c.c. of the pure water, and this followed by the series 2 c.c. + 30 c.c.; 1 c.c. + 30 c.c.; a drop y of this showed considerable adjustment. A single experiment will be sufficient.

¹⁴ Drop y contained six paramecia.

Experiment 4 (a). $D \longrightarrow 1$.

D = the original culture.

May 19. 1 = drop x of culture D put suddenly into 30 c.c. of pure water
($k = 2.7 \times 10^{-6}$).

Result: Death and beginning disintegration, thirty minutes.

(b). $D \longrightarrow 1 \longrightarrow 2 \longrightarrow 3 \longrightarrow 4$.

D = the original culture.

May 19. 1 = 3 c.c. of culture D + 30 c.c. pure water.

" 21. 2 = 2 " " dilution 1 + " " " "

" 22. 3 = 1 " " " 2 + " " " "

" 23. 4 = drop y of " 3 + " " " " ($k = 3.5 \times 10^{-6}$),

Result: Three swimming one hour, fifteen minutes; two still alive two hours, fifteen minutes — one of these slow, the other moving at a normal rate.

VI. SUMMARY.

This paper is the outgrowth of a series of experiments that had for its purpose the preparation of a pure standard medium in which to test the adjustment of organisms. The medium used was distilled water, and the organism studied a unicellular animal — paramecium.

The history of experimentation upon distilled water shows that water re-distilled in glass, though of a low electrical conductivity, is often unfit for physiological purposes. This is interpreted by investigators in one of two ways: (1) that pure water is itself the agent of destruction; (2) that destruction is brought about by slight traces of toxic substances passing into the distillate.

As a method for testing these two propositions I have shaken down once distilled water with thoroughly washed carbon-black and afterwards re-distilled the same in Jena glass. In this way a water both of a low conductivity and of physiological purity was readily obtained.

With water of this kind four strains of paramecia, reared under essentially different conditions, were tested, with the result that two types, designated as B and C, when added in the proportion of 1 drop to 30 c.c. of pure water, succumbed, — one in an hour's time, the other at the end of two hours. At the extremes of these

were two other types, one (Type D) dying within thirty minutes, the other (Type A) living for days in a third transfer. These strains varied so much in behavior as to make it not improbable that the differing results recorded by various observers are largely due to a study of the organisms under different physical and environmental conditions.

The destructiveness of distilled water has been attributed to its low content of inorganic salts. This was tested by diluting two drops with equal amounts of pure distilled water, the first drop having the high original salt content of the natural culture and containing many paramecia; the second having a very low salt content and containing few organisms. The paramecia in the first drop died quickly, while those in the second drop lived for days.

That death in distilled water is caused by the sudden change in media is shown by the fact that organisms transferred gradually lived for days without food in a purer water than it took to kill the control animals suddenly subjected to it.

The adjustment of animals to the conditions under which they live, and their power of becoming adjusted by a slow process to new conditions is one of the most important matters requiring consideration in dealing with the effects of environmental agents.

EXPERIMENTS ON THE ABSORPTION OF FAT FROM
AN ISOLATED LOOP OF SMALL INTESTINE IN
HEALTHY DOGS.

By O. H. PLANT.

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MOST experiments on the absorption of fats from the intestines have been made on dogs soon after some severe operative procedure, as the formation of anastomosis, removal of the pancreas, or the ligation of pancreatic and bile ducts. With the exception of some experiments by V. Harley¹ on the absorption of milk in dogs before and after removal of the pancreas, no quantitative determinations of fat were made; the appearance of the lacteals was taken as the index to the degree of fat absorption.

The experiments described here were made during 1904 and 1905. The plan followed was to isolate a loop of small intestine in dogs, in such a way as to eliminate the influence of the digestive juices (*i. e.*, bile and pancreatic juice) and at the same time not to interfere with the blood supply of the loop or the general nutrition of the animal. After waiting a considerable interval (10 days to 3 weeks), to make sure that the animal had recovered from the operation, and that separating a part of its intestine from the digestive tract had not interfered with the general nutrition, fats in various states, with or without bile, could be placed in the loop and the amount of absorption determined.

Cunningham² reports some experiments of this sort, in which he placed neutral cottonseed oil in such a loop two or three days after the operation, and after waiting a number of hours (6 to 18), killed the animal in order to inspect the lacteals.

He states that in experiments where the fat was placed in the loop a few days (2 or 3) after its isolation, the lacteals showed that the neutral oil was absorbed; but when the animals were allowed to

¹ VAUGHAN HARLEY: *Journal of physiology*, 1895, xviii, p. 1.

² R. H. CUNNINGHAM: *Journal of physiology*, 1898-99, xxiii, p. 209.

live as long as four weeks, no such absorption could be detected. He explains this by the statement that the loop of intestine in such experiments "undergoes fatty degeneration and becomes greatly atrophied."

I have found that this was not the case in the dogs used in my experiments. In one dog that lived two and one-fourth years after the operation, microscopic sections of the loop show no atrophy or fatty degeneration. Further, it was found that fats in various states were absorbed quite as readily twelve and eighteen months after the formation of the loop as immediately after the operation.

For these experiments, young (about half grown) female dogs were selected. Under aseptic precautions a piece of gut, from 55 to 75 cm. in length, beginning from 25 to 30 cm. below the pylorus was separated from the rest of the intestines, without interfering with its blood supply or mesenteric attachment. The continuity of the bowel was then established by an end to end anastomosis.

The ends of the resected portion were sutured in the abdominal wound, 5 to 7.8 cm. apart. The muscular layers of the abdominal wall were stitched to the serous coat of the ends of the loop, the skin to the mucous membrane. By this procedure the muscles of the abdominal wall served as a sort of sphincter to the openings of the loop.

Three dogs were successfully prepared in this way; two others died a few days after the operation from obstruction at the point of anastomosis. The three successful cases recovered rapidly from the operation, and continued to grow and increase in weight, showing that the separation of approximately one-fourth of the small intestine from the digestive tract had no effect on their nutrition.

Dog II. — Female coach dog, weight 9.54 kg. Dec. 30, 1903, operation for the formation of loop of intestine was performed. Length of loop, approximately 75 cm., beginning 25 cm. below the pylorus. March 5, 1904, the dog weighed 12.72 kg.; wound healed completely and dog in good condition. This dog increased in weight to 16.36 kg. and remained in good condition until it was killed (March 7, 1906, two and one-fourth years after operation) by chloroform, in an experiment during the course of another research. Post-mortem examination showed the length of small intestine to be 292.5 cm.; length of loop, 67 cm.

Dog III. — Black female, weight 10.22 kg. June 30, 1904, operation for formation of loop of intestine was performed. Length of loop,

55 cm., beginning 30 cm. below the pylorus. August 12, 1904, dog weighed 11.36 kg., and continued in good health until death, April 25, 1905. The dog was kept in a kennel on the roof of an out-building, and its death resulted from a fall from the top of the kennel to the ground.

Dog V. — Brown female, weight 8.63 kg. Aug. 2, 1905, the operation to form a loop of intestine was performed. Length of loop, 50 cm., beginning 30 cm. below the pylorus. Sept. 20, 1905, dog weighed 9 kg. and remained in good condition until its death, Feb. 26, 1906, when it was killed by chloroform in the course of another research.

Animals prepared in this way may be kept indefinitely and used repeatedly. By placing them on their backs in a trough-shaped dog-holder, the absorption of fats in various states, as emulsions, soaps, neutral oil, etc., can be studied in a normal animal, and under conditions in which the action of the pancreatic juice is eliminated. I found that after the first few experiments the dogs became accustomed to lying on their backs, and usually remained quiet throughout the six hours that the experiment lasted. When they became restless, a small dose of chloral hydrate, 0.2 to 0.4 gm. was administered by mouth and served to keep them quiet.

The greatest difficulty met with in these experiments was to devise some means of closing the ends of the loop during the time the fats were left in it. Various forms of glass and of distensible rubber cannulas were tried; but with all of these there was more or less leakage. The first 15 experiments performed were discarded and are not reported in this paper because the results were probably vitiated to some extent by this leakage, although it was carefully mopped up on cotton and saved. This difficulty was finally overcome by using a double metal cannula. (See diagram.)

This consisted of an inner tube (*a*) with a circular flange (*b*) at the end, which was placed inside the opening of the loop beyond the muscular sphincter. Over the inner tube an outer tube was fitted (*c*), also bearing a flange (*d*) at the end. When the two flanges (*b*)

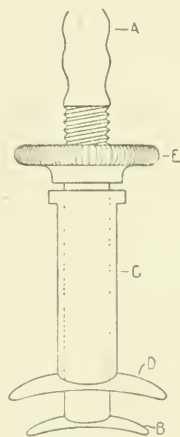


Diagram of metal cannula used to close the openings of the loop of intestine (actual size).

and (*d*) were brought together by a thumb-screw (*e*), the edges of the opening of the loop were grasped between them. The flanges of both inner and outer tubes were protected by rubber tubing, and by releasing the thumb-screw for a few minutes every one-fourth hour during the experiment the bad effects of constant pressure on the tissues about the openings of the loop were avoided.

One of these cannulas was placed in each end of the loop, and the ends connected by glass and rubber tubing. In this way all leakage was prevented, and when the content of the loop was forced to the lower end by peristalsis, it was driven through the cannulas and tubing into the upper end.

This circulation from end to end of the loop was observed to take place frequently throughout all the experiments, and I believe was an important factor in the results obtained.

The loop was always washed out thoroughly with large quantities of warm physiological saline solution before each experiment, care being taken to distend the loop by hydrostatic pressure so as to remove any secretion remaining in the folds of the mucous membrane. This procedure insures complete removal of the intestinal contents before and after each experiment. This is very important where quantitative determinations are made, and can only be done in an isolated loop.

Six series of experiments were made, each series including two groups. In one group the fatty substance under consideration was placed alone in the loop; in the other group the same fatty substance, together with bile or a solution of bile salts, was used. In all the experiments the fat was allowed to remain in the loop for six hours. Whenever practicable, the same quantity of the various fatty substances was used in the different series, so that comparisons could be made of the absorption of fats in different states in parallel experiments.

Series I. Cream.— Sweet, gravity cream was used. All the samples experimented with were acid in reaction to litmus. The percentage of fat in each sample was determined by the Adams process, in which a known weight of cream is taken up by a fat-free capsule of bilulous paper, dried in an oven until it ceases to lose weight, and is then extracted with ether in a Soxhlet apparatus. The amount of fat is determined by the loss in weight after the ether extraction.

In the first group of this series a weighed amount of cream was placed in the loop and allowed to remain six hours. The loop was then thoroughly washed out with from 200 to 300 c.c. of warm phys-

iological saline solution; the washings were evaporated on a water-bath to a small bulk and mixed with fat-free kaolin. This kaolin residue was placed in a paper capsule and dried. It was then extracted with ether in a Soxhlet apparatus. After again drying in an oven, the amount of fat unabsorbed in the experiment was determined by the loss in weight in the capsule.

The results in this group of experiments are given in Table I.

TABLE I.

CREAM.

Exp. No.	Date.	Dog.	Amt. of cream used.	Amt. of fat in cream used.	Fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
	'04		gm.	gm.	gm.	gm.	
1	June 4	II	20	4.976	4.448	0.519	10.2
2	June 6	II	20	4.074	3.915	0.158	3.8
3	June 7	II	20	5.665	5.425	0.240	4.2

In the second group of this series a weighed quantity of cream mixed with 10 c.c. of a six per cent aqueous solution of bile salts was placed in the loop and allowed to remain six hours.

The washings from the loop at the end of the experiment were treated exactly as in the first group of this series.

The bile salts used in the experiments reported in this paper were obtained from ox bile by the following method: Fresh ox bile was evaporated to dryness on a water-bath, powdered and mixed with animal charcoal, and percolated with alcohol. The alcoholic solution was shaken repeatedly with animal charcoal, until it showed only slight coloration from bile pigments; it was then evaporated and the residue dried and powdered. This gave a crystalline powder, entirely soluble in water, and of a very light yellow color.

The results of the experiments in which cream and bile salts were used are given in Table II.

Series II. Soap Emulsion. — A soap emulsion of neutral cottonseed oil was used. The emulsion was made by mixing pure oleic acid 4 gm., neutral cottonseed oil 40 gm., 0.5 per cent aqueous solution, sodium carbonate enough to make the mixture weigh 200 gm. This made a perfectly smooth emulsion, that was permanent and contained 22 per cent by weight of fatty bodies.

In the first group of this series a weighed amount of the emulsion was placed in the loop and allowed to remain six hours. The loop was then washed out as in the first series. The washings were poured into an equal volume of warm 20 per cent solution of sulphuric acid, in order to liberate the fatty acid from the soap. The mixture was then filtered. The filtrate was cooled and shaken with ether in a separator funnel; then the aqueous solution run off and

TABLE II.
CREAM AND SIX PER CENT SOLUTION OF BILE SALTS.

Exp. No.	Date.	Dog.	Amt. of cream used.	Amt. of fat in cream used.	Fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
	'04		gm.	gm.	gm.	gm.	
4	June 9	II	15	3.933	3.040	0.893	22.7
5	June 10	II	15	4.147	2.074	2.073	49.2
6	June 15	II	15	3.011	1.620	1.391	46.1

the ether solution containing the fat placed in an evaporating dish together with the filter paper and residue from the filtration.

After the ether had evaporated spontaneously the residue was mixed with kaolin and placed in an extraction capsule. The capsule was extracted with ether in a Soxhlet apparatus after it had been dried in an oven at 40° C.; and the amount of fat in the washings calculated from the loss in weight.

To determine the accuracy of this method of recovering fat from a soap emulsion, 20 gm. of the emulsion used in the experiments was mixed with 200 c.c. of water, and then treated in the same way as described for the washings.

Twenty gm. of the emulsion should contain 4.4 gm. of fatty bodies, and in the first check experiment 4.389 gm. were recovered; in the second, 4.386 gm.

The results obtained in this group of experiments are given in Table III.

In the second group of this series a weighed amount of the same soap emulsion used in the first group, mixed with 2 c.c. of a five per cent aqueous solution of bile salts, was placed in the loop and allowed to remain six hours. The washings at the end of the experiments were treated in the same manner as in the first group of this series. The results are given in Table IV.

Series III. Soap Solution.—In this series of experiments a solution of soap of definite composition was used. The solution was made by adding one-fortieth of the molecular weights of pure oleic

TABLE III.
SOAP EMULSION.

Exp. No.	Date.	Dog.	Amt. of emulsion used.	Amt. of fat in emulsion used.	Fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
7	'04 Aug. 8	II	gm. 20	gm. 4.4	gm. 4.18	gm. 0.220	5.0
8	Aug. 9	II	20	4.4	4.075	0.325	7.39
9	Aug. 10	II	20	4.4	4.235	0.165	3.75
10	Aug. 11	II	20	4.4	4.040	0.360	8.18

acid and sodium hydroxide to 100 c.c. of distilled water, *i. e.*, pure oleic acid 7.050 gm., sodium hydroxide 1 gm., distilled water enough to make the mixture weigh 100 gm. This made a clear amber-colored solution that was alkaline to litmus, that frothed on shaking

TABLE IV.
SOAP EMULSION AND SOLUTION OF BILE SALTS.

Exp. No.	Date.	Dog.	Amt. of emulsion used.	Amt. of fat in emulsion used.	Fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
11	'04 Sept. 5	III	gm. 10	gm. 2.2	gm. 1.39	gm. 0.810	36.81
12	Sept. 7	II	10	2.2	1.775	0.425	19.31
13	Sept. 9	III	10	2.2	1.780	0.420	19.09

and each gram of which contained 0.0705 gm. of oleic acid in combination as soap.

In the first group of this series a weighed quantity of the soap solution was placed in the loop and allowed to remain six hours. At the end of that time the loop was washed out as in the other experiments, and the washings poured into warm 20 per cent sulphuric acid, to decompose the soap and liberate the fatty acid. After cooling, the mixture was shaken with an equal volume of ether in a

separator funnel, the aqueous fluid run off, and the ether solution evaporated. The residue was mixed with kaolin and dried in an extraction capsule at 48° C. The amount of fatty acid in the washings was determined by extraction with ether in a Soxhlet apparatus.

The results are given in Table V.

TABLE V.
SOAP SOLUTION.

Exp. No.	Date.	Dog.	Amt. of soap solution used.	Amt. of fatty acid in soap sol. used.	Fatty acid recovered from washings.	Fatty acid (as soap) absorbed.	Percentage absorbed.
	'04		gm.	gm.	gm.	gm.	
14	Sept. 15	III	10	0.705	0.433	0.272	38.72
15	Sept. 17	III	10	0.705	0.605	0.100	14.1
16	Sept. 22	II	10	0.705	0.390	0.315	44.68

In the second group of this series the same soap solution, mixed with 2 c.c. of a five per cent aqueous solution of bile salts, was placed in the loop and allowed to remain six hours. The washings from the loop at the end of the experiments were treated as in the first group of this series. The results are given in Table VI.

TABLE VI.
SOAP SOLUTION AND BILE SALTS.

Exp. No.	Date.	Dog.	Amt. of soap solution used.	Amt. of fatty acid in soap sol. used.	Fatty acid recovered from washings.	Fatty acid (as soap) absorbed.	Percentage absorbed.
	'04		gm.	gm.	gm.	gm.	
17	Sept. 26	II	10	0.705	0.180	0.525	74.46
18	Sept. 28	III	10	0.705	0.061	0.644	91.48
19	Sept. 30	II	10	0.705	0.109	0.596	84.53

In the experiments of both groups of this series peristalsis was very active. It was so much increased that in one experiment of the first group and in two of the second group some of the contents of the loop were forced past the cannula when the thumb-screw was loosened to relieve the pressure on the tissues about the

openings of the loop. The experiments in which leakage occurred are not included in the tables given above.

Series IV. Oleic acid dissolved in solution of bile salts.—In this series 0.5 gm. pure oleic acid was dissolved in 10 c.c. of a five per cent aqueous solution of bile salts. The resulting solution was placed in the loop and allowed to remain six hours. The washings from the loop at the end of the experiments were evaporated to a small bulk, the residue mixed with kaolin and dried in an extraction capsule at 48° C. The amount of fatty acid recovered was determined by extraction with ether in a Soxhlet apparatus. The results of this series are given in Table VII.

TABLE VII.

OLEIC ACID DISSOLVED IN SOLUTION OF BILE SALTS.

Exp. No.	Date.	Dog.	Amount of oleic acid used.	Amt. of oleic acid recovered from washings.	Amount of oleic acid absorbed.	Percentage absorbed.
			gm.	gm.	gm.	
20	Sept. 30	III	0.5	0.204	0.296	59.02
21	Oct. 10	II	0.5	0.224	0.276	55.2
22	Oct. 14	III	0.5	0.295	0.205	41.0

It was found impracticable to place pure oleic acid alone in the loop. When this was tried it caused the animal so much pain that the experiment had to be abandoned, for fear that the acid would do permanent injury to the lining of the loop.

Series V. Neutral oil.—In this series neutral cottonseed oil was used. The oil was first rendered free from fatty acid by shaking it repeatedly with hot baryta water in a separator funnel, and finally with baryta water and ether. The ether solution was drawn off with a pipette, the ether allowed to evaporate, and the oil filtered. This gave an oil that showed no emulsification or clouding when shaken with 0.5 per cent solution of sodium carbonate. In the first group of this series a weighed quantity of neutral oil was placed in the loop and allowed to remain six hours. The washings from the loop at the end of the experiments were evaporated to a small bulk, mixed with kaolin and dried in an extraction capsule at 55° C. The amount of fat recovered in the washings was determined by

extracting the capsule with ether in a Soxhlet apparatus. The results are given in Table VIII.

TABLE VIII.
NEUTRAL COTTONSEED OIL.

Exp. No.	Date.	Dog.	Amount of oil used.	Amt. of fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
	'04		gm.	gm.	gm.	
23	Oct. 15	II	5	3.215	1.785	35.7
24	Oct. 17	III	5	2.803	2.198	43.96
25	Oct. 24	II	5	2.676	2.324	46.48

In the second group of this series a weighed quantity of neutral cottonseed oil, mixed with 5 c.c. of a ten per cent aqueous solution of bile salts, was placed in the loop and allowed to remain six hours. The washings were treated in the same way as in the first group of this series. The results are given in Table IX.

TABLE IX.
NEUTRAL COTTONSEED OIL AND BILE SALTS.

Exp.	Date.	Dog.	Amount of oil used.	Amt. of fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
	'04		gm.	gm.	gm.	
26	Nov. 5	II	5	2.236	2.764	55.28
27	Dec. 3	III	5	3.272	1.727	34.54
28	Dec. 10	III	5	2.473	2.527	50.54

In this series of experiments the washings did not show any visible emulsion, although they were not examined microscopically; most of the fat floated on the surface in fair-sized droplets, mixed with mucus that probably contained considerable fat. The addition of bile salts to the neutral oil did not increase the peristalsis to the same extent that it did when other forms of fat were used, as the soap solution or soap emulsion. Circulation of the contents of the loop, however, was observed to take place frequently in all the experiments of this series.

Because such a large amount of neutral oil was absorbed in these experiments, and because the addition of bile salts did not increase the absorption to any great extent, it was decided to repeat the series so as to verify the results and also to determine if there was any marked change in the reaction of the oil during the time it remained in the loop.

Neutral cottonseed oil was prepared in the same way as before. To 5 c.c. of this oil 10 drops of an alcoholic solution of phenolphthalein was added and the mixture shaken. The color was unchanged. Decinormal NaOH solution was then added, drop at a time, and it required but two drops (less than 0.1 c.c.) of the alkali to produce permanent marked alkaline color in the mixture.

Before repeating the experiments on the absorption of this oil, the following observations were made to determine whether a change in reaction occurred while the oil remained in the loop:

Phenolphthalein was added to a weighed quantity of the neutral oil, and decinormal NaOH solution added from a burette until the mixture, on being thoroughly shaken, showed a permanent alkaline color. It was then placed in the loop. After varying intervals the contents were allowed to escape from the lower end of the loop, no fluid being added to force them out. The amount of change in reaction was then determined by titration.

Experiment 1. June 27, 1905. Dog II. — 10 gm. neutral oil required 3 drops of $n/10$ NaOH to produce alkaline reaction. Placed in the loop for three hours. 8 c.c. contents of loop collected; acid in reaction and required 3.1 c.c. $n/10$ NaOH to bring to alkaline reaction.

Experiment 2. June 28, 1905. Dog II. — 12 gm. neutral oil required 5 drops $n/10$ NaOH to produce alkaline reaction. Placed in loop for two and one-half hours. 9 c.c. of contents of loop collected; acid in reaction and required 3.6 c.c. $n/10$ NaOH to produce alkaline reaction.

Experiment 3. June 30, 1905. Dog II. — 10 gm. neutral oil required 4 drops of $n/10$ NaOH to produce alkaline reaction. Placed in the loop for one hour. 7.6 c.c. of contents collected; acid in reaction and required 1.8 c.c. of $n/10$ NaOH to bring to alkaline reaction.

These results show that neutral oil, placed in a loop of intestine such as described in this paper, becomes markedly acid in as short a period as one hour. The secretions from the loop, as obtained in

the first of the washings at the beginning of the experiments, were usually faintly alkaline, and when not alkaline, required not more than five or six drops of $n/10$ NaOH solution to produce an alkaline reaction.

TABLE X.
NEUTRAL COTTONSEED OIL.

Exp. No.	Date.	Dog.	Amount of oil used.	Amt. of fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
29	'05 July 12	II	gm. 10	gm. 7.230	gm. 2.770	27.7
30	July 13	II	10	7.640	2.360	23.6
31	Aug. 2	II	10	7.892	2.208	22.08

Series VI. Neutral oil. — The experiments of series V were repeated, using a larger quantity of oil. A weighed quantity of neutral cottonseed oil was made slightly alkaline in reaction to phenolphthalein by adding a few drops of $n/10$ NaOH solution. Enough of the alkali was added to produce a permanent color in the mixture;

TABLE XI.
NEUTRAL COTTONSEED OIL AND FRESH BILE.

Exp. No.	Date.	Dog.	Amount of oil used.	Amt. of fat recovered from washings.	Amt. of fat absorbed.	Percentage absorbed.
32	'05 July 31	II	gm. 10	gm. 7.598	gm. 2.402	24.02
33	Aug. 4	II	10	6.657	3.343	33.43
34	Sept. 22	II	10	6.602	3.398	33.98

this never required more than six or seven drops. The oil remained in the loop for six hours, and the amount unabsorbed was determined as in series V. The results of the first group of this series are given in Table X.

The conditions in the experiments of the second group of this series were the same as in the first group, except that 2 c.c. of fresh bile, obtained from the gall bladder of dogs immediately after death, was mixed with the neutral oil. The results are given in Table XI.

In the experiments of this series (VI) the contents of the loop were seen to circulate frequently from the lower to the upper end through the cannulas and tubing; and in both groups the alkaline color of the contents disappeared in from fifteen to twenty minutes after the fat was placed in the loop. The washings showed much free fat floating as globules on the surface; also some rather thick clumps of what appeared to be mucus and fat mixed together.

The absolute amount of fat absorbed in the experiments of series VI was practically the same as in series V. The relative proportion of fat absorbed in series V was approximately twice as great as in series VI. This difference in the percentage absorbed is due to the fact that in the second series of experiments with neutral oil (series VI), twice as much fat was used as in the first (series V), while the amount absorbed was practically the same. In other words, increasing the amount of neutral oil placed in the loop did not increase the amount of absorption that occurred in six hours.

Table XII is a summary of the results obtained in testing the absorption of the various fatty substances under consideration. The figures represent the averages from each of the foregoing tables.

In all the experiments where a solution of bile salts was added to the fatty substance, peristalsis was more active than where fat alone was used, except in the experiments with neutral oil (series 5 and 6). This increase in peristalsis was shown by the frequency with which the contents of the loop passed through the glass tubing that connected the cannulas. I believe the increase in peristalsis contributed to the increase in absorption that occurred when bile salts were added to the fat: the arrangement of the cannulas so that the fatty substance could pass into the upper end of the loop after it had been forced to the lower end, and in this way be presented repeatedly to the absorbing surface, undoubtedly increased the absorption over what would have occurred had the fat been held at the lower end of the loop.

Reference to Table XII shows that bile salts very greatly increased the amount of fat absorbed in the experiments where cream, soap emulsion, or soap solution was used. The greatest increase occurred with cream (39 per cent as compared with 6 per cent); with soap emulsion the increase was not so great (25 per cent as compared with 6 per cent). When neutral oil was used, bile or bile salts increased the amount of absorption very little. These facts

seem to indicate that the part taken by bile is connected either with fatty acid (as in cream) or with soap (as in soap emulsion or soap solution).

When we compare the amount of absorption that occurred from an emulsion (either cream or soap emulsion) and from a soap solu-

TABLE XII.

SUMMARY.

Fatty substance used.		Average amt. of fat placed in soap.	Average amt. of fat absorbed in 6 hours.	Average percentage absorbed.
		gm.	gm.	
Series No. 1	Sweet cream	4.902	0.306	6.06
	Sweet cream and sol. of bile salts	3.697	1.450	39.3
Series No. 2	Soap emulsion	4.4	0.242	6.08
	Soap emulsion and sol. of bile salts	2.2	0.552	25.07
Series No. 3	Soap solution	0.705	0.296	32.5
	Soap solution and sol. of bile salts	0.705	0.588	83.49
Series No. 4	Oleic acid dissolved in sol. of bile salts	0.500	0.259	51.74
Series No. 5	Neutral oil. (First series.)	5.0	2.102	42.04
	Neutral oil and sol. bile salts	5.0	2.339	46.79
Series No. 6	Neutral oil. (Second series.)	10.0	2.446	24.46
	Neutral oil and fresh bile	10.0	3.047	30.47

tion (6 per cent against 32.5 per cent), it is evident that soap is more readily absorbed than emulsified fat.

Comparison between the absorption of an emulsion and the absorption of a neutral oil (where practically the same quantities of fat were used) shows that, in the absence of pancreatic juice, an emulsion possesses no advantages over a neutral oil; *i. e.*, from 4.9 gm. of fat as cream 0.3 gm. fat was absorbed, while from 5 gm. of neutral oil 2.1 gm. was absorbed. This indicates that the importance of emulsification in the intestine is digestive rather than absorptive, and that the purpose of this process, when it occurs, is to present a

greater surface upon which the steapsin of the pancreas juice can act.

The results in series IV, where oleic acid dissolved in a solution of bile salts was used, confirms the report of other investigators that fatty acid can be absorbed from the intestines.

The large amount of neutral oil absorbed, together with the fact that it became acid in reaction, may be explained in two ways: either the bacteria present in the loop were able to decompose the neutral oil into fatty acid and glycerine, or the secretion of the intestinal mucosa contains some ingredient (probably an enzyme) that is able to bring about the decomposition of the neutral oil. Loevenhart³ has shown that an enzyme, intestinal lipase, can be extracted from the mucosa of pigs' intestine that is capable of decomposing ethyl butyrate into butyric acid and ethyl alcohol. The absorption of the oil as neutral oil could hardly have taken place, for in the experiments with emulsions (where the fat was separated into fine particles and in what must have been a more favorable state for the absorption of fat as neutral fat) there was less absorbed than from the neutral oil. Furthermore, the change in reaction of the neutral oil argues against its absorption in an unaltered form.

As fatty acids are no more soluble than neutral oil, and as the addition of bile salts did not increase the absorption of it very much, the question arises: How are neutral oils absorbed from an isolated loop of intestine?

This could be explained by a combination of fatty acid with sodium carbonate to form a soap that is absorbed as rapidly as it is formed. As soaps and fatty acids are synthesized by the intestinal mucosa into neutral fats, the sodium carbonate set free by the decomposition of these soaps may be secreted in the intestinal juice to again combine with more fatty acid. In this way a small amount of sodium carbonate could combine with a large amount of fatty acid. The amount of sodium carbonate in the intestinal juice at any time is not great; but it is always present in sufficient quantity to give the secretion an alkaline reaction. If this explanation be correct, a circulation of sodium carbonate takes place between the intestinal contents and the intestinal mucosa similar to that of bile salts between the intestine and the liver.

³ A. S. LOEVENHART: This journal, 1901-02, vi, p. 334.

CONCLUSIONS.

1. Bile salts greatly increase the absorption of fats from a mixture that contains free fatty acid or soap. They only slightly increase the absorption of neutral oil.
2. Solutions of soap, in the absence of other fat, are absorbed from a loop of intestine in greater percentage than emulsified fats; this is also true of fatty acid dissolved by bile salts.
3. Neutral oil can be absorbed without the action of either bile or pancreatic juice from a loop of intestine where both these secretions are excluded. Under such conditions the neutral oil becomes markedly acid in reaction.
4. Taken as a whole, the results of these experiments favor the theory that fats are absorbed in solution rather than as emulsified fats.

HYDROLYSIS OF FISH MUSCLE.¹

BY THOMAS B. OSBORNE AND FREDERICK W. HEYL.

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

CONTINUING the comparison of the proportion of the several amino-acids yielded by hydrolyzing different food proteins, we have analyzed the muscle substance of the halibut (*Hippoglossus vulgaris*).

In preparing the material for hydrolysis, the skin, bones, and larger pieces of connective tissue were carefully removed from a large piece of fresh halibut meat, purchased in the market, and the muscle substance was then reduced to a pulp with a meat chopper. By extracting with water, alcohol, and ether as described for preparing the chicken meat already hydrolyzed,² a product was secured which was comparable with that obtained from the chickens.

The material thus prepared contained 13.32 per cent of moisture, 0.61 per cent of ash, and 16.40 per cent of nitrogen.

The results of the hydrolysis of this material are given in the table on page 82, together with those obtained with the chicken muscle.

From these figures distinct differences in the proportion of glycocoll, alanine, valine, and glutaminic acid appear between these two muscle substances. Although great care was taken and persistent efforts were made to separate glycocoll, none was obtained, and there is every reason to believe that none was yielded by the halibut muscle. While over two per cent of alanine was easily obtained from the chicken muscle, only doubtful traces of this substance were found among the products of hydrolysis of fish muscle. On the other hand, a relatively considerable quantity of valine was separated from the fish muscle, but none from the chicken muscle. The greatest quantitative difference was found for glutaminic acid,

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

² OSBORNE and HEYL: This journal, 1908, xxii, p. 433.

less than two thirds as much being obtained from the fish as from the chicken.

That practically all of the glutaminic acid produced by hydrolysis was obtained by the direct determinations is made almost certain by the fact that the quantity obtained from the esters and distillation residue formed 8.87 per cent of the part of the muscle substance hydrolyzed from which the glutaminic acid had not previously been separated. If it is assumed that by esterification about

	Halibut per cent.	Chicken per cent.
Glycocoll	0.00	0.68
Alanine	?	2.28
Valine	0.79	?
Leucine	10.33	11.19
Proline	3.17	4.74
Phenylalanine	3.04	3.53
Aspartic acid	2.73	3.21
Glutaminic acid	10.13	16.48
Serine	?	?
Tyrosine	2.39	2.16
Arginine	6.34	6.50
Histidine	2.55	2.47
Lysine	7.45	7.24
Ammonia	1.33	1.67
Tryptophane	present	present
Total	50.25	62.15

80 per cent of the total glutaminic acid present is obtained, the above quantity would be equal to 11 per cent of the muscle substance, a result in good agreement with the 10.3 per cent found by the direct determination. We are therefore justified in considering that a marked difference actually exists between the proportion of glutaminic acid yielded by the muscle substance of these two species.

The proportion of the other amino-acids obtained from each of these muscles is substantially the same. One of the most striking features of both of these analyses is the very large amount of lysine found. The results of these analyses are shown in the above table.

HYDROLYSIS OF HALIBUT MUSCLE.

Three portions of the air-dry halibut meat, weighing, respectively, 100, 100, and 290 gm., together equal to 421.7 gm. ash and mois-

ture free substance, were separately dissolved by warming on the water bath with three times their weight of hydrochloric acid, sp. gr. 1.10, and then hydrolyzed by boiling for twenty-three hours in an oil bath at $130^{\circ} - 135^{\circ}$.

Glutamic acid was carefully separated from the two 100 gm. portions, and 10.88 gm. and 10.14 gm. of the hydrochloride, respectively obtained, equal to 10.13 and 9.33 per cent of glutamic acid.

The glutamic acid hydrochloride decomposed at 198° .

Chlorine, 0.3215 gm. subst., gave 0.2523 gm. AgCl.

Calculated for $C_5H_{10}O_4NCl = Cl$ 19.35 per cent.

Found = Cl 19.40 " "

The free glutamic acid gave the following analysis:

Carbon and hydrogen, 0.2224 gm. subst., gave 0.3363 gm. CO_2 and 0.1225 gm. H_2O .

Calculated for $C_5H_9O_4N = C$ 40.81; H 6.12 per cent.

Found = C 41.24; H 6.11 " "

The filtrate and mother liquors from the glutamic acid hydrochloride were joined to the *third* portion, from which the glutamic acid had not been separated, and the whole solution concentrated to a syrup at a low temperature under strongly diminished pressure. This syrup was then subjected to two esterifications in the usual manner. After removing the ether, by distilling from a water bath at 760 mm., the esters were distilled under low pressures with the following result:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	100°	10.00 mm.	71.11 gm.
II	100°	0.35 "	37.23 "
III	150°	0.26 "	52.83 "
IV	190°	0.21 "	58.90 "
Total			220.07 gm.

The undistilled residue weighed 33 gm.

Fraction I. — This fraction was saponified by boiling for seven hours with water. The solution was evaporated to dryness and proline extracted by boiling with absolute alcohol. The amino-acids insoluble in alcohol were dissolved in water and separated into

twenty-seven fractions, of which the first eight were practically pure leucine. The eighth fraction gave the following analysis:

Carbon and hydrogen, 0.1169 gm. subst., gave 0.2337 gm. CO₂ and 0.1025 gm. H₂O.

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

Found = C 54.52; H 9.74 " "

The fifth and sixth fractions, when united and recrystallized from dilute alcohol, had the composition of leucine:

Carbon and hydrogen, 0.1579 gm. subst., gave 0.3188 gm. CO₂ and 0.1419 gm. H₂O.

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

Found = C 55.06; H 9.98 " "

After separating 24.47 gm. leucine, the following thirteen fractions weighing 13.75 gm. appeared to be an inseparable mixture of leucine and valine, the percentage of carbon being 53.8 in the tenth fraction and 52.3 in the twenty-first. These were all joined and racemized by heating in an autoclave for twenty-four hours at 175° with an excess of baryta. The baryta was removed, and by a systematic fractional crystallization 4.72 gm. of leucine and 3.32 gm. of valine were obtained.

The valine showed, after crystallization from dilute alcohol, the following composition:

Carbon and hydrogen, 0.1460 gm. subst., gave 0.2761 gm. CO₂ and 0.1225 gm. H₂O.

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

Found = C 51.57; H 9.32 " "

By recrystallization from water, the valine was obtained in beautiful kite-shaped plates having the composition of pure valine.

Carbon and hydrogen, 0.1300 gm. subst., gave 0.2442 gm. CO₂ and 0.1076 gm. H₂O.

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

Found = C 51.23; H 9.20 " "

This racemic valine was coupled with phenylisocyanate and yielded the phenylhydantoic acid, which crystallized in perfect hexagonal plates which melted at 158°-159°.

Carbon and hydrogen, 0.1225 gm. subst., gave 0.2747 gm. CO₂ and 0.0765 gm. H₂O.

Calculated for C₁₂H₁₆O₈N₂ = C 61.02; H 6.78 per cent.

Found = C 61.15; H 6.94 " "

The remaining fractions of the original twenty-seven which contained about 6 gm. of amino-acids were joined and repeatedly esterified in order to separate glycocoll as the ester hydrochloride, but none was found. The amino-acids, which when regenerated weighed 3.61 gm., were converted into copper salts which were fractionally crystallized. The final fraction, which should have contained the copper salt of glycocoll, contained only 24.9 per cent of copper; calculated for C₄H₈O₄N₂Cu = Cu 32.18 per cent. The ether removed from the esters at atmospheric pressure contained no glycocoll. It seems, therefore, practically certain that the halibut muscle yields no glycocoll.

In fractionally crystallizing the amino-acids composing this substance a few needles resembling alanine were seen under the microscope, but too little was present to be identified. The greater part of the substance appeared to consist chiefly of valine, but this could not be obtained sufficiently pure to be weighed. As this mixture formed less than one per cent of the substance hydrolyzed, the amount of alanine actually present was very small.

Fraction II. — This fraction was saponified and the proline removed as from Fraction I. By direct fractional crystallization of the part insoluble in alcohol, 14.01 gm. of leucine were obtained, and from the mother liquor of the leucine, 1.03 gm. of copper aspartate and 0.49 gm. of leucine copper.

The free leucine was analyzed as follows:

Carbon and hydrogen, 0.1404 gm. subst., gave 0.2820 gm. CO₂ and 0.1260 gm. H₂O.

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

Found = C 54.77; H 9.97 " "

The copper aspartate, which crystallized in the characteristic sheaves, gave the following analysis:

Copper, 0.2150 gm. subst., gave 0.0613 gm. CuO.

Calculated for C₄H₈O₄N Cu 4 1/2 H₂O = Cu 23.57 per cent

Found = Cu 22.78 " "

The leucine copper had the following composition:

Copper, 0.2101 gm. subst., gave 0.0514 gm. CuO.

Calculated for $(C_6H_{12}O_2N)_2Cu = Cu$ 19.65 per cent.

Found = Cu 19.54 " "

The alcoholic solutions containing the proline yielded by the usual treatment 0.60 gm. of racemic proline copper, and 16.40 gm. of lævo-proline copper, dried at 110° , equal to 12.94 gm. of proline.

Water, 0.1457 gm. subst., air dried, gave 0.0160 gm. H_2O at 110° .

Calculated for $C_{10}H_{16}O_4N_2Cu \cdot 2 H_2O = H_2O$ 10.99 per cent.

Found = H_2O 10.98 " "

Copper, 0.1282 gm. subst. (dried at 110°), gave 0.0347 gm. CuO.

Calculated for $C_{10}H_{16}O_4N_2Cu = Cu$ 21.81 per cent.

Found = Cu 21.62 " "

The lævo-proline was converted into the phenylhydantoine, which crystallized in the characteristic prisms, melting at 142° .

Nitrogen, 0.1937 gm. subst., required 18 c.c. N/10 HCl.

Calculated for $C_{12}H_{12}O_2N_2 = N$ 12.96 per cent.

Found = N 13.00 " "

Fraction III. — This fraction yielded 6.53 gm. of phenylalanine hydrochloride, 4.38 gm. of aspartic acid, and 6.22 gm. of glutamic acid hydrochloride. The aspartic acid, which reddened at 300° but did not melt, gave the following analysis:

Carbon and hydrogen, 0.2130 gm. subst., gave 0.2850 gm. CO_2 and 0.1045 gm. H_2O .

Calculated for $C_4H_7O_4N = C$ 36.09; H 5.26 per cent.

Found = C 36.49; H 5.45 " "

Fraction IV. — This fraction yielded 7.90 gm. phenylalanine hydrochloride, which, when converted into the free acid, decomposed at 270° .

Carbon and hydrogen, 0.1329 gm. subst., gave 0.3187 gm. CO_2 and 0.0846 gm. H_2O .

Calculated for $C_9H_{11}O_2N = C$ 65.45; H 6.66 per cent.

Found = C 65.40; H 7.07 " "

There were further obtained 16.03 gm. glutamic acid as the barium salt, and 1.27 gm. phenylalanine hydrochloride. The filtrate

from the latter, united with the corresponding solution from Fraction III, yielded 13.77 gm. of copper aspartate. The glutaminic acid decomposed at $202^{\circ} - 203^{\circ}$.

Carbon and hydrogen, 0.2117 gm. subst., gave 0.3196 gm. CO_2 and 0.1207 gm. H_2O .

Calculated for $\text{C}_6\text{H}_9\text{O}_4\text{N} = \text{C } 40.81$; $\text{H } 6.12$ per cent.

Found = $\text{C } 41.17$; $\text{H } 6.33$ " "

The copper aspartate gave the following analytical data:

Copper, 0.1454 gm. subst., gave 0.0416 gm. CuO .

Nitrogen, 0.5003 gm. subst., required 18.3 c.c. $\text{N}/10$ HCl .

Calculated for $\text{C}_4\text{H}_5\text{O}_4\text{NCu } 41/2 \text{ H}_2\text{O} = \text{Cu } 23.07$; $\text{N } 5.08$ per cent.

Found = $\text{Cu } 22.85$; $\text{N } 5.12$ " "

THE RESIDUE AFTER DISTILLATION.

The residue after distillation was dissolved in hot alcohol and 0.5 gm. diketopiperazines separated on cooling. After removing the alcohol the residue was saponified with baryta, and 1.4 gm. of glutaminic acid hydrochloride obtained in the usual way.

From 200 of the 490 gm. of fish muscle hydrolyzed the glutaminic acid had been separated directly, and the filtrates and mother liquors added to the hydrolysis solution of the remaining 290 gm. equal to 249 gm. ash and moisture free. From the esters of Fractions III and IV, 20.87 gm. of glutaminic acid were separated, which, with that from the distillation residue, 1.12 gm., makes 22.08 gm. in all from the esters obtained from the 249 gm., or 8.87 per cent. Assuming that only 80 per cent of the glutaminic acid was thus obtained from the esters, the total glutaminic acid originally present was equivalent to about 11 per cent of the fish muscle, thus confirming the substantial accuracy of the direct determinations of this substance.

TYROSINE.

A quantity of ash and moisture free halibut meat weighing 43.03 gm. was hydrolyzed by boiling for eighteen hours in a mixture of 150 gm. of sulphuric acid and 300 c.c. of water. After removing sulphuric acid quantitatively with baryta, the filtrate and washings of the barium sulphate were concentrated on the water bath to

crystallization. The substance thus separated, when decolorized with animal charcoal and recrystallized, yielded 1.03 gm. of pure tyrosine, equivalent to 2.39 per cent.

Carbon and hydrogen, 0.1559 gm. subst., gave 0.3410 gm. CO₂ and 0.0873 gm. H₂O.

Nitrogen, 0.1630 gm. subst., required 9.2 c.c. N/10 HCl.

Calculated for C₉H₁₁O₃N = C 59.67; H 6.08; N 7.73 per cent.

Found = C 59.64; H 6.22; N 7.90 " "

The filtrate and washings from the tyrosine were examined according to the method of Kossel and Patten for bases. The results follow:

HISTIDINE.

The solution of the histidine = 500 c.c.

Nitrogen, 50 c.c. sol. required 2.98 c.c. 5/7 N HCl = 0.2980 gm. N in 500 c.c.
= 1.10 gm. histidine = 2.55 per cent.

The histidine was identified as the dichloride which melted at 232°.

Chlorine, 0.1094 gm. subst., gave 0.1374 gm. AgCl.

Calculated for C₆H₁₁O₂N₃Cl₂ = Cl 31.14 per cent.

Found = Cl 31.07 " "

ARGININE.

The solution of the arginine = 1000 c.c.

Nitrogen, 50 c.c. sol. required 4.11 c.c. 5/7 N - HCl = 0.8220 gm. N in 1000 c.c. = 2.5540 gm. arginine + 0.1750 gm. = 2.7290 gm. arginine = 6.34 per cent.

The arginine was converted into the copper nitrate double salt, which gave the following analysis:

Copper, 0.1104 gm. subst., air dry, gave 0.0150 gm. CuO.

Calculated for C₁₂H₂₅O₄N₃Cu(NO₃)₂ 3 H₂O = Cu 10.79 per cent.

Found = Cu 10.85 " "

LYSINE.

The lysine picrate weighed 8.2260 gm. = 3.2023 gm. lysine = 7.45 per cent.

The lysine picrate gave the following analysis :

Nitrogen, 0.1664 gm. subst., required 21.9 c.c. N/10 — HCl.

Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3 = N$ 18.67 per cent.

Found = N 18.42 “ “

DISTRIBUTION OF NITROGEN.

The different forms of nitrogen yielded by hydrolyzing the halibut muscle were determined by the method of Hausmann as modified by Osborne and Harris.³ The results were:

	Per cent.
Nitrogen as ammonia	1.10
Basic nitrogen	4.95
Non-basic nitrogen	9.96
Nitrogen in magnesium oxide precipitate	<u>0.39</u>
Total nitrogen	16.40

The nitrogen contained in the histidine, arginine, and lysine is equal to 4.16 per cent, or 0.79 per cent less than the basic nitrogen precipitated by phosphotungstic acid. This difference is nearly the same as that similarly found for chicken muscle, and is probably largely caused by basic substances of non-protein origin contained in the muscle substance.

³ OSBORNE and HARRIS: *Journal of the American Chemical Society*, 1903, xxv, p. 323.

BLOOD PRESSURE WITH SPECIAL REFERENCE TO HIGH ALTITUDES.

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HISTORICAL.

THE study of the influence of altitude or low atmospheric pressure on blood pressure is of interest from the fact that a large percentage of our total population live at an altitude of from 3000 to 10,000 feet, corresponding to a reduction of atmospheric pressure of from one tenth to one third of the pressure at sea level.

The problem has been approached by two methods, — the pneumatic chamber and by mountain ascensions. The first is under easier experimental control, but cannot be regarded as giving perfectly normal conditions. Thus in the pneumatic chamber there is more abrupt change in pressure than would occur in mountain climbing. In using animals an element of uncertainty is introduced because of the operation and the use of anesthesia. At best such experiments cannot be said to be under strictly normal conditions. The study of blood pressure by climbing to higher altitudes is obviously attended with more or less inconvenience and difficulty, but on the whole admits of perfectly normal conditions of experimentation.

The pneumatic chamber method. — Paul Bert¹ was the first to study, by use of the pneumatic chamber, the effect of reduced atmospheric pressure on blood pressure. He found a slight diminution in blood pressure on lowering the atmospheric pressure. In 1883 Frankel and Geppert² showed a rise in blood pressure equal to 20 mm. of mercury for considerable changes in atmospheric pressure.

¹ BERT, PAUL: *La pression barometrique*, Paris, 1878.

² FRANKEL and GEPPERT: *Ueber die Wirkungen der verdünnte Luft*, Berlin, 1883, p. 65.

Lazarus and Schrymunski³ made a study of human blood pressure in a cabinet in which the pressure could be reduced to one half the normal value. They used von Bacl's sphygmomanometer and took two readings in each case. They report a fall of blood pressure of about 15 mm. of mercury on reducing the air pressure to one half. When the air pressure was restored to normal, the blood pressure did not, however, return to the normal value within the time limits of their experiment. Their work on dogs and sheep confirmed these results.

Mosso⁴ in a number of observations on dogs in a pneumatic cabinet reduced the atmospheric pressure to correspond with that of Mt. Everest — 228 mm. of mercury — and obtained a slight fall in blood pressure. G. Liebig,⁵ working with the Mosso sphygmomanometer, found for man a reduction of blood pressure in two cases under reduced atmospheric pressure, and an increase in two other cases. Dietrick⁶ and Rollet Sommerbradt and J. Schreiber obtained a slight increase in blood pressure in animals subjected to lowered atmospheric pressure.

Among the latest investigations by the use of this method are those of Lucien Camus⁷ in 1903, who used an apparatus so arranged that the animal alone was confined in the pneumatic chamber, the sphygmomanometer being outside. He records a striking parallelism between the atmospheric and blood pressures for various values of atmospheric pressure.

In 1904 John M. Cowan⁸ published the results of a series of observations in which he used practically the same method. He found that changes in atmospheric pressure influenced blood pressure decidedly. A decrease gave a rise in blood pressure, and an increase in atmospheric pressure a fall. Furthermore the effect was marked only when changes in external pressure were rapid. He found no constant relation between the two.

Crile⁹ in a number of experiments on dogs obtained an elevation

³ LAZARUS and SCHRYMUNSKI: *Zeitschrift für klinische Medizin*, 1883, vii.

⁴ MOSSO: *Archives italiennes de biologie*, 1905, xliii.

⁵ LIEBIG, G.: *Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München*, 1896, xii, p. 37.

⁶ DIETRICK: *Zeitschrift für klinische Medizin*, ii.

⁷ CAMUS, LUCIEN: *Journal de physiologie et pathologie générale*, 1903, v.

⁸ COWAN, JOHN M.: *Comptes rendus de la Société de biologie*, 1903, iv.

⁹ CRILE, GEORGE W.: *Blood pressure in surgery*, Philadelphia, 1903.

in blood pressure when the surrounding pressure was increased; similarly he obtained a fall of blood pressure with a lowering of external pressure, and the two seemed to rise and fall proportionally.

Bartlett,¹⁰ working in Professor Kronecker's laboratory, experimented with rabbits which were made to breathe air under reduced pressure. He found regularly a fall of blood pressure with reduced atmospheric pressure, the effect being more pronounced when the air pressure in the respiratory apparatus was reduced rapidly. The fall in blood pressure varied in different animals, the greatest fall being 40 mm. for an atmospheric pressure of 20 mm. of mercury less than normal.

We thus see that the results obtained by different investigators are not consistent. The greater part of the evidence, however, points to a fall of blood pressure both in man and animals when under reduced pressure in a pneumatic cabinet and in animals when made to breathe rarefied air.

Mountain ascensions. — Only a few studies of the influence of high altitudes on blood pressure have been recorded. Gorbatschew¹¹ in 1891 published the results of a few observations on the effect of mountain ascensions on blood pressure. He used the von Bach sphygmomanometer and made his observations on young soldiers. He reports an increase of pressure, due to elevation, of about 35 mm. and a fall to normal on returning to the lower altitude.

Mosso,¹² using his own instrument, made a few observations on Mt. Rosa. He writes: "On Mt. Rosa, at an elevation of nearly 15,000 feet, my blood pressure was the same as at Turin."

Oliver¹³ gives a few observations. He says: "Observations show that altitude may raise the mean arterial pressure for a time. Twenty-four observations made daily of the blood pressure on two subjects at an altitude of 5800 feet suggests that altitude, for a time at least, raises the blood pressure. During the fourteen days of our stay at this altitude the mean arterial pressure was raised in twelve days above the highest point recorded at home."

¹⁰ BARTLETT, FREDERICK H.: *Journal of physiology*, 1903-1904.

¹¹ GORBATSCHEW, P. K.: *Centralblatt für die medicinischen Wissenschaften*, 1891, ii.

¹² MOSSO: *Man in the High Alps*.

¹³ OLIVER: *A contribution to the study of blood pressure*, London, 1901.

In 1905 Gardiner and Hoagland¹⁴ published the results of a series of observations on blood pressure at Colorado Springs, altitude 6000 feet, and on Pike's Peak, altitude 14,109 feet. They found for men and women who had resided at an altitude of 6000 feet for a year or more an average blood pressure slightly lower than that usually given for sea level. Their data at 14,000 feet were secured from 22 college men who were taken to the summit of Pike's Peak by the Pike's Peak and Manitou Railway. Two observations were made on each man, — the first on arriving, and the second three and one-half hours later. By the Hall-Quetelet¹⁵ method of averaging they found the pressure to average 121 mm. at the first reading and 118 mm. at the second, whereas they give 126 as an average reading at an altitude of 6000 feet.

It is thus seen that only a few studies of the influence of altitude have been made. Further, the results obtained by the work that has been done are not consistent. This is not surprising in view of the lack of agreement that is found throughout the general literature on blood pressure, due almost entirely to faulty technique and placing reliance in untrustworthy instruments. Thus Gorbatschew used the earlier imperfect von Bach sphygmomanometer, and the stay at the higher elevation was brief. Mosso records only a few observations made on himself, and they, as well as those made by Oliver, were made with an imperfect sphygmomanometer. Gardiner and Hoagland compared their average at 6000 feet with one sometimes given for sea level. Their observations at 14,000 feet were made with the Riva Rocci sphygmomanometer, with which the limits of experimental error may be as great or greater than the differences in results which they obtained. Further, some of their men suffered severely from mountain sickness throughout the stay on the summit. Some of the men ate a hearty lunch, others ate nothing. Some took no exercise whatever, while others exercised vigorously by climbing among the boulders. The most serious shortcoming, in addition to all these, was the shortness — three and one-half hours — of the stay on the summit and the fact that only two readings were made on each man.

¹⁴ GARDINER and HOAGLAND: Transactions of the American Climatological Association, 1905.

¹⁵ Journal of the American Medical Association, Dec. 21, 1901.

METHOD OF STUDY.

All observations recorded in this paper were obtained with J. Erlanger's¹⁶ improved sphygmomanometer. The criteria used to detect systolic pressures were the method of V. Recklinghausen¹⁷ and the new method of Erlanger.¹⁸ Diastolic pressures were determined by Erlanger's¹⁹ method.

The technique was the same throughout. The subject was always seated, the cuff adjusted in the same position on the right arm. Attempts were always made to take observations under as normal conditions as possible, and any disturbing factor, such as eating, exercise, indisposition, etc., was always carefully noted and recorded. Furthermore all observations on a subject were made by the same observer. This was done to insure greater reliability of records. All data presented have been secured by repeated observations at two altitudes. This method of collecting data was found necessary because of the great discrepancy in the figures given for normal pressures at lower altitudes. Thus Mosso²⁰ considers 80 to 100 mm. as normal pressure in a sitting posture. Jackson²¹ says, "The normal pressure may vary from 100 to 150 mm." Erlanger and Hooker²² give 110 mm. Dr. S. Jellinck²³ made a series of observations with different instruments on 532 normal soldiers between 20 and 23 years of age, living under the same conditions of exercise, diet, and general daily routine. He found their pressure to vary between 80 and 185 mm., whereas in the great majority it fluctuated between 100 and 160 mm., even when disturbing influences of diet and exercise were eliminated. Schilling and Murnberg²⁴ studied a large number of normal men with Erlanger's

¹⁶ ERLANGER, J.: The Johns Hopkins Hospital reports, 1904, xii; also JANEWAY: The clinical study of blood pressure, p. 93.

¹⁷ RECKLINGHAUSEN, V.: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xvi.

¹⁸ ERLANGER: This journal, 1908, xxi, Proceedings of the American Physiological Society, p. xxiv.

¹⁹ ERLANGER: This journal, 1901, vi, Proceedings of the American Physiological Society, p. xxii.

²⁰ MOSSO: *Loc. cit.*

²¹ JACKSON, J. M.: Boston Medical and Surgical Journal, 1903, cxviii.

²² ERLANGER and HOOKER: The Johns Hopkins Hospital reports, 1904, xii.

²³ JELLINCK, S.: Zeitschrift für klinische Medizin, 1900, xxxix.

²⁴ SCHILLING and MURNBERG: Münchener medicinische Wochenschrift, 1906.

sphygmomanometer. In 37 per cent of these men the average systolic pressure was below 125 mm.; in 31 per cent it varied between 125 and 148 mm.; in 27 per cent it varied between 145 and 206 mm.

OBSERVATIONS.

Our observations may, for convenience, be divided into seven groups to correspond to the time and altitude at which they were made.

Group I. — Three women and one man were studied. Normal pressures were ascertained in Iowa at an altitude of 1700 feet. Readings were made at intervals throughout two and a half summer months. These observations were followed by studies at Colorado Springs, altitude 6000 feet. In two cases repeated observations have been made at the higher altitude during a period of three years. The other subjects were in residence at the higher altitude from two to four weeks.

The differences in these records are clearly shown in the averages of all the normal pressures. In preparing the averages we have eliminated all readings in which disturbing factors were active. Table I gives these averages for both altitudes, and also the highest and lowest readings secured from each subject regardless of cause.

The fall in systolic pressure is clearly marked for each subject. On the other hand our records for diastolic pressures show a decided fall in two cases and a rise in two others. The explanation for the fall in pulse rate is probably found in the influence of the external temperature. The records at the low altitudes were taken during the oppressive heat of midsummer.

Group II. — Two sound college men served as subjects. The normal blood pressure was determined at Colorado Springs. Then following a journey of fifteen hours to Nebraska, to an altitude of 2000 feet, a number of readings were made at the lower altitude during a period of four days. The records secured are misleading because of irregular and heavy eating, loss of sleep and psychic influences attending the excitement of a short visit among friends. The records for the two subjects run parallel throughout the systolic pressures, but do not show corresponding changes in the diastolic pressures. At the lower altitude the average systolic pressure

TABLE I.

Altitude 1700 feet.					
Person.	Sex.	Reading.	Pressure in mm. of Hg.		Pulse.
			Systolic.	Diastolic.	
1	Female	average	116.0	77.0	77.6
		highest	188.0	89.0	96.0
		lowest	102.0	66.0	60.0
2	Female	average	113.5	67.5	80.0
		highest	144.0	71.0	92.0
		lowest	100.0	62.0	70.0
3 ¹	Female	average	109.0	67.5	75.0
4	Male	average	125.0	68.0	79.3
		highest	176.0	75.0	94.0
		lowest	108.0	60.0	70.0
Altitude 6000 feet.					
1	Female	average	109	70	70.7
		highest	144	83	90.0
		lowest	102	63	58.0
2	Female	average	107	70	68.5
		highest	126	76	83.0
		lowest	98	60	56.0
3	Female	average	98	60	65.0
4	Male	average	122	77	76.1
		highest	168	90	108.0
		lowest	100	67	62.0
Amount of change at 6000 feet.					
1	Female	average	-7.0	-7.0	-6.9
2	Female	average	-6.5	+2.5	-11.5
3	Female	average	-11.0	-7.5	-10.0
4	Male	average	-3.0	+9.0	-3.2
¹ Six and five readings at each altitude.					

was materially raised in both men, while the diastolic pressure remained unchanged for one and was clearly lowered in the second subject. All readings secured from one of the men are given in Table II, and the averages at both altitudes for the two men in Table III.

TABLE II.
SUBJECT NO. 1.

Day.	Time.	Pressure.	
		Systolic.	Diastolic.
1	1.50 P. M.	152	77
	2.30 P. M.	136	85
	3.45 P. M.	140	80
2	9.00 A. M.	142	83
	10.00 A. M.	146	81
	10.30 A. M.	142	83
	2.30 P. M.	150	64
	4.30 P. M.	162	86
	5.30 P. M.	156	90
	9.30 P. M.	156	78
3	9.00 A. M.	198	78
	10.00 A. M.	179	78
4	10.00 A. M.	146	82
	11.00 A. M.	146	87
	4.00 P. M.	185	86

Group III. — In this series of observations two men remained twenty-four hours with a camping party at a mountain cabin at an altitude of 9000 feet. The records show a decided increase in systolic pressure, a fall in diastolic pressure and a rise in pulse rate. The changes, however, cannot be attributed to the influence of the higher altitude in that the subjects used tea and coffee freely and ate frequently. We also believe that the psychic factor, mirth

and argument, was in large measure responsible for the changes observed. The records for the day follow in Table IV, and with that given for each man is his average at 6000 feet.

TABLE III.

Person.	Altitude 2000 feet.		Altitude 6000 feet.	
	Systolic, av. pressure.	Diastolic, av. pressure.	Systolic, av. pressure.	Diastolic, av. pressure.
1	155.7	81.2	135.0	81.0
2	155.9	75.1	138.4	85.4

Group IV. — Seven normal college men constituted this group. Their pressures were first determined for the altitude of Colorado Springs. A leisurely tramp was then taken to the mountain cabin previously referred to, altitude 9000 feet. Their pressures were taken at frequent intervals during a stay of two days. The men exercised vigorously and ate heartily of the camp fare, including large quantities of strong coffee; hence changes of blood pressure produced by the change in altitude are obscured by factors similar to those operating in Group III. The results showed an average rise of systolic pressure of about 4 mm. and diastolic pressure of 1 mm. and an average increase of heart beats of 11 per minute. The records for Groups III and IV suggest that a comparison of readings at different altitudes is of little value unless the environment and habits of the subject are much alike at each altitude.

Group V. — In this study two men and one woman spent ten days in a mountain camp at an altitude of 7900 feet. Records taken at this altitude fail to show changes in systolic and diastolic blood pressures and give only a slight increase in the pulse rate.

Group VI. — Two men (one colored) who spend the entire summer on the summit of Pike's Peak, altitude 14,109 feet, were studied. They go up with the first train about May 1st and remain on the summit continuously until the last of October. Both were perfectly normal; one of the two was kindly examined and pronounced so by a physician. The other holds the time record for the climb to the summit of the "Peak" from Manitou, which

fact alone is sufficient guaranty of a sound physique. These two men were subjected to a critical study of pressures at the altitude of 6000 feet, for a period of five days in the spring prior to the

TABLE IV.

Person A. at 9000 feet.			
Time.	Systolic pressure.	Diastolic pressure.	Pulse.
3.00 P. M.	150.0	72.0	98.0
4.00 P. M.	142.0	80.0	104.0
10.00 P. M.	166.0	70.0	92.0
9.30 A. M.	138.0	75.0	84.0
11.30 A. M.	170.0	76.0	96.0
4.00 P. M.	150.0	70.0	92.0
Average	152.6	73.8	94.3
Av. at 6000 feet .	122.0	77.0	76.1
Person B. at 9000 feet.			
3.00 P. M.	152.0	74.0	84.0
4.00 P. M.	136.0	80.0	82.0
10.00 P. M.	156.0	72.0	84.0
9.30 A. M.	160.0	86.0	76.0
11.30 A. M.	174.0	76.0	84.0
4.00 P. M.	148.0	78.0	76.0
Average	154.3	77.6	81.0
Av. at 6000 feet .	135.0	81.0	74.2

time of ascending the "Peak." They had spent the winter at the lower altitude. All disturbing factors were noted and eliminated in so far as was possible, and each determination of pressure was made in duplicate to eliminate chance of error. The men left for the summit about the middle of May, where they remained as usual all summer. Their pressures were determined on the summit at

intervals during June, July, and September. In Table V the results are expressed as averages for all the readings at the altitudes 6000 feet and 14,109 feet. Table VI gives all observations on subject No. 1.

TABLE V.

Altitude 6000 feet.			
Person.	Systolic pressure.	Diastolic pressure.	Pulse.
1	125.6	85.6	77.7
2	136.8 ¹	86.9	79.0
Average	131.2	86.2	78.3
Altitude 14,109 feet.			
1	118.6	84	79.7
2	127.0	86	82.4
Average	122.8	85	81.0
Changes at altitude 14,109 feet.			
1	-7.0	-1.6	+2.0
2	-9.8	-0.9	+3.4
Average	-8.4	-1.2	+2.7
¹ Had been drinking during this time, although not enough to intoxicate.			

As seen from Tables V and VI, there was a decided fall in systolic pressure, a slight fall in diastolic pressure, and a slight increase in the rate of heart beat.

Group VII. — These observations were made on nine college men during a continuous stay on the summit of Pike's Peak of from three to five days. The expedition was made to the summit on March 27 on foot. The summit was reached after some hardship due to cold and snow and mountain sickness in the case of one of the men. Throughout the stay they were comfortably quartered in

the Summit House, a substantial stone building, and had plenty of fuel and provisions. The men were very much fatigued im-

TABLE VI.

COMPLETE RECORD OF NO. 1.					
Date.	Time.	Altitude.	Systolic pressure.	Diastolic pressure.	Pulse.
April 26	3.30 P. M.	6000 feet ¹	126	87	72
April 27	9.00 A. M.	" "	127	82	76
April 27	10.45 A. M.	" "	126	84	76
April 27	2.00 P. M.	" "	118	86	84
April 27	3.00 P. M.	" "	118	90	78
April 29	10.30 P. M.	" "	120	90	78
April 30	2.30 P. M.	" "	130	84	76
April 30	4.00 P. M.	" "	127	86	79
May 1	10.00 A. M.	" "	132	87	80
May 1	11.30 A. M.	" "	132	80	78
June 6	12.00 Noon	14,109 feet ²	116	90	68
June 6	12.45 P. M.	" "	116	84	68
June 6	2.00 P. M.	" "	115	84	68
July 28	9.30 A. M.	" "	122	86	88
July 28	4.00 P. M.	" "	122	84	72
Sept. 10	12.30 P. M.	" "	118	80	88
Sept. 10	2.30 P. M.	" "	122	84	96
Sept. 10	3.40 P. M.	" "	118	80	90

¹ All readings at 6000 feet were taken while subject was quiet or after an easy walk.

² All readings at 14,109 feet were taken on subject just after he had been serving as a waiter at a lunch counter.

mediately on reaching the summit, and several suffered from headache, but all of these symptoms disappeared after the first day. After this none of them were in the least afflicted by the altitude,

except during vigorous exercise, when they suffered from breathlessness and a tendency to headache if the exercise was prolonged. Two of the men remained three days, the other seven five days. Their pressures had been determined, as had those in the other groups, for an altitude of 6000 feet and were again measured after returning to this altitude. In all of these tests the conditions were favorable for comparative results. The weather was such that the men remained inside the house reading, etc., much as they would in their college work, and the daily routine was much the same.

The results of the observations are tabulated in Table VII. The figures represent the averages of all normal readings of each individual while on the summit and of a number of readings at the lower altitude.

It is seen from these tables that there is a fall in blood pressure for the higher altitude in all cases but one, and that the one exception shows a slight fall in the records of the later observations. The average fall in systolic pressure varies between 0.8 and 21.9 mm. of mercury and in diastolic pressure between 0.9 and 11 mm. of mercury. The average fall for the eight men is 6.9 mm. of mercury for systolic and diastolic pressures. It seems significant that the case that was most affected by the altitude shows much the greatest fall in systolic pressure, the least fall in diastolic pressure, and more than twice the average acceleration in the rate of heart beat.

Throughout the records there is a rough correspondence in the fall between the systolic and diastolic pressures. We also find that the pressure tends to fall as the pulse rate increases at the higher altitude, but that this relation is not constant.

On comparing the figures obtained in the last two groups it will be seen that the results are the same, varying only in quantity. The average changes, both in diastolic pressure and heart rate, are least marked in the men who remained on the summit all summer. This is especially marked in case of the heart beat, showing an average increase of only about three beats in Group VI, while in the other group the acceleration was 20.9 beats per minute.

CONCLUSIONS.

1. A considerable elevation in altitude tends to lower systolic and diastolic blood pressure and to increase the rate of heart beat.

TABLE VII.

Altitude 6000 feet.			
Name.	Systolic pressure.	Diastolic pressure.	Pulse.
C. A.	135.0	81.0	74.2
E. E.	138.4	85.4	86.3
M.	132.0	85.0	80.4
T.	130.0	82.0	65.0
H. ¹	134.0	86.6	67.0
L. ²	120.0	74.6	74.5
L. ³	(132.0)	(82.0)	(76.0)
N.	129.3	78.1	71.2
D. ⁴	156.0	80.2	63.6
E.	131.6	86.0	84.8
Average ⁵	135.7	83.0	74.0
Altitude 14,109 feet.			
C. A.	132.2	78.0	101.8
E. E.	127.6	76.0	103.3
M.	124.5	74.0	93.1
T.	126.6	76.5	82.0
H.	131.9	79.0	84.2
L.	131.2	77.4	102.1
L.	.	.	.
N.	126.0	70.0	95.2
D. ⁴	134.1	79.3	104.7
E.	127.5	76.0	96.0
Average ⁵	128.8	76.1	95.0
Changes at 14,109 feet.			
C. A.	-2.8	-3.0	+27.6
E. E.	-10.8	-9.4	+17.0
M.	-7.5	-11.0	+12.7
T.	-3.4	-5.5	+17.0
H.	-2.1	-7.6	+17.2
L. ⁶	+11.2	+2.8	+27.6
L. ⁶	(-0.8)	(-4.6)	.
N.	-3.3	-8.1	+24.0
D.	-21.9	-0.9	+41.1
E.	-4.1	-10.0	+11.2
Average ⁵	-6.9	-6.9	+20.9
<p>¹ We have very few readings at 6000 feet for H.</p> <p>² Less number of normal pressure readings were taken for L. at 6000 feet than any other.</p> <p>³ Average for L. of repeated readings taken at a much later time.</p> <p>⁴ Troubled with mountain sickness in the ascent and most affected by the altitude.</p> <p>⁵ L. has been omitted from the average.</p> <p>⁶ Not included in the average.</p>			

2. The fall in systolic pressure is slightly greater and more certain to occur than the fall in diastolic pressure.

3. A rise in diastolic pressure occurs in some individuals.

4. The influence of such factors as psychical states, eating, and exercise may obscure the influence of altitude on the blood pressure.

5. The fall in blood pressure and increase in heart beat is most marked in the early part of the residence at the higher altitude.

6. On a prolonged stay at high altitude the heart rate probably returns more nearly to the normal than the blood pressure.

7. High altitudes do not affect to the same degree the blood pressure of all individuals.

8. Small elevation in altitude does not appreciably influence the blood pressure.

9. Those individuals most affected by high altitude seem to sustain the greatest fall in systolic blood pressure and the greatest acceleration in the rate of heart beat.

10. The heat of the summer season probably accelerates the pulse rate.

THE ACID CLOSURE OF THE CARDIA.

By W. B. CANNON.

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

THAT the cardia exhibits an alternating increase and decrease of its contracted state was known to Magendie early in the last century.¹ These variations of contraction at the cardia, according to Schiff, are not actually localized there, but result from a ring of constriction moving up and down the lower œsophagus and periodically involving the cardia.² Schiff's observations were made on dogs and cats. In 1860 Basslinger described rhythmic pulsations of the cardia in the excised stomach of the rabbit,³ a phenomenon sometimes designated as "Basslinger's pulse." The cardia of the normal rabbit Kronecker and Meltzer⁴ found usually quiet, but in a freshly bled rabbit they saw the spontaneous movements described by Basslinger.

The proper normal function of the cardia without doubt is to prevent regurgitation of swallowed food into the œsophagus. If Schiff's conception of peristalsis and antiperistalsis in the lower œsophagus is correct, such regurgitation could take place only slowly and to a slight extent; but if, as Magendie stated, a true diminution of the contracted state occurs, leaving an easily forced passage, gastric contents might suddenly be forced backward throughout the gullet. The difference between the views of Magendie and Schiff,

¹ MAGENDIE: Précis élémentaire de physiologie, fourth edition, Paris, 1836, ii, pp 81, 82. The original report was made in 1813.

² SCHIFF: Leçons sur la physiologie de la digestion, Florence and Turin, 1867, ii, p. 333.

³ BASSLINGER: MOLESCHOTT'S Untersuchungen zur Naturlehre des Menschen und der Thiere, 1860, vii, p. 359.

⁴ KRONECKER and MELTZER: Archiv für Physiologie, 1883, Supplement-Band, p. 347.

and the possibility that after all their and Basslinger's observations might have resulted, as Kronecker and Meltzer's study suggests, from abnormal conditions, make it desirable to investigate the action of the cardia under more natural conditions.

About six years ago, during an attempt to see the movement of particles of food in the stomach when the gastric contents were fluid, repeated regurgitation of the fluid from the stomach into the œsophagus was observed.⁵ The fluid consisted of 2 gm. potato starch boiled in 100 c.c. water, with 5 gm. subnitrate of bismuth added. It was given by stomach tube. The animal lay comfortably on a holder, unanæsthetized, and was examined by means of a screen made fluorescent by the X-rays. The regurgitation was unattended by any signs of nausea or retching, and when the animal was lifted from the holder she acted quite as a cat normally acts. The periodically lessened contraction of the cardia would therefore appear to be a natural phenomenon. Since the fluid on emerging from the stomach at once passed quickly up the œsophagus to the level of the heart or even to the base of the neck, it is clear that Magendie's conception is correct, and that Schiff's idea of an oscillating peristalsis and antiperistalsis in the lower œsophagus must be discarded.

Each regurgitation is followed at once by a peristaltic wave, which pushes the escaped fluid back again into the stomach. Soon after the fluid is thus restored, the cardia again relaxes and the fluid rushes out into the œsophagus, only to be restored again to the stomach by another peristaltic wave. Thus the process continues. The peristaltic wave is seldom started by voluntary deglutition, but is stimulated by the presence of the material in the œsophagus.⁶

The regurgitation and restoration of the fluid may thus recur fairly periodically for twenty or thirty minutes. The periods are shorter at first than later. The following figures show the time taken by these periodic movements in a large cat given 180 c.c. of fluid boiled starch at 3.20 P. M. The figures under "Out" indicate the moment when the fluid emerged into the œsophagus; those under "In," when the last of the fluid disappeared into the stomach.

⁵ A notice of these observations was published in the Proceedings of the fifteenth annual meeting of the American Physiological Society. See this journal, 1903, viii, p. xxii.

⁶ Compare MELTZER: *Zentralblatt für Physiologie*, 1905, xix, p. 994.

Out	In
3.21- 6	3.21-12
17	24
32	38
48	54
22- 2	22- 8
19	28
44	51
23- 2	23- 8
21	29
43	49

The regurgitation continued thus, but became gradually less frequent. Twenty minutes after the first observation appearance and disappearance were as follows:

Out	In
3.41-14	3.41-27
42-26	42-42
43-45	43-59
45- 8	45-16

During the eighteen minutes of observation that followed, the food emerged into the œsophagus only three times.

In this instance there was a fairly rhythmic appearance of food in the œsophagus, beginning at the rate of four times a minute, gradually falling to three and two times a minute, and ceasing almost entirely soon after a rate of about once per minute was reached. It is noteworthy that in this and other animals, bodily movements indicating excitement were attended by cessation of the regurgitations.

Two questions are suggested by these observations: Under what circumstances do the regurgitations occur? and, Why, once begun, do they cease?

In answer to the first question the fluidity of the gastric contents must be regarded as a prime factor in the regurgitations. When the food escapes into the œsophagus, it escapes quickly in a thin stream. If the stomach is full of more or less gross fragments of food, it is quite conceivable that a slight weakening of the contraction of the cardia would not permit such semi-solid material to pass back into the œsophagus. A second factor in the regurgitation is intragastric pressure. Into the stomach of the cat that furnished

the records given above were introduced on one occasion 60 c.c. of the fluid starch, with no regurgitation during the next five minutes; 60 c.c. more were introduced, with no regurgitation during the five minutes that followed; then 60 c.c. more were introduced, making in all 180 c.c., and regurgitations at once began and continued. In order to demonstrate the rhythmic relaxations of the cardia, therefore, the gastric contents must be fluid, and must be under sufficient pressure to pass through the cardia when the contraction weakens.

At first it seemed as if the two factors just mentioned were the only factors concerned. The cessation of the regurgitations might then be explained by a slow accommodation of the stomach to the volume of its contents, or by the escape of material into the duodenum until the intragastric pressure was insufficient to press the fluid through the only slightly relaxed cardia. These explanations are, however, not adequate. Kelling has shown that, within limits, intragastric tension is readily adjusted to varying amounts of food, and that for this adjustment only a few moments are required;⁷ the normally rapid adjustment of intragastric tension therefore would not explain the cessation of the regurgitations after their continuance for twenty or thirty minutes. And observations on the intestinal contents of animals in which the regurgitations have ceased have shown only a small amount of the fluid starch in the intestine. A diminution of intragastric pressure does not therefore account for the disappearance of the regurgitations.

Since the repeated passage of fluid food into the œsophagus is dependent on a periodic lessening of the contraction of the cardia and on an intragastric pressure sufficient to force the gastric contents through the weakened barrier, and since intragastric pressure has probably not materially diminished at the time when the regurgitations cease, the explanation of the cessation must lie in a change at the cardia. Either the rhythmic relaxations might be stopped, or the tonus of the sphincter might be increased. With an increased tonus the cardia might still undergo rhythmic contractions and relaxations, but on a level so much higher than before that the intragastric pressure would now be unable to overtop it. Thus the cardia would perform its normal function of preventing the passage of food backward into the œsophagus during the course of gastric digestion.

⁷ KELLING: *Zeitschrift für Biologie*, 1903, xliv, p. 235.

What new agencies are developed in the stomach during gastric digestion that might affect the cardia? The agencies might be of two orders, mechanical or chemical, — the actual stretching of the stomach might cause closure of the cardia, as Magendie suggested; or the new condition developed in the stomach during digestion, an acid reaction, might have that effect. Evidence has been presented (see page 108) that the rhythmic relaxations of the cardia are made manifest, by the method used in this investigation, only as the content of the stomach is increased. And, furthermore, the gastric wall certainly does not become gradually more stretched as the food lies in the stomach during twenty or thirty minutes. The cessation of the regurgitation is therefore not explained by any increase of intra-gastric pressure. Is the chemical agency, acid in contact with the gastric mucosa, capable of changing the contraction of the sphincter?

That acid continuously injected into the duodenum may prevent the discharge of food through the pylorus for an unlimited period is well known;⁸ that this effect is due to closure of the pylorus is also known.⁹ That the response of the pylorus to acid in the duodenum is like the response to a stimulus at any point in the intestine has already been pointed out¹⁰ — the stimulus causes a contraction above the stimulated point. And just as acid below the pylorus keeps the pylorus closed, so likewise acid in the stomach (below the cardia) may keep the cardia closed. Thus an essential condition for digestion in the stomach, the development of an acid reaction, would automatically hold a barrier against a return of the gastric contents into the œsophagus.

That a marked acidity of the gastric contents does promptly check regurgitation through the cardia is proved by such observations as the following:

A cat with an empty stomach was given by stomach tube 200 c.c. fluid starch with 10 gm. bismuth subnitrate at 2.55 P. M. The regurgitations occurred as follows:

Out	In
2.56- 1	2.56-11
16	28
32	42

⁸ See PAWLOW: The work of the digestive glands, London, 1902, p. 164.

⁹ See CANNON: This journal, 1907, xx, p. 289.

¹⁰ See CANNON: This journal, 1907, xx, p. 319.

TABLE (continued).

Out	In
46	57
57-8	57-18
29	39
48	60
58-12	58-22
38	49
58	59-10
59-30	40 cat excited
3.00-35 cat excited	3.01- 2
01-15	25
44	54
02- 2	02-12

At this time no food had passed through the pylorus. The gastric contents were now as much as possible removed (about 180 c.c.). The reaction was very faintly acid. Fresh fluid starch was added to make 200 c.c., and then 4 c.c. of 25 per cent hydrochloric acid was mixed with the fluid, making approximately a 0.5 per cent acidity, which is normal for the carnivora. The fluid was then reintroduced into the same animal, at 3.12 P. M., with the following results:

Out	In
3.13-45	3.13-53
14-17	14-39

The fluid passed from the stomach into the œsophagus these two times in a very thin stream. Thereafter there was no regurgitation whatever during ten minutes of observation. The cardia was now holding tightly enough to retain gastric contents amounting to 220 c.c., although previous to the acidification it did not withstand the pressure of 200 c.c.

This observation has been repeated on normal animals and on an animal whose splanchnic nerves had previously been severed, with the same results.

The effect of acid in the stomach on the tonus of the cardia can be demonstrated also in the anæsthetized operated animal.

A cat was etherized, and given subcutaneously 1 c.c. of 1 per cent morphine sulphate. While the animal was under the effects of the ether the spinal cord was destroyed below the brachial region, the abdomen was opened, a tube was introduced into the cardiac end of the stomach,

and a ligature, passed around the stomach, was tied tightly about the tube. Another tube was introduced through the cervical œsophagus as far as the upper thorax and tied in place. Each tube was connected by rubber tubing with a long upright thistle tube. Warm physiological salt solution was now introduced until the level in each tube was 9 cm. above the cardia. At once the fluid in the œsophageal tube began to disappear and reappear at fairly regular intervals, precisely as in the X-ray observations on regurgitation. Oscillations of the fluid in the gastric tube were seen corresponding reciprocally to the oscillations in the œsophageal tube. During this period the ether administration was largely suspended, since the morphine was an effective narcotic.¹¹

After the rhythmic regurgitation had proceeded for several minutes the salt solution was removed. It was replaced by a similar solution with 0.5 per cent hydrochloric acid, poured into the thistle tube connected with the stomach. The acidulated salt solution was added until the meniscus in the tube stood 19 cm. above the cardia. For several minutes it stood at that point with no relaxation of the sphincter. The stomach was now compressed, and the fluid rose 33 cm. above the cardia before the sphincter relaxed. The fluid that then passed into the œsophagus was immediately pushed back into the stomach by peristalsis and held there. Pressure again applied to the stomach forced the column of salt solution to 42 cm. above the cardia before relaxation again occurred. No rhythmic regurgitation was observed.

Now the acidulated salt solution was removed from the stomach and replaced by 1 per cent sodium bicarbonate, poured into the stomach tube until 9 cm. above the cardia. Almost immediately regurgitations began and continued rhythmically during ten minutes of observation.

In this instance the regurgitations were seen when the intragastric pressure was little more than 9 cm. of water, the regurgitation ceased and the cardia supported a pressure of 30 and 40 cm. of water after the gastric contents were made acid, and the regurgitations began again in the presence of a pressure of 9 cm. of water when a nearly neutral fluid was again introduced into the stomach.

This effect of intragastric acidity on the cardia can be registered graphically by connecting the œsophageal tube, described in the foregoing experiment, with a recording tambour. The regurgitations into the œsophagus cause the writing lever of the tambour to rise,

¹¹ It is commonly stated that morphine has a strychnine effect on cats. For the information that this effect is due to too large doses, and that 1 or 2 c.c. of a 1 per cent solution of morphine sulphate narcotizes a cat I am indebted to Dr. John Auer.

and as the regurgitated fluid is carried back into the stomach the lever falls. Figure 1 presents a record of such regurgitations. The glass tube tied into the cardiac end of the stomach was short, and connecting it with a thistle tube was a piece of rubber tubing. Through the rubber tubing a hollow needle was introduced into the gastric cavity. Thus the stomach was not disturbed in the subsequent experimental manipulation. During the period indicated by

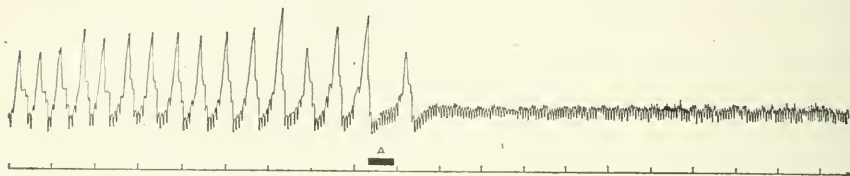


FIGURE 1.—Record showing cessation of rhythmic regurgitations of fluid from the stomach into the œsophagus on acidulation of gastric contents; the upstroke of the larger oscillations represents the outflow, the downstroke the return of the fluid to the stomach by œsophageal peristalsis. At *A* enough HCl (2 c.c.) was introduced into the stomach to give the contents an acidity of 0.5 per cent. The small oscillations are due to respiration. The time is marked in half-minute intervals.

the broad black line at *A*, sufficient hydrochloric acid (2 c.c.) was introduced through the needle into the stomach to render the salt solution acid to 0.5 per cent. After one more regurgitation the cardia closed. The time is registered in half-minute intervals.

The question arises as to whether the effect of the acid on the cardia is, as in the duodeno-pyloric reflex, a local effect, or mediated through the vagus or splanchnic nerves. That regurgitations continue after splanchnic section and may be caused to stop by rendering the gastric contents acid has already been noted (see page 110). The task of eliminating the vagus nerves is more difficult, because only the lowest few centimetres of the œsophagus remain capable of peristalsis after vagotomy, and this portion has not given clear records of a restoration of regurgitated fluid into the stomach. The effect of the acid can be tested, however, by observing the intragastric pressure required to open the cardia before and after the acidulation of the fluid.

A cat with the right vagus nerve severed below the recurrent laryngeal branch seven days before, and the left severed five days before (both operations under complete anæsthesia), was etherized, given 1 c.c. 1 per cent morphine sulphate, and pithed from the lower end of the cord to the brachial region. Tubes were tied into the œsophagus and

stomach. Warm normal salt solution was poured into the thistle tube connected with the stomach until the pressure above the cardia was 14 cm., rising to 19 cm. during inspiration. Only then did the cardia relax. A second determination resulted in the same figures.

The salt solution, which proved to be neutral, was now removed and replaced by the same solution with 0.5 per cent hydrochloric acid. The acid fluid was poured into the tube tied into the stomach until the pressure was 17 cm. (rising to 22 cm. during inspiration) before the cardia relaxed. The fluid was now removed and immediately again poured into the stomach; this time the pressure rose to 19 cm. (24 and 25 cm. during inspiration) before the cardia opened. Another immediate repetition gave 21 cm. rising to 26 and 27 cm., as threshold pressures. In a fourth trial the pressure was raised to 53 cm., and the sphincter gave way only when still more fluid was poured into the tube.

In this experiment, as well as in those in which regurgitations were observed and registered, a more or less prolonged latent period intervened between the application of the acid and its full effect in closing the cardia. But the fact that the liminal pressure gradually rose in this instance and finally became about four times as great with acid gastric contents as it was with neutral gastric contents, proves that the effect of the acid is not produced through extrinsic nerves but by the local reflex in the wall of the gut. This result has been confirmed by similar observations made immediately after destruction of the lumbar and thoracic cord and severance of the vagus nerves.

The question may be raised as to whether the prolonged period of regurgitation observed when fluid starch was given (frequently 20 or 30 minutes after its introduction) does not indicate that the acid mechanism of the cardia is rather defective. In this connection it should be remembered that boiled starch has very little effect in exciting the flow of gastric juice,¹² and that the cardia therefore probably exhibits relaxations for a much longer period when fluid starch is given than when foods more favorable to gastric secretion are fed.

The fluid character of the boiled starch is also favorable to the early closure of the cardia, for the acid secreted is not kept in contact with the wall of the stomach, but is diffused into the fluid; and each movement of the fluid to and fro between stomach and cesophagus serves to mix the secreted acid with the total contents. For this

¹² PAWLOW: *Loc. cit.*, p. 97.

reason it has been found impossible to get consistent results in determining the acidity of the gastric contents under these circumstances. When the food is less fluid, the acid reaction of the contents of the cardiac end of the stomach is found solely on the surface, near the mucosa, for a considerable period after digestion has commenced.¹³ Under these circumstances the conditions for closure of the cardia are most favorable.

It should not be forgotten that although the evidence points to the acid control of the cardia through a local reflex, the cardia is nevertheless under the influence of extrinsic nerves, and that in abnormal states these nerves may cause the cardia to open and permit regurgitation of acid food. The regurgitation of gases might be due to their effect in keeping the acid gastric contents away from the stomach wall in the region of the cardia. As the cardia relaxes and permits the regurgitation of gas, acid fluid may also escape before the sphincter again closes. All these conditions, however, cannot be regarded as normal. Normally we are quite unconscious of the nauseating odor and the highly disagreeable taste of the gastric contents, and for this pleasant security the closed cardia is responsible.

It is a pleasure to acknowledge here the help I have received at various times during this investigation from my students, James Archer O'Reilly, Harold H. Smith, and Carl A. Hedblom.

SUMMARY.

If the stomach is well filled with neutral fluid, the fluid is rhythmically regurgitated into the œsophagus. The regurgitations result from rhythmic relaxations of the contracted cardia.

If the fluid in the stomach is made normally acid, the regurgitations, after a more or less brief latent period, cease. They may be restarted by rendering the fluid again neutral.

The effect of acidity of the gastric contents in keeping the cardia closed is seen after severance of the vagus nerves and destruction of the lumbar and thoracic portions of the spinal cord. The effect is therefore due to a local reflex in the wall of the alimentary canal.

¹³ CANNON: This journal, 1898, i, p. 379.

THE NATURE OF THE CONDUCTION OF NERVE IMPULSE.

By WILLIAM SUTHERLAND.

IN two communications to this journal¹ I have given a physical theory of the propagation and electric properties of nerve impulse. That theory is a deduction from the general physical principle which I have sought to establish, namely, that all rigidity is of electric origin. In nerve there are two broad classes of electric action to be distinguished, — first, that in which, for instance, a wireless telegraphic signal passes unnoticed through the body of a living animal, nerve behaving like any ordinary substance; and, second, that occurring when the electricity of a nerve is displaced so as to displace also the protein molecule with which the displaced electricity is associated. In this second case the main electric disturbance and the associated elastic disturbance in the nerve are propagated together as two aspects of the same occurrence. In this way the conclusion is reached that the velocity of propagation of nerve impulse is that of a shear in the substance of the nerve. It follows, then, that to a first approximation the change in the velocity of nerve impulse with temperature ought to be equal to the change in the velocity of a shear. But with lowering of temperature most substances, including protein jellies, become more rigid and therefore transmit a shear more quickly. Hence it appears from this physical theory that nerve impulse ought to travel faster at lower temperatures than at higher. But within the range of temperatures physiologically permissible, say from 0° to 40°, the change to be expected on the physical theory must be small, because the change in the rigidity of nerve within these temperatures is small. The original observation of Helmholtz on the effect of temperature on nerve velocities seemed to contradict this theoretical deduction flatly, but in my first paper I mentioned that Weiss claimed to have shown,

¹ SUTHERLAND: This journal, 1905, xiv, p. 112, and 1906, xvii, p. 297.

by cooling nerve and muscle separately, that from 20° to 0° nerve velocity diminishes not more than 9 per cent, in contrast to the 90 per cent of Helmholtz. A smallish diminution like this, rather than an increase, is not excluded by my physical theory, because the physical assumptions are close enough to the actualities of nerve to furnish only a first approximation.

But recent experimental work gives the seeming result that nerve velocity diminishes very much with falling temperature. C. D. Snyder² and S. S. Maxwell³ have found so large a temperature change that they have rejected the physical theory and declared for the propagation of nerve impulse as a chemical process. I propose to show that their very acceptable experimental results are capable of a different interpretation from that which they give them, supplying, indeed, a useful instalment of confirmation and extension of the physical theory. In my previous papers I did not include the effects of viscosity in the investigation, I merely referred to them, as the experimental material then in existence was not definite and abundant enough to afford a test of a more complete theory. Thanks to these recent American experiments, it becomes profitable to carry the physical theory a stage farther.

To get an idea of the way in which viscosity may profoundly affect the phenomena of elasticity, compare the behavior of a steel wire under torsion with that of a lead one. When released from torsion, the steel wire will execute a number of torsional vibrations of slowly decreasing amplitude, whereas the lead one will quickly come to rest. A steel tuning-fork will sound for a long time, when a lead one will not yield a recognizable note. This difference is connected with the greater plasticity of the lead, which causes it under stress to flow like a liquid at the same time that it is being strained like a solid. Strain is frittered away by internal relaxation. In the case of nerve and all tissues and jellies the effect of viscosity upon elastic behavior is very marked. It will now be shown that if nerve impulse is propagated as a shear in the protein framework of the nerve substance, the effect of viscosity will be particularly pronounced. To fix ideas let us make a simple schematic graphical picture of the behavior of a nerve-muscle preparation in the experimental measurement of the velocity of a nerve impulse

² SNYDER: This journal, 1908, xxii, p. 179.

³ MAXWELL: Journal of biological chemistry, 1907, iii, p. 359.

by the method of Helmholtz. Let A be the first point at which the nerve ABC is stimulated, B the second point, and C the point at which our simplified nerve enters our simplified muscle. At C the nerve undergoes some change which it transmits to the muscle. Let us then imagine the nerve AC prolonged to D instead of passing into muscle at C , and let the point D of the nerve be supposed to be kept changeless in such a way that the amount of change reaching C from A is the same as that which occurs when C is the common end point of nerve and muscle. We shall use freely

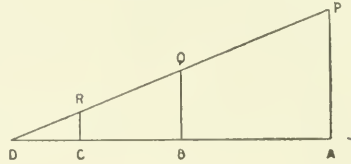


FIGURE 1.

the analogy of treating AD as a wire held fast at D and subject to torsion at A or B . Suppose the end A of the wire to receive a twist of amount represented by AP . Join DP . Then the amount of the twist at B is represented by BQ and at C by CR . In the case of nerve let AP measure that physical change which is produced at A by a stimulus. As the end D is supposed changeless, the simplest mode of transition from the condition of the nerve at A to that at D is given by the straight line PD , as in the analogous case of the wire. Now, the most important phenomenon in the case of nerve is that the amount of change at C must reach a certain value before it elicits muscular response. In the experiments, then, we produce by stimulation at A such change through the whole length AD that CR , the particular amount at C , is equal to y_m , this necessary minimum. It seems to me that it has not been sufficiently recognized in nerve physiology that stimulus at A may have to dominate the whole nerve before it produces its effect, and not merely travel like an explosive sound along a speaking-tube. Returning to the analogy of a wire in torsion, we know that to produce at C a twist $CR = y_m$ we must at A produce a definite related twist of amount AP . Now, the torsional strain in the wire is measured by PA/AD . If the change in the nerve causing muscular stimulation at C is the displacement CR , then y_m is a most important physiological quantity, as also the shear PA/AD .

We have now to consider the way in which viscosity will affect the time required to produce the shear PA/AD . This leads us to a consideration of the "damping" of vibrations as studied in acoustics or in the theory of the galvanometer. The typical instance of a damped vibration is that of a particle attracted to a

point with a force proportional to its distance from the point, and subject to a resistance proportional to the velocity. The importance of the theory of damped vibrations in general nerve physiology is demonstrated in the admirable popular lecture on "La vibration nerveuse" delivered by Ch. Richet before the British Association.⁴ For a discussion of the problem the reader is referred to "A Treatise on Electricity and Magnetism" by J. Clerk Maxwell.⁵ It will suffice to give here the essential results. First, the differential equation for the motion of such a particle at distance x from the origin, the centre of attraction being at distance a , is

$$\frac{d^2x}{dt^2} + 2k \frac{dx}{dt} + w^2(x - a) = 0 \quad \dots \quad (1)$$

Here $2k dx/dt$ is the resistance proportional to the velocity, and $w^2(x-a)$ is the attraction proportional to distance. There are three forms of solution for this equation according to the conditions $k \leq w$. When k is less than w , the solution is

$$x - a = Ce^{-kt} \cos(t\sqrt{w^2 - k^2} + \beta) \quad \dots \quad (2)$$

which represents a harmonic motion of frequency $\sqrt{w^2 - k^2}/2\pi$ and having an amplitude which dies away with time towards extinction in the manner expressed by the factor e^{-kt} ; that is to say, the amplitude of each swing is always the same fraction of the previous one. But when a vibration is transmitted along a line like a wave in a canal or sound along a speaking-tube, there is a corresponding diminution of amplitude with distance x , and a damping factor e^{-hx} comes in. When $k = w$, the solution is

$$x - a = (A + Bt) e^{-kt} \quad \dots \quad (3)$$

Here the motion ceases to be oscillatory. At the end of a time $-A/B$ the particle reaches the attracting point and passes through it, swings to the other side, reaches a maximum distance from the attracting point, and then begins to return slowly towards it. It may be said to execute one peculiar vibration, the two halves of which are very different in character. For the evidence in support of the existence of this peculiar type of single uneven vibration in nerve as well as that of ordinary damped oscillations

⁴ RICHEL: Nature, 1899, lx, p. 625.

⁵ MAXWELL: Vol. ii, ch. xvi.

the reader is referred to the lecture of Richet. When k is greater than ω , the motion is of similar general character to that when $k = \omega$, but is expressed by a more complicated formula. The transition from the one type to the other can be seen by vibrating a pendulum with its bob in water, its oscillations being rapidly damped. If, then, the water is made more and more viscous by the addition of a substance like molasses, a point is reached at which true vibratory motion ceases and only one uneven swing is accomplished.

To apply these considerations to our schematic nerve AD and the analogous wire in torsion, let us denote the shear PA/AD by s and AD by l , and write for the magnitude y of AP the terminal displacement generated in time t the formula

$$y = Cts e^{-Ks} \dots \dots \dots (4)$$

where K is a quantity expressing viscous resistance to the establishment of the shear. If the shear s is very small, e^{-Ks} is nearly 1, and for small shears $y = Cts$ and so $y/s = l = Ct$; that is to say, l/t , which is the velocity of propagation of the shear, $= C$. Small shears travel with a constant velocity characteristic of the particular nerve or wire AD . We can represent this state of affairs for small shears graphically by supposing shear in the nerve of small amount s , or torsion in the wire, started at A at time O and propagated as far as X in time t . DXS represents the state of affairs over the whole nerve at time t , DX being undisturbed and XA all distorted by the small shear $s = SA/AX$.

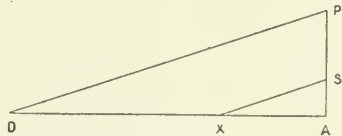


FIGURE 2.

When the shear just reaches D , the state of affairs is recorded by DP parallel to XS . This is the history of the simplest establishment of a state of torsion in a finite wire. Advancing now to the case in which s is no longer small, let us first consider (3) a little more closely. In it put $A = 0$; that is to say, let us take the case of a pendulum whose bob is immersed in molasses and receives a sudden blow displacing it from its position of rest. Equation (3) expresses the fact that the bob begins to move with constant velocity B , which is afterwards reduced at a rapidly increasing rate by the damping factor e^{-kt} . In the same way in (4) we introduce a damping

factor e^{-Ks} , depending not upon time, but upon shear. The reason for this lies in the very nature of the viscosity of solids. If we attempt to produce a large shear in lead, it begins to flow like a very viscous liquid. With large shear large viscous forces appear. In this case, then, the damping depends primarily on s . We have now to apply (4) to experiments on nerve. Returning to Fig. 1, we see that if for excitation of the muscle $CR = y_m$, then, since for a given preparation DC is a constant, say l_3 , $y_m/l_3 = s$ is also a constant, which may be denoted by s_m . If, then, at A at time 0 we apply an electric stimulus which in time t throws the nerve into such a state as is represented by PD when $CR = y_m$, we have for AP , denoted by y_1 , the value $Ct_1s_m e^{-Ks_m}$. In the same way, when we stimulate at B , the necessary displacement to elicit the same muscular response is $y_2 = Ct_2s_m e^{-Ks_m}$. Hence we have, since $y_1/s_m = AD = l$, say, and $y_2/s_m = BD = l_2$, say,

$$l_1 - l_2 = C(t_1 - t_2)e^{-Ks_m} \dots \dots (5)$$

$$\therefore \text{the experimentally measured velocity, } \frac{l_1 - l_2}{t_1 - t_2} = Ce^{-Ks_m} \dots \dots (6)$$

Thus, then, we have to do with the physically simple velocity C for very small stimuli, but for stimuli that must reach a certain minimum corresponding with y_m before they produce their observed physiological effect we find a velocity Ce^{-Ks_m} in which the viscosity parameter K has produced a damping effect. The velocity C is the one which should increase a little with falling temperature on account of increasing rigidity. But until greater experimental refinement is attained, within physiological limits of temperature we can assume C to be practically independent of temperature. Thus, in dealing with the velocity Ce^{-Ks_m} of physiological experiment, we trace its large diminution with temperature, as found in an exaggerated form by Helmholtz, to the large diminution of e^{-Ks_m} with temperature.

We must now investigate the probable nature of viscous resistance in nerve to the establishment of an impulse. The first objection to be considered is that the nerve impulse seems to be propagated with a remarkably inconspicuous amount of damping. Indeed many experimental physiologists have expressed the opinion that an impulse in nerve often becomes stronger as it travels. But the present theory does not contemplate the damping of an impulse as it travels; that would imply the presence of a damping factor

e^{-kt} involving the time, and this was specially excluded. The damping expressed by e^{-Ks} applies to the original establishment of the shear, not to its propagation. It appears from our figures that a greater displacement AP is needed at A than BQ at B to obtain muscular response. Experimentally the effect would be much the same as if PA had been damped down to BQ at B and CR at C , but this linear drop is very different from true damping. Still it is a good subject for experimental research to find the connection between stimulus and distance. It must be remembered that nerve impulse travels both ways in a nerve from the point of excitation. Hence, while APD represents the state of affairs when the muscle is excited from the end A , AQD represents it when excited from B on the supposition that A behaves as if fixed. Stimulus may appear to be always nearly the same, because it has generally to control the same amount of nerve, the effect QA requiring

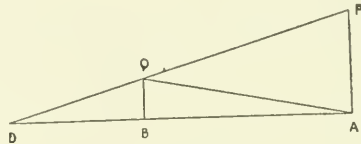


FIGURE 3.

nearly as much stimulus as QP . We can help ourselves to understand the position if we imagine a steel rod clamped rigidly at its upper end and dipping vertically into molasses, the immersed end carrying a large flat disk. Suppose, now, that we try to bend the rod quickly in a direction at right angles to the plane of the disk. The resistance of the molasses to a quick motion of the disk through it may be enormous, and control the time taken to deflect the rod through any desired displacement. When the rod is released, its motion will be damped during the return to its equilibrium position, but only to a slight extent if the motion is slow. The greater part of the damping action takes place during the establishment of the displacement, and only a small fraction during the subsequent motion.

Having disposed of these preliminary considerations, we can now devote our attention to the main point, namely, the physical law of the damping exponent Ks in e^{-Ks} . Imagine the water of a nerve evaporated so as to leave its protein framework unaltered in position, and suppose each thread of the framework to receive a shear, which may be of the nature of a separate torsion in each thread. This strain will propagate itself along the dry framework of the nerve like a strain in an ordinary solid, that is, with a small or quite moderate damping effect. But now imagine the water

restored to its place, some of its molecules being intimately associated with those of the protein and some of the protein dissolving in the water. If the protein threads are now subjected to the same shear as before, in receiving the shear s the threads will drag their associated molecules of water through a similar displacement, and these will experience powerful viscous resistance from the surrounding water or protein solution. By this reasoning we find that K is proportional to the viscosity either of water or of the fluid protein solution in the nerve. Fortunately for the further simplification of our complicated problem these alternatives reduce to the single condition, that as regards temperature changes K changes proportionally to η the viscosity of water. This simplification is due to the fact that, although a protein solution like egg white has an enormously greater viscosity than water, the relative changes of its viscosity with temperature are almost the same as those of water until the temperature of coagulation is approached. The evidence for this important fact is supplied in the following table from A. J. Ewart's "Protoplasmic Streaming in Plants," giving the viscosity of egg white and water at different temperatures. I have added the ratio of the viscosity of egg white to that of water.

Temp.	3°	10°	18°	27°	45°	60°	63°	65°
Egg white . . .	0.107	0.085	0.073	0.062	0.049	0.040	0.053	0.112
Water	0.0162	0.0133	0.0107	0.0086	0.0061	0.0049	0.0047	0.0045
Ratio	6.6	6.4	6.8	7.2	8.0	8.2	11.3	25.0

This shows that within the physiological limits of temperature the viscosity of egg white is proportional to that of water. It is very probable that the viscosity of egg white and other aqueous solutions of protein is directly derived from that of water in the following way. The protein molecules are continually capturing and releasing their neighbors through their latent valencies, so that the water molecules are partly confined in meshes of a protein framework which is always breaking and always mending itself. The framework has both the properties of a solid and those of a fluid. In itself it is somewhat viscous, but during its displacements the confining action of the protein framework causes the water molecules to exercise powerful frictional forces on one another. It amounts to this, that the viscosity of mud is derived from that of water and must vary proportionally with that of water during alterations of temperature. A mud made with ethyl oxide under

similar conditions would be less viscous than a mud made with water, in the proportion of the viscosity of ethyl oxide to that of water. A small allowance would have to be made for the direct grinding action of the solid particles of the mud on one another. These considerations, then, justify us in writing $K = c\eta$, where c is a constant for a given nerve substance and η is the viscosity of water. So we have arrived at the very simple result that the velocity of nerve impulse V as measured under the usual conditions of physiological experiments is given by the formula

$$V = Ce^{-cs_m\eta} \dots \dots (7)$$

This has now to be tested by the experimental data, especially those of Snyder and Maxwell. They themselves accentuate the large variations in the values of V found under seemingly identical physical conditions. I would attribute these chiefly to large unknown variations in the electrical conditions of stimulation, especially at the contact of nerve and electrode. On the physical theory of nerve impulse we regard the electricity as producing a displacement of the electrical charges in the protein molecule with an associated elastic strain similar to that constituting the main phenomenon in piezoelectricity. As the inflow of electricity has to control the strain of the nerve from the electrode down to the muscle and has to produce at the muscle a certain minimum displacement, it is plain that the conditions are eminently favorable to the production of erratic results on account of uncertainty in the control of the electrical conditions at the electrodes. It is possible that in Weiss's experiments making velocity of nerve impulse nearly independent of temperature the uncertainty gave rise to a systematic error. With the large erratic variations occurring in Snyder's and Maxwell's individual measurements the risk of considerable systematic error must be remembered. But in the absence of experimental tracing of the erratic uncertainties to their source, which is probably a difficult electrical investigation, Snyder and Maxwell have kept the probable erratic error of their averages down by making a large number of determinations. The method which I have used for handling their large mass of data is the following. In the case of Snyder's measurements all the observations on one specimen of frog's motor nerve are recorded on squared paper with temperature for abscissa and V for ordinate. Amongst these points the best average curve is drawn for the range

of temperature 0° to 30° . The ordinates at intervals of 5° on this curve give the best obtainable smoothed values of V at these regularly spaced temperatures. Then we write (7) in the form

$$\log_e V = \log_e C - c_s m \eta \quad \text{or} \quad \log_{10} V = \log_{10} C - 0.4343 \cdot c_s m \eta \quad \dots \quad (8)$$

which states that the logarithm of the smoothed V is to be linear in η , the viscosity of water at the same temperature. The values of η at the regular temperatures are the following taken from Thorpe and Rodger⁶ to the nearest third or second effective digit:

Temp.	0°	5°	10°	15°	20°	25°	30°	35°
$10^6 \eta$. . .	178	151	130	113	100	89	80	72

From Snyder's experiments of November 9, 1907, we get for V in metres per second

$$\log_{10} V = 2.65 - 113 \eta \quad \dots \quad (9)$$

with the following comparison:

Temp.	0°	5°	10°	15°	20°	25°	30°
V smoothed exper. .	5	9	14	21	30.5	45	65
V calcul. by (9) . .	4.4	8.5	15.1	23.4	33.1	44.7	56.2

The experiments of November 13 yield the same curve and similar results. Those of December 18 furnish the equation

$$\log_{10} V = 2.07 - 75 \eta \quad \dots \quad (10)$$

and the comparison

Temp.	0°	5°	10°	15°	20°	25°	30°
V smoothed exper. .	6.0	9.0	12.0	15.5	20.0	25.0	32.5
V calcul. by (10) . .	5.5	8.5	12.6	16.6	20.9	25.1	29.5

The experiments of December 23 are represented by the same curve as those of November 9 and 13, but the results obtained with the second preparation of that date yield the slightly different equation

$$\log_{10} V = 2.65 - 122 \eta \quad \dots \quad (11)$$

giving the following comparison:

Temp.	0°	5°	10°	15°	20°	25°	30°
V smoothed exper. .	2.5	6.4	11.2	17.0	23	30.0	(40)
V calculated by (11) .	2.7	5.6	10.5	16.6	24	33.1	(43)

⁶ THORPE and RODGER: Philosophical transactions, 1894, clxxxv A, p. 449.

In the first two comparisons it will be seen that the calculated values of V fall below the smoothed experimental at the lowest and the highest temperature, while in the third comparison the calculated exceed the experimental at these extreme temperatures. On the whole, then, the theoretical result agrees with experiment in a satisfactory manner, the selected experiments being typical of the whole of Snyder's measurements. He quotes some determinations of Nicolai on a sensory nerve, the olfactory, of the pike. These, upon treatment in the manner explained above, give the equation

$$\log_{10} V = 1.94 - 67 \eta \dots \dots (12)$$

with the comparison

Temp.	0°	5°	10°	15°	20°	25°
V smoothed exper. . .	5.5	8.5	11.6	15.3	18.5	22.0
V calculated by (12) . .	5.6	8.3	11.7	15.1	18.6	21.9

Here the agreement is far better than one would expect possible on *a priori* grounds, because in theorizing at all we are forced to make so many simplifying assumptions to render the complex physiological conditions tractable. There seems to be little doubt as to the truth of the main theoretical conclusion, that the velocity of nerve impulse is one damped by the viscosity of the water in the nerve. It is interesting to turn to the experiments of S. S. Maxwell upon the pedal nerves of the giant slug. Here the physical difficulties of the measurements are further complicated by the tendency to spontaneous rhythmical contraction. At temperatures near 12° Maxwell found velocities ranging from 0.18 to 0.43 metres per second, the average for his 43 sets of experiments being 0.31, say 0.28 at 10°. We can avoid the labor of smoothing his experimental results at other temperatures by adopting his conclusion that a rise of 10° multiplies the velocity by 1.78 on the average. Hence we may summarize all his work in the smoothed experimental values of V given below which lead to the equation

$$\log_{10} V = 0.335 - 65 \eta \dots \dots (13)$$

and the comparison

Temp.	0°	10°	20°
V smoothed experimental . .	0.157	0.28	0.50
V calculated by (13)	0.150	0.31	0.48

The most interesting point about the numbers in equations (9) to (13) is that the coefficient of η has the value 65 in a motor nerve of the giant slug, 67 in a sensory nerve of the pike, 75 in one specimen of a frog's motor nerve, and 113 and 122 in other specimens. The total range in these values is not great; so we may conclude that the viscous resistance offered by the water in these various nerves to the shear of nerve impulse in the protein framework is rather nearly the same, the tendency in these limited data being to show a smaller coefficient of η , and therefore a smaller resistance, the softer and more watery the nerve becomes. This is a result that the physical theory leads us to expect. Next, as to the values of the first number on the right-hand side of these equations, for example, 2.65 in (9). By (8) we see that this is $\log_{10} C$, so that $C = 447$ metres per second in certain specimens of motor nerve in frog. Equation (10) gives only 117 metres per second for another specimen, while (11) gives 398. In the olfactory of the pike C by (12) is 87 metres per second, and by (13) in the pedal of the giant slug it is only 2.2 metres per second. These are all velocities with which nerve impulse would be found to travel if measured under conditions making viscosity inoperative, that is, with stimuli much smaller than those required to produce the responses used in the usual experiments. These undamped velocities are the ones discussed in my previous papers as calculable from the rigidity and density of nerve. It was shown that the rigidity determined by Wertheim in the human sciatic implies a velocity of 220 metres per second for a shear in such nerve, while another value of the rigidity implies 59 metres per second. Here we have magnitudes of the same order as those of the values of C found above for frog's motor nerve and pike's sensory. The small value of C in the giant slug's pedal nerve points to a small rigidity in that nerve. The small nerve velocities, such as 8 metres per second in electrical fish, 4 in octopus, 0.01 in anodon, and 0.001 in eledon, are affected by unknown amounts of damping as well as by small values of C due to low rigidity in the nerve.

These physical principles lead to a possible elucidation of the advantage of a high temperature to warm-blooded animals. It is obvious that high speed of nervous signalling in an animal would be an element of fitness for surviving. The speed of the nerve impulse is small compared with that of light and even of sound.

The lowness of nerve velocity is due to the fact that the nerve impulse is an electrical displacement in the protein molecule associated with a material displacement as well. If it were an almost purely electrical affair, it would travel with the speed of light. But the inertia and the material connections of the protein molecule bring the velocity down to one of the order C just found. And then the viscosity of the fluid medium in which the protein molecule is displaced causes this velocity to be still further reduced to $Ce^{-csm\eta}$. Suppose, now, that certain limits are placed upon the constitution of the protein molecule, as for instance a temperature above which it cannot be raised without coagulation. Then the higher the temperature of a nerve, short of its feeling the incipient effects of approaching coagulation, the quicker will a nerve impulse travel along it, because of the increase of $Ce^{-csm\eta}$ through the diminution of η caused by rise of temperature. The warm-blooded animals seem to have pushed their temperature up as high as it can safely be carried without undue risk of coagulation, in order that the velocity of nerve impulse may be the highest possible. It is true that a rise of temperature reduces also the degradation of muscular energy into heat through the viscosity of the muscle substance, but the losses of heat by radiation and convection due to high temperature are so great that the warm-blooded animal, on the whole, suffers large loss of energy on account of its high temperature. It seems, then, as if the warm-blooded animal found the advantage of the quickest obtainable nervous signalling worth purchasing at the cost of the large amount of energy wasted on account of a high body temperature. In the animal body economy of fuel is a consideration secondary to nervous efficiency. It would be interesting to ascertain whether birds have the highest nerve velocity on account of their highest temperature, and whether also the temperature of coagulation of their most coagulable proteins is raised above that for other warm-blooded animals in order to leave them a similar margin of safety. It is probable that mild fever increases the nerve velocity in an animal, but that nearer approach to the temperature of coagulation of nerve protein causes the nerve velocity to diminish.

Concerning the chemical theory which has been advanced to explain the large variation of nerve velocity with temperature, the following criticism is offered. The argument of that theory is this: the velocity of many chemical reactions is doubled by a rise of 10°

at ordinary temperature, the velocity of nerve impulse is about doubled by a rise of 10° , therefore it is very probable that nerve impulse is transmitted by a chemical process. As this argument stands, it involves the assumption of a direct relation between a physical velocity and what is metaphorically called a chemical velocity. The danger of confusion from these two uses of the term "velocity" is pointed out in some text-books of physical chemistry. In order that the chemical theory of nerve impulse may prove helpful it must show in some reasonable way how the velocity of propagation of a chemical reaction along a nerve can be proportional to the metaphorically so-called velocity of reaction at any point in the nerve. Now, the chemical velocity of reaction is the number of gram-molecules of substance per cm^3 undergoing some definite chemical change in a second. If this change is such that its occurrence at one place tends to promote its occurrence at adjacent places, then the velocity of propagation of the reaction may be a function of the velocity of reaction, but will depend on many other conditions. Some very special set of conditions would have to exist to make the velocity of propagation proportional to the velocity of reaction. Until the chemical theory supplies this missing premise, it is almost meaningless. Nevertheless to its latest advocates we owe the valuable experimental contributions which I have sought to interpret in the present extension of my previous physical theory.

SUMMARY.

Small nerve impulses are propagated with the velocity C of a mechanical shear in nerve substance. But with those impulses which must reach a certain minimum strength in order to produce the observed response there is a special damping of this velocity to the smaller value $Ce^{-cs_m\eta}$ in which c is a constant characteristic of different samples of nerve, s_m is the minimum shear eliciting the observed response, and η is the viscosity of water. This viscosity is the only one of these quantities which varies markedly with temperature, and so the variation of the velocity of nerve impulse with temperature is traced entirely to the variation of the viscosity of water with temperature. This simple conclusion is fully borne out by the experimental results of Nicolai, Snyder, and S. S. Maxwell, which thus strengthen and extend the physical theory of nerve impulse.

MELBOURNE, August, 1908.

POSTSCRIPT.

Immediately after the above was finished and posted Professor Osborne of Melbourne drew my attention to the experimental work of Lucas and that of Woolley described in the "Journal of Physiology" for June 30 which had just arrived.⁷ This should be discussed in connection with the foregoing theory. That of Lucas relates to the temperature coefficient of the rate of conduction in the sciatic, tibial, and sural nerves in large specimens of *Rana esculenta* by a method which aims at making the electrical conditions of excitation more uniform than usual. Nevertheless he still encounters marked irregularities amongst individual measurements of nerve velocity, the most important being indicated by his result that in one group of experiments the velocity at 18.5° is 1.92 times that at 8.5°, and in another 1.64 times. The average outcome is that he finds 16.3 metres per second for the velocity at 8.5° and 28.6 at 18.5°. With the viscosity of water as 0.0136 and 0.0104 at these temperatures, (8) takes the form

$$\log_{10} V = 2.2465 - 76 \eta \dots \dots (14)$$

Here the coefficient of η is 76, almost identical with that in (10) derived from Snyder's data. The value 2.2465 for $\log_{10} C$ gives 176 metres per second for the value of C , which falls within the limits of Snyder's separate results.

The paper of Woolley treats experimentally the temperature coefficient of the rate of conduction and of the latent period in muscle. This is a different subject from that discussed above, but on account of its obvious relation to it I shall briefly discuss the applicability of the form (8) to the experiments of Woolley, who has already smoothed out his experimental results in two curves which give times in an arbitrary unit. In the conduction experiments these are the times taken at different temperatures by the contraction wave in a frog's sartorius to travel a fixed unspecified length. Call these times t and the unknown length x , then $V = x/t$ and we may write $C = x/T$, so that (8) becomes

$$-\log_{10} t = -\log_{10} C - .4343 c s^m \eta \dots \dots (15)$$

Applying this to Woolley's curve for conduction in muscle, we get

$$-\log_{10} t = -1.107 - 79 \eta \dots \dots (16)$$

⁷ LUCAS and WOOLLEY: Journal of physiology, 1908, xxxvii, pp. 112, 122.

with the comparison

Temp.	5°	10°	15°	20°
t smoothed experimental . . .	194	138	100	77.0
t calculated by (16) . . .	198	135	99	78.7

Here we have a satisfactory indication that a similar theory applies to muscle. It is noteworthy that γ_9 , the coefficient of η , is nearly the same as that found in nerve. Woolley quotes from Burdon Sanderson the results for the velocity of propagation of contraction in frog's sartorius 3.2 metres per second at 17° and 1.4 at 7°. These give, with 0.0142 and 0.0107 for the viscosity η ,

$$\log_{10} V = 1.607 - 103\eta \quad (17)$$

in which, again, the coefficient of η , namely, 103, has the same sort of value as before, while 1.607 for $\log_{10} C$ denotes the value 40 metres per second for C , the velocity with which muscular contraction would travel along the sartorius of the frog in the absence of viscous resistance.

Of course there is no reason to expect that (15) should apply to Woolley's measurements of the latent period of frog's muscle at different temperatures, because the latent period does not present itself simply as a time spent in the propagation of a disturbance through some as yet unknown region. But it occurred to me to try whether some constant time added to the latent periods at different temperatures would make them proportional to the time taken by the muscular contraction at these temperatures to travel the fixed unknown length in Woolley's experiments. I have found that this is so, the constant time being 28.4 in the arbitrary units of Woolley's curves, from which the following data are taken. Let us call the latent period τ , and the time of conduction of contraction t ; then the last row of the table gives the ratio $t (\tau + 28.4)$.

Temp.	5°	10°	15°	20°
t	194.0	138.0	100.0	77.0
$\tau + 28.4$	114.4	81.4	59.4	45.4
Ratio	1.696	1.695	1.684	1.696

Here we have an indication of a simple relation between latent period of muscle and the velocity of propagation of contraction along muscle. It would be interesting to work out the absolute value of this constant time which is measured in arbitrary units by the number 28.4, but it might lead too far from the present subject.

A COMPARATIVE STUDY OF THE VASOMOTOR REFLEXES.¹

BY W. T. PORTER AND R. RICHARDSON.

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

I.

THE functions of living tissues may be divided into groups, which may be termed fundamental and accessory. The respiration, reproduction, and the maintenance of the body temperature are fundamental; skilled movements are accessory. Existence depends on the first group, civilization on the second. The fundamental functions are of ancient origin; the accessory functions are comparatively recent. The fundamental functions are in the main alike in all the higher animals; the accessory functions differ widely in different species.

We bring in this paper evidence that the blood pressure is to be classed with the fundamental phenomena. We find that the reflex rise of blood pressure upon stimulation of the sciatic and the brachial nerves is quantitatively almost the same in the rabbit, cat, guinea pig, rat, and hen,² — animals differing greatly in structure. It is improbable that animals so diverse as those here stimulated would give quantitatively the same response were not the maintenance of blood pressure, like the maintenance of body heat, a fundamental phenomenon developed to the same high efficiency in many, perhaps in all, warm-blooded animals.

II.

The rise in blood pressure was produced by stimuli of the same intensity applied to the sciatic and the brachial nerves of the rabbit,

¹ A preliminary account of this investigation was given in a Harvey lecture, delivered Nov. 17, 1906; published in the Boston medical and surgical journal, 1908, clviii, pp. 73-79, and The Harvey lectures, 1906-07, pp. 98-116. J. B. Lippincott Co.

² Observations were also made upon the dog; these will be mentioned separately.

cat, guinea pig, rat, hen, and dog. The animals were etherized. A mercury manometer was connected with the carotid artery or, in the case of the hen, with the femoral artery. Just enough curare to prevent muscular reflexes was injected through a vein.³ The nerves were then stimulated with induction currents of uniform intensity and the consequent rise in blood pressure recorded with the kymograph. The figures thus obtained give the absolute rise in blood pressure upon stimulation of an afferent nerve.

At this point it was necessary to adopt the principle of percentile values. The absolute change in blood pressure obtained at one level cannot be compared directly with that obtained at a different level. For example, in one series the stimulation of the sciatic nerve in the rabbit, while the blood pressure was 100 mm. Hg, caused a rise of 35 mm. Hg; and when the blood pressure was 50 mm., a stimulus of equal intensity still caused a rise of 35 mm. The absolute change was the same in both, but in the first instance the change was 35 per cent, while in the second it was 70 per cent. It is necessary, then, in measuring vasomotor reflexes to take into account the level of the blood pressure at the beginning of stimulation, and this is done by expressing the change in blood pressure as a percentage of this level.

To the percentile values obtained in the present experiments were added records of brachial and sciatic stimulation obtained from the rabbit, cat, and dog in previous investigations.⁴ The total material available was as follows:

Animal.	Sciatic records.	Brachial records.
Rabbit	101	37
Cat	165	167
Guinea pig	31	33
Rat	25	22
Hen	40	25
Dog	30	45
Total	<u>392</u>	<u>329</u>

In order to diminish the influence of accidental errors, the observations were treated in groups. Tables I to VI present the arithmetical mean of all observations recorded when the blood pressure

³ The technique of these experiments is described in this journal, 1907, xx, p. 399.

⁴ W. T. PORTER: This journal, 1907, xx, p. 401.

at the beginning of stimulation was between 11 and 20, 21 and 30, 31 and 40 mm. Hg, and thus by increments of 10 up to 160 mm. Hg. There are obvious difficulties in this method. Thus in Table II, in the group in which the blood pressure in the cat at the beginning

TABLE I.

THE ABSOLUTE AND PERCENTILE RISE IN THE BLOOD PRESSURE OF THE RABBIT ON STIMULATING THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure before stimulation.	Number of observations.		Absolute rise on stimulation.		Percentile rise on stimulation.	
	Sciatic.	Brachial.	Sciatic.	Brachial.	Sciatic.	Brachial.
mm.Hg. 111-120	2	4	mm.Hg. 39	mm.Hg. 36	per cent. 34	per cent. 31
101-110	5	3	38	41	36	39
91-100	8	6	43	40	45	42
81-90	10	3	37	39	44	46
71-80	13	5	39	39	52	52
61-70	17	7	33	37	50	57
51-60	12	6	31	27	56	49
41-50	20	3	34	29	75	64
31-40
21-30	11	..	15	..	60	..
11-20	3	..	4	..	27	..
Average percentile rise in rabbit					47.9	47.5

of stimulation was from 71 to 80 mm., the percentile rise in stimulation of the sciatic nerve was 77 per cent, which is clearly too high. On inquiry, it appears that in three of the observations the blood pressure at the beginning of stimulation was 80 mm., the highest possible figure in this group, and in a fourth instance the beginning blood pressure was 78 mm. As the absolute and therefore the percentile rise was calculated from 75 mm., midway in the group, the average absolute and percentile values were raised by this occurrence of four observations at the highest levels. Notwithstanding

these difficulties, the method seems the best that could be used for the purpose in hand. It must, however, always be borne in mind that the compensation of accidental errors requires first of all a

TABLE II.

THE ABSOLUTE AND PERCENTILE RISE IN THE BLOOD PRESSURE OF THE CAT ON STIMULATING THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure before stimulation.	Number of observations.		Absolute rise on stimulation.		Percentile rise on stimulation.	
	Sciatic.	Brachial.	Sciatic.	Brachial.	Sciatic.	Brachial.
mm.Hg. 151-160	3	4	mm.Hg. 44	mm.Hg. 44	per cent	per cent. 28
141-150	3	5	41	40	28	28
131-140	8	3	38	38	28	28
121-130	10	10	46	56	37	45
111-120	10	10	54	45	47	39
101-110	7	9	41	43	39	41
91-100	7	4	45	52	47	55
81-90	7	5	42	29	50	34
71-80	7	6	58	48	77	64
61-70	6	10	39	48	60	73
51-60	17	19	34	31	62	56
41-50	16	10	29	20	64	44
31-40	18	17	18	15	51	43
21-30	32	31	10	9	40	36
11-20	14	24	7	10	47	67
Average percentile rise in cat					47.0	45.4

sufficient number of observations. In all, 721 records were used in the present investigation, a number requiring much labor, but it is to be regretted that still more were not available. Nevertheless, the uniformity of the results obtained with this, statistically speaking, scanty material is surprising.

III.

The percentile rise of blood pressure following the stimulation of the sciatic and the brachial nerves in the rabbit, cat, guinea pig, rat, and hen may be summarized as follows:

Animal.	Sciatic per cent.	Brachial
Rabbit	47.9	47.5
Cat	47.0	45.4
Guinea pig	40.2	41.5
Rat	54.6	45.2
Hen	46.2	49.6
Average	47.2	43.8

The results with the dog (Table VI) were not so favorable, the sciatic percentile rise being 30 and the brachial 24.8 per cent. We ascribe this chiefly if not wholly to a difference in the effect of curare upon the dog as compared with the other animals employed. In the

TABLE III.

THE ABSOLUTE AND THE PERCENTILE RISE IN THE BLOOD PRESSURE OF THE GUINEA PIG ON STIMULATING THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure before stimulation.	Number of observations.		Absolute rise on stimulation.		Percentile rise on stimulation.	
	Sciatic.	Brachial.	Sciatic.	Brachial.	Sciatic.	Brachial.
mm. Hg. 81-90	3	..	mm. Hg. 24	..	per cent. 28	..
71-80	7	7	27	27	36	36
61-70
51-60	..	2	..	29	..	52
41-50	4	4	19	14	42	31
31-40	4	4	14	20	40	57
21-30	9	11	12	10	48	40
11-20	4	5	7	5	47	33
Average percentile rise in the guinea-pig. . .					40.2	41.5

TABLE IV.

THE ABSOLUTE AND PERCENTILE CHANGE IN THE BLOOD PRESSURE OF THE RAT ON STIMULATING THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure before stimulation.	Number of observations.		Absolute rise on stimulation.		Percentile rise on stimulation.	
	Sciatic.	Brachial.	Sciatic.	Brachial.	Sciatic.	Brachial.
mm.Hg. 71-80	5	4	mm.Hg. 28	mm.Hg. 18	per cent. 35	per cent. 24
61-70	3	3	22	15	33	22
51-60	6	4	35	26	58	49
41-50	6	6	34	24	72	60
31-40	5	5	31	30	75	71
Average percentile rise in the rat					54.6	45.2

TABLE V.

THE ABSOLUTE AND PERCENTILE CHANGE IN THE BLOOD PRESSURE OF THE HEN ON STIMULATING THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure before stimulation.	Number of stimulations.		Absolute rise on stimulation.		Percentile rise on stimulation.	
	Sciatic.	Brachial.	Sciatic.	Brachial.	Sciatic.	Brachial.
mm.Hg. 111-120	3	mm.Hg. 39	mm.Hg.	per cent. 34	per cent.
101-110	9	4	40	24	38	23
91-100	5	2	45	38	47	40
81-90	8	7	40	24	47	28
71-80	8	7	38	27	51	36
61-70	7	5	39	47	60	71
Average percentile rise in the hen					46.2	39.6

face of the reactions obtained from the five other animals we do not believe the lower values observed in the dog impair the demonstra-

TABLE VI.

THE ABSOLUTE AND PERCENTILE RISE IN THE BLOOD PRESSURE OF THE DOG ON STIMULATING THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure before stimulation.	Number of observations.		Absolute rise on stimulation.		Percentile rise on stimulation.	
	Sciatic.	Brachial.	Sciatic.	Brachial.	Sciatic.	Brachial.
mm. Hg. 151-160	..	5	mm Hg. ..	mm Hg. 19	per cent. ..	per cent. 12
141-150	6	7	24	23	17	16
131-140	7	13	37	20	27	17
121-130	6	6	34	27	27	22
111-120	2	4	23	20	20	17
101-110
91-100	2	2	29	35	30	37
81-90	2	..	30	..	35	..
71-80	..	2	..	30	..	40
61-70
51-60	..	2	..	8	..	14
41-50	2	..	15	..	33	..
31-40	3	4	10	12	29	48
21-30	2	13	..	52	..
Average percentile rise in the dog					30	24.8

tion that animals widely differing in structure are alike in this important function.

IV.

It is characteristic of the fundamental phenomena that they are little disturbed by afferent impulses. They are long practised against the swarms of stimuli that strike upon the periphery. The respira-

tory rhythm goes on without significant variation, and the balance between heat production and heat loss is maintained in spite of all vicissitudes. The accessory functions, on the contrary, have not the poise of countless generations, nor can they so readily defend themselves against the afferent stream, which now rises against them as Scamander rose against Achilles. For we have entered a new environment. Compared with the ages that have gone in the development of function, civilization is of yesterday. Stimuli in our urban life have increased until they drive against us like a storm. At the same moment, the effect of these stimuli has been heightened by the growth in reflex irritability which has followed the habitual use of lately discovered poisons, the housing of skins made for the open air, and all the unfavorable influences of our crude life in towns. The accessory functions resist these onslaughts with difficulty. Already, physicians are concerned over the increasing frailty of the higher nervous system, to cite but one example. It is therefore a matter of importance to determine the developmental position of all functions. The present investigation makes it probable that vasomotor functions belong with those we have termed fundamental, and we should therefore expect to find them little affected by the increasing complexity of our environment. This view is supported by the fact that the prolonged stimulation of afferent nerves does not produce a significant fall in blood pressure.⁵

The present demonstration is interesting from another point of view. Experimentation on man is properly very limited. Human conditions make it usually impossible to subdivide a general problem like the vasomotor relations into those simple constituents which alone can be made the subject of exact experimentation. To secure the necessary factorial simplicity, the investigator must inevitably choose the higher animals for all or almost all the exact measurements that he requires. The measurements made on any one species, for example, the rabbit, will primarily be useful as regards that particular animal, but it does not follow that they will be applicable to man and other animals. To apply to man the blood pressure data obtained from the rabbit or from any other species, it must first be shown that the phenomena in question are quantitatively the same in all the higher animals, or at least the limits of variation must be quantitatively determined. It will then be possible to fix the degree

⁵ PORTER, MARKS, and SWIFT: This journal, 1907, xx, pp. 444-449.

of probability that man will follow the law proved true for other animals. The present research shows that the vasomotor reflexes are broadly identical in the rabbit, cat, guinea pig, rat, and hen. The difference in structure between the cat and the hen, for example, is greater than the difference between the cat and man. We may therefore justly conclude that the vasomotor reactions in man are essentially like those in other high vertebrates.

IS THE ANESTHESIA AND MOTOR PARALYSIS CAUSED BY MAGNESIUM SALTS DUE TO ASPHYXIA?¹

BY S. J. MELTZER AND JOHN AUER.

[From the Department of Physiology and Pharmacology of the Rockefeller Institute
for Medical Research.]

IN our studies upon the effects of magnesium salts² we assumed that their action was exercised directly upon the nervous system, causing temporary sensory and motor depression (inhibition) in anesthetic doses, and respiratory paralysis with its consequences in larger doses. In a recent article in this journal, entitled "Control of Spasms by Asphyxiation," A. H. Ryan and C. C. Guthrie³ criticize adversely our theory and offer instead the view that relaxation and anesthesia are only the secondary effects of asphyxiation. The authors are so convinced of the plausibility of their view that they critically say that the factor of asphyxiation "should be constantly borne in mind in interpreting the results obtained by the injections of such solutions," assuming, accordingly, that the offering of another theory for our facts was simply due to our oversight of the factor of asphyxiation. Ryan and Guthrie extend their criticism also to the statement of S. A. Matthews,⁴ that "the convulsions produced in tetanus could be inhibited by the intravenous injection of a salt solution in which calcium was present," and to the findings of Mac-

¹ A reply to A. H. RYAN and C. C. GUTHRIE.

² MELTZER and AUER: This journal, 1905, xiv, p. 366; 1906, xv, p. 387.

³ RYAN and GUTHRIE: This journal, 1908, xxii, p. 440.

⁴ S. A. MATTHEWS: Journal of the American Medical Association, 1903, xli, p. 565. The solution which MATTHEWS used in a case of tetanus was composed as follows: Sodium chloride 7.3, sodium sulphate 10.0, sodium citrate 3.36, calcium chloride 0.13 to 1000 water. The small amount of calcium chloride in this solution was evidently converted into insoluble calcium sulphate (or citrate) and became inert. Why MATTHEWS (This journal, 1905, xii, p. 174) and others should ascribe any inhibitory effects to the calcium content of this solution is unintelligible to us.

Callum and Voegtlin,⁵ who "inhibited tetany caused by the removal of the parathyroids by the injection of a solution of calcium chloride."

In the first part of their paper the authors deal with the abolition of strychnin spasms by exposure of animals to CO₂. It is therefore evident that, when the authors speak of the factor of asphyxiation as the primary cause, they mean the asphyxiation which is produced by the presence of an excess of CO₂ within the blood.

Before bringing forward some old and new facts in support of our theory we shall look first into the validity of the statements of Ryan and Guthrie, which served them as a basis for their adverse criticism. At first glance it would appear that they have fortified their position by experimental observations and by *a priori* logical evidence. Let us first examine the weight of their experimental proof. They made one experiment, the protocol of which they give. A sheep, showing symptoms of tetanus (whose weight was only estimated), died of respiratory paralysis twenty-five minutes after a subcutaneous injection of a 25 per cent solution of MgSO₄, the dose of the salt being equal (estimated) to about 1.25 gm. per kilo body weight. Five minutes before death the muscles were well relaxed and the tongue was blue. Since, in the strychnin experiments, the tetanic symptoms were inhibited by CO₂, and since this animal had a blue tongue and died of respiratory paralysis, it became probable to Ryan and Guthrie that the primary cause of these effects was CO₂, asphyxiation.

The authors made only one experiment, and it would certainly be an inadequate evidence, even if that experiment were capable of proving the author's contention. However, the authors did not need to make even this one experiment, since we ourselves made numerous experiments of that kind. We have repeatedly stated that certain doses of the magnesium salts are fatal, the doses varying with the species of the animals. We have experimented upon seven species; sheep were not included among these, but cats died with doses smaller than 1.25 gm. per kilo body weight. But one experiment or many — in which way can they serve as evidence that our theory is wrong? Our assumption is that the salts paralyze the centre of respiration; the animals will then die from respiratory paralysis, and it is tacitly understood that there will be cyanosis shortly before death. The point of contention is whether the centre of respiration

⁵ MACCALLUM and VOEGTLIN: Johns Hopkins Bulletin, 1908, p. 1.

is affected primarily by the salt or by CO_2 ; in either way symptoms of asphyxia will set in. The presence of these symptoms can therefore not decide the question as to the primary cause.

Briefly, then, Ryan and Guthrie made only one experiment, which contained nothing new, but was simply a repetition of our own numerous experiments; but above all an experiment which is entirely incapable of deciding the question whether the primary effect of the salt is upon the centre of respiration or upon the blood.

In further support of their view Ryan and Guthrie bring forward an argument which appears to them to be very convincing, and which we shall restate in their own words: "On the basis of 1.5 gm. per kilo of body weight (the approximate anesthetic dose in our experiments) the concentration of the magnesium salt in the blood would be about 5 per cent. But assuming that only half of it were absorbed, it is conceivable that even then the character of the blood would be altered to such an extent that the normal process of respiration would be interfered with." By alterations of the blood the authors apparently mean an increase of the CO_2 content of the blood. We should first call attention to the fact that the authors do not adduce any physical, chemical, or physiological facts in support of the assumption that blood which contains 5 per cent of magnesium salt will retain an excess of CO_2 . They simply state: it is conceivable. The mere conceivableness of an idea does not yet raise it to the dignity of even an hypothesis, not to speak of a well-founded theory.

Furthermore, a miscalculation crept into the above quoted statement: 1.5 gm. per kilo body weight makes the concentration of the magnesium salt within the blood only 3 and not 5 per cent, calculating the blood even as low as 5 per cent of body weight.

The most surprising statement, however, is the assumption of Ryan and Guthrie, that the whole or half of the quantity of the salt solution which was injected subcutaneously could be present within the blood at any time. Those who have made subcutaneous injections ought to know that it takes hours before the solutions disappear from the subcutaneous tissues. On the other hand, it is an established fact that crystalloid solutions, leave the blood very rapidly into the lymph spaces and through the kidneys, so that, even when injected directly into the circulation, at the end of the injection there is very little change in specific gravity or osmotic pressure of the blood (Sherrington and Copeman, Leathes, Lazarus-Barlow and others).

The truth of the matter is that after subcutaneous injections only a very small fraction of the injected fluid is present within the blood at any one time. As to the subcutaneous injections of magnesium salts, we may definitely state from our experience that in the experiments in which the animals were in deep anesthesia and recovered spontaneously, the concentration of the magnesium salt within the blood could not be even one third of 1 per cent. In our experiments with intravenous injections of magnesium sulphate in rabbits we found that, when 1 c.c. of a 25 per cent solution was injected in twenty seconds, the respiration stopped before the injection was finished, and it required continuous artificial respiration for half an hour before spontaneous respiration returned. This means that 0.25 of $MgSO_4$ in 75 c.c. of blood (rabbits of 1500 gm.) stops the respiration completely; and we have to bear in mind besides that at the end of twenty seconds a part of the salt had left the blood again and that, furthermore, the respiration stopped long before the injection was finished. It is therefore evident that in the experiments in which subcutaneous injections of $MgSO_4$ lead to a profound but only temporary anesthesia, the concentration of this salt within the blood must be very dilute indeed.

We may therefore state that the argument of Ryan and Guthrie is based upon erroneous premises, and, like their experiment, carries no weight either in support of their view or in justification of their adverse criticism of our theory.

We shall now show the reason why we did not accept the hypothesis that asphyxiation is the primary cause of the anesthetic and paralyzing effects of magnesium salts, uncovering at the same time the chief source of the error of Ryan and Guthrie.

These authors discuss in their paper only the paralyzing action of CO_2 , entirely overlooking the fact that in asphyxia the paralytic stage is *preceded by a state of excitation*. They were led into this error probably by the fact that the animals which they were studying in their present investigation were already in a state of excitement, convulsions from strychnine, etc., before they were subjected to the influence of CO_2 . In asphyxia of mammals the exciting stage is the dominating feature; it is manifested by rapid and labored respiration, by the rise of blood pressure, and frequently also by the presence of convulsions. One of the authors (Guthrie⁶) in

⁶ GUTHRIE and PIKE: This journal, 1906, xvi, p. 475; 1907-1908, xx, 452.

agreement with Bienfait and Hogge⁷ insisted that the increase of CO₂ within the blood leads to an increase in the rate of respiration. In the above-mentioned protocol of the experiment with the subcutaneous injection of MgSO₄ the authors report that the respirations gradually came down to 48 per minute. Did they ever notice an increase in rate under the influence of magnesium salts?

In our various papers we have repeatedly stated that the chief reason for our assumption that the magnesium salts exert an inhibitory influence upon the nervous system was the complete absence of phenomena of excitation. We may give here the following quotation from one of our papers:⁸ "*Under no circumstances did the injection of magnesium salts ever cause an increase of the respirations in depth and frequency. In other words, magnesium salts do not excite the respiratory function; on the contrary, if present in the blood in sufficient quantity, they are capable of rapidly and completely inhibiting all the excitation phenomena of asphyxia.*" If Ryan and Guthrie had read our papers with some care, they would have found sufficient evidence that we did not overlook the factor of asphyxia.

However it was only for the fatal doses of magnesium salts that we needed calling attention to the fact that the asphyxia was incomplete and that just the dominating sign of mammalian asphyxia, the characteristic excitation, was absent. For the anesthetic effect of magnesium salts the reason for assuming that it was not due to asphyxia was simply this, that in most of the cases *not one symptom of asphyxia was present during anesthesia*. Besides, in some protocols we have stated expressly that *the blood was bright red*. There was no excitation, no dilated pupils, no cyanosis, — on what basis could it then be assumed that the anesthesia was a secondary effect of asphyxiation?

For further elucidation of our position we shall append here an abbreviated protocol of an experiment with a fatal dose in which the independence of anesthesia and paralysis from asphyxiation is clearly demonstrated.

Gray male rabbit, 1629 gm. Ether, tracheotomy; recovered from ether and removed from the board.

2.10. Injected subcutaneously 16.2 c.c. MgSO₄ in M/1 solution = 2.25 gm. per kilo. Animal lively.

⁷ BIENFAIT and HOGGE: Archives de biologie, 1890, x, p. 139.

⁸ MELTZER and AUER: This journal, 1905-1906, xv, p. 393. Italics in the original.

- 2.25. Animal quiet, does not move.
- 2.35. Can be placed on side, respiration 48 per minute, lid reflex reduced, feels pinch of tail; *mucous membranes pink color.*
- 2.45. Animal can be placed in any position, lid reflex slight, no reaction on pinching tail; 36 respirations to the minute; *mucous membranes still pink.*
- 2.47. Started artificial respiration, 48 per minute.
- 3.00. No lid reflex, no sign of motion on pinching any part of the body; *mucous membranes pink.*
- 3.05. Stopped artificial respiration. No spontaneous respiration, *mucous membranes become bluish*; started artificial respiration again.
- 3.15. No lid reflex, no reaction whatsoever to pinching, heart beat strong 100 per minute; *mucous membranes pink.* Artificial respiration continued for 32 minutes longer, condition remained the same.
- 3.47. Artificial respiration stopped. After a few minutes very slight, hardly visible movements of the abdomen appeared, *pupils became dilated, mucous membranes became bluish*; *no convulsions*; heart became gradually slower and then stopped.

This animal received a large, surely fatal dose of the magnesium salt, and it would have been surely dead twenty-seven minutes after the injection if it had not been for the artificial respiration which was then instituted. *For one hour, while the artificial respiration continued, the mucous membranes remained pink, the pupils were of normal size and the heart continued to beat strongly, although the animal was completely anesthetized and paralyzed.* As soon as the artificial respiration was discontinued, cyanosis appeared, the pupils became dilated, and the animal died without convulsions. Evidently the prolonged anesthesia and paralysis were due to some other primary cause than asphyxiation, and the partial asphyxia (cyanosis and dilatation of the pupils) which made their appearance after stopping the artificial respiration were only secondary phenomena due to paralysis of respiration.

Similar experiments were made in which artificial respiration was carried on for many hours, until the animal finally recovered its spontaneous respiration and thus survived the effect of a surely fatal dose. During these long hours the animals are completely paralyzed and anesthetized; the pupils, however, retain their normal width. But as soon as the artificial respiration is discontinued for a few minutes, the pupils become temporarily very wide, indicating the onset of asphyxia.

To recapitulate briefly: with anesthetic doses not a single sign of asphyxia is present; the blood is bright red, the mucous membranes are pink, and the pupils are of normal size. Also after fatal doses of magnesium salts the dominating feature of asphyxia, excitation, is absent, and by artificial respiration any and every sign of asphyxia can be completely removed without changing the anesthesia, the relaxation of the muscles, and the abolition of reflexes. It is therefore evident that asphyxiation is not the primary cause of the anesthesia, muscular relaxation, and abolition of reflexes which follow the injection of efficient doses of magnesium salts.

We may be permitted to add a few words with regard to the contents of the first part of the paper by Ryan and Guthrie. The experiments from which the positive conclusion was drawn that CO₂ abolishes strychnine convulsions consisted in observations upon one cat and two frogs. This is surely an unpermissibly small number of experiments to serve as a basis for a scientific statement. The conclusions, however, so far as the effect of carbon dioxide is concerned, are probably nevertheless correct, because *exactly similar experiments were made a few years ago by H. Winterstein*.⁹ Against the statement, however, that oxygen favors the development of the effects of strychnine (two frogs) stand the experiments of Osterwald¹⁰ and of Czyhlartz,¹¹ who state that they have suppressed the effects of strychnine by the inhalation of oxygen, and also the numerous experiments by many authors on the suppression of strychnine convulsions by artificial respiration,¹² which many investigators consider as due to the effect of oxygen.

⁹ H. WINTERSTEIN: Archiv für Anatomie und Physiologie, 1900, Suppl. Band, p. 177.

¹⁰ OSTERWALD: Archiv für experimentelle Pathologie und Therapie, 1899, xlv, p. 451.

¹¹ CZYHLARTZ: Zeitschrift für Heilkunde, 1901, p. 160.

¹² See GIES and MELTZER: This journal, 1903, ix, p. 1.

ON THE DIASTASES IN THE BLOOD AND THE BODY FLUIDS.

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THE observations of Carlson and Ryan¹ on the diastatic ferment in the saliva of cats and dogs led to the conclusion that the salivary glands of these animals do not "manufacture" any ptyalin, but that the diastase or the diastases found in their saliva are simply the blood and lymph diastases passed into the saliva from the blood and lymph along with other constituents. Their results also showed that the diastases in the cat's saliva become more concentrated during ether anæsthesia. The reason for this difference was not investigated by them. It may be due to change in the gland activity by the ether, or it may be due to an increase in the concentration of the diastases in the blood and lymph. This raises the question of the source or sources of the blood and lymph diastases.

The fact that the salivary glands of dogs and cats do not produce ptyalin renders impossible any adaptation of the ptyalin-producing

¹ CARLSON and RYAN: This journal, 1908, xxii, p. 1.

function of the salivary glands to the diet in these animals, as reported by Nielson and Terry for the dog.² The possibility remains, however, that a carbohydrate diet may increase the concentration of the blood and lymph diastases, in which case there may be an actual increase of the salivary diastases, owing to their greater concentration in the blood and lymph.

The aim of the present experiments was the solution of these two questions. The source of the blood and lymph diastases is not a virgin field, but our results seem to contradict some of the recent findings of other observers. Our experiments seem to show that the concentration of the blood and serum diastases cannot be varied by variation in the diet, the health, and general conditions of the animal remaining the same. Moreover, the blood and lymph diastase is neither amylopsin nor ptyalin absorbed from the alimentary canal, but in all probability "by-products" of tissue metabolism of no essential importance in the body economy, because in some animals they are practically absent.

I. LITERATURE.

The presence of starch-splitting ferments in the blood and lymph has been known since the early researches of Magendie and of Claude Bernard. The more important recent contributions on the subject are those of Bial, Röhmann, Hamburger, Borchardt, Ascoli and Bonfanti, and Schlesinger. In his first studies Bial³ arrived at the conclusion that the blood diastase differs from ptyalin and pancreatic amylopsin in that the former splits starch to dextrose, while the action of the latter ferments stop at the maltose stage. Hamburger⁴ showed that this conclusion is erroneous, dextrose being one of the end products of the digestive action of all three ferments.

Röhmann and Bial⁵ showed that the thoracic lymph usually contains less diastase than the serum, and that intravenous action of peptone increases the contraction of the diastase in the lymph. The experiments of Hamburger led him to the conclusion that the blood and lymph contain at least two ferments acting on carbohydrates, —

² NIELSON and TERRY: This journal, 1906, v, p. 406.

³ BIAL: *Archiv für die gesammte Physiologie*, 1892, lii, p. 137.

⁴ HAMBURGER: *Archiv für die gesammte Physiologie*, 1896, lx, p. 543.

⁵ RÖHMANN and BIAL: *Archiv für die gesammte Physiologie*, 1894, lv, p. 469.

a diastase splitting starch into dextrine and maltose and another ferment (glucase) splitting these to dextrose. This view is supported by Borchardt.⁶ Ascoli and Bonfanti⁷ go still further in this direction. According to these observers the blood and lymph diastases of different animals act with varying rapidity on different starches, which would indicate that we are not dealing with a single ferment, but with a number of them varying in structure in different animals.

Borchardt showed that the diastatic properties of the blood and lymph ferments are identical with those of the ferments extracted from the liver and other organs. He concludes that the blood diastases are simply the organ diastases passed into the blood and lymph.

Bainbridge and Beddard⁸ record an almost complete absence of the blood diastases in two diabetic patients, while other diabetic patients exhibit no diminution in the concentration of the blood diastases. They also record that in pancreatectomy in cats (Case 1) there is no disappearance of the blood diastases.

The most recent observations on the subject are those of Schlesinger.⁹ This observer ligatured the duct of Wirsung and found that this procedure increased the concentration of the diastase in the blood. He also extirpated the pancreas in two dogs and one cat, and reports that this operation is followed by complete disappearance of the diastase from the blood. Hence he concludes that the blood diastase is the pancreatic amylopsin absorbed from the pancreas or from the intestines. Schlesinger also found that the concentration of the diastase in dog serum is eight to ten times greater than that in the rabbit serum. He found that ox serum and human serum were less active than rabbit serum.

II. EXPERIMENTAL METHODS.

The concentration of the diastases in the serum and lymph was measured by (1) the rate of clearing and (2) by the rate of complete disappearance of erythrodextrin in the starch solution. The

⁶ BORCHARDT: *Archiv für die gesammte Physiologie*, 1904, c, p. 259.

⁷ ASCOLI and BONFANTI: *Zeitschrift für physiologische Chemie*, 1904, xliii, p. 156.

⁸ BAINBRIDGE and BEDDARD: *Biochemical journal*, 1907, ii, p. 89.

⁹ SCHLESINGER: *Deutsche medizinische Wochenschrift*, 1908, no. 14.

starch solution and serum or lymph were usually mixed in the proportion of 5 c.c. to 0.1 c.c. and kept in thermostat at 38° C. In a few series on the pigeon's serum, which has a very strong diastatic action, the test tubes were kept at room temperature. Three to five duplicates were run on each sample of serum or lymph, so as to exclude accidental errors in measurements, etc.

The serum or lymph was added to all the samples of boiled starch practically simultaneously, and the starch tubes shaken for the same length of time and with the same intensity. That our procedure did not introduce any material error is shown by the fact that the rate of clearing and of disappearance of the erythrodextrin in the three or five duplicate tests made of each sample was practically the same.

It occurred to us that the manner and rapidity of coagulation of the blood and lymph might cause variations in the concentrations of the diastases. It is well known that the digestive ferments are carried down with, or clinging to, precipitates in the medium. The proteolytic ferments of the blood and lymph cling to fibrin. The same may be true for the diastases. In that case we reasoned that the serum secured by centrifugalizing defibrinated blood ought to contain less diastase than the serum pressed out of a clot not agitated, as the latter would present a greater absorption surface. To test this hypothesis, three series of comparisons with serum from the same animal (3 dogs) were made, one sample being defibrinated by whipping and then centrifugalized, the other sample being allowed to clot undisturbed, and the serum pressed out by the contraction of the clot. In all three series the serum pressed from the clot had a slightly stronger diastatic action than the sample obtained by defibrination and centrifuging. The difference was not great, but it was constant. The cause of this difference is not obvious. It is known that the erythrocytes contain no diastases. The leucocytes and possibly the blood plates contain diastase. Extensive solutions of the leucocytes may thus produce a difference in the percentage of the diastase, but it is difficult to see why a greater number of the leucocytes should break down in the undisturbed clot than in the whipped blood. On the other hand, it is not obvious why more of the diastatic ferments should adhere to the fibrin when removed by whipping than when allowed to form spontaneously throughout the whole blood. But, as just stated, the difference is slight. Hence variations in the manner and rate of the coagulation do not constitute material errors.

Securing the blood and lymph. — It is altogether probable that the blood and lymph from different organs contain varying concentrations of the diastases. It is therefore to be regretted that in the experiments on the comparative side of the question absolute uniformity in the method of securing the blood could not be maintained. All the samples of pig, ox, sheep, rat, and pigeon blood were arterial blood. The same is true of many samples of the dog, cat, and rabbit blood. Some of the goat blood samples were arterial, and some were had by bleeding from a cut in the ear. All the chicken blood was from bleeding the combs. The blood from the cats, dogs, and rabbits used in a series of tests, was secured from the ear or from a cut in the tail.

III. THE SOURCE OF THE DIASTASES IN THE BLOOD.

According to the data in the literature already referred to, this is still an open question. The blood and lymph diastases may come from the tissues in general (including the leucocytes), or they may be salivary and pancreatic ferments absorbed from the digestive tract, or both of these sources may contribute. The literature is conflicting, Bainbridge and Beddard finding diastase in pancreatectomized animals, while Schlesinger states that pancreatectomy in cats and dogs removes the diastases from the body fluids.

I. *The relative concentration of the diastases in the blood, the lymph, and the other body fluids.* — The experiments under this head were made on 5 dogs and 2 cats. It is known, since the work of Röhmman and Bial, that the lymph from the thoracic duct contains less diastase than the serum of the same animal. This point we can confirm; the thoracic lymph from normal dogs and cats collected under ether anæsthesia has a weaker diastatic action than the serum. The difference is not very great. In our two experiments on cats the difference appeared less than in the case of the dogs.

Again, the lymph collected from the neck lymphatics has a weaker diastatic power than the thoracic lymph. This difference between neck lymph and thoracic lymph, again, is less marked in the cat than in the dog, but our series is not extensive enough to establish the point.

The neck lymph and the lymph collected from the lymphatics of the forelimb exhibit practically the same concentration of the dias-

tases. In the dog the other body fluids tested were the pericardial and the cerebro-spinal fluids. The diastatic power of the pericardial fluid (normal dog) is considerably less than that of the neck lymph, while the cerebro-spinal fluid has the least of all. In fact, the cerebro-spinal fluid added to starch in the proportion of 1 c.c. to 5 c.c. and kept at 38° C. clears the solution only after ten to twenty hours, and some starch remains unchanged for at least three days. It is therefore obvious that only traces of diastases are found in the cerebro-spinal fluid.

The various body fluids, then, exhibit the following descending series in the concentration of the diastases: serum, thoracic lymph, neck lymph = lymph from limb, pericardial fluid, cerebro-spinal fluid. This series is identical with that shown by the concentration of the hemolysin and bacterio-agglutinins.¹⁰ It is, furthermore, evident that the order of these fluids based on concentration of their proteins would normally be the same.

But these data give no definite answer to the question of the source of the diastases. The diastases of the lymph pass into the blood, and some are eliminated into the urine. The diastases may pass back again to the lymph through the walls of the blood and the lymph capillaries. In fact, that would be the only way to account for the presence of the diastases in the neck lymph and the lymph from the extremities, if Schlesinger is right in his contention that the blood diastase is simply resorbed amylopsin. On the other hand, if these diastases are the products of the tissues in general (excluding the leucocytes), the greater concentration in the blood may involve several factors. They may be poured into the blood slightly faster than they are eliminated in the urine, or destroyed in some other manner. They may pass directly into the blood capillaries as well as into the lymph. Or, assuming that the leucocytes contribute to the diastase content, there may be a greater destruction of the leucocytes in the blood than in the other body fluids. It is well known that the leucocytes are practically absent from the cerebro-spinal fluid.

2. *The effect of lymphagogues on the concentration of the diastases in blood and lymph.* — Röhmann and Bial found that intravenous injection of peptone increases the concentration of diastase in the thoracic lymph, while the concentration in the serum remains

¹⁰ HUGHES and CARLSON: This journal, 1908, xxi, p. 236; BRAUDE and CARLSON: *Ibid.*, p. 221.

unchanged. Our five experiments (dog) on this point did not give uniform results, as will be seen by Table I. In three of the experiments the peptone injection was followed by an increase in the diastatic power of the thoracic lymph, and in two of them a slight increase in that of the serum; in the other experiment the normal

TABLE I
THE EFFECTS OF INTRAVENOUS INJECTION OF PEPTONE ON THE DIASTATIC POWER OF SERUM AND LYMPH.

No. of experiment.		Serum.	Thoracic lymph.	Neck lymph.
1	Normal	>	>
	35' after peptone	∧	∧	same
2	Normal	>	>
	20' after peptone	same	∧	same
	30' after peptone	same	same	same
	60' after peptone	same	same	same
3	Normal	>	>
	40' after peptone	∧	∧	same
4	Normal	>	>
	20' after peptone	same	same	same
5	Normal	∧	>
	20' after peptone	∨	same	same

serum and the peptone serum exhibited the same diastatic power. In the remaining two experiments one exhibited no difference in the diastatic action after the peptone injection, while the other showed a slight diminution in the serum and no change in the lymph.

The peptone injections have no effect on the diastatic power of the neck lymph. The results on this point are uniform. Our results on the thoracic lymph seem to parallel the findings of Hughes and Carlson and Braude and Carlson on the effect of peptone injections on the concentration of hemolysins and bacterio-agglutinins in the lymph. The lymphagogues of the first class usually do, but sometimes do not, increase the lysins in the thoracic lymph.

The actual reason for the occasional failure of peptone to increase

the diastase in the thoracic lymph will probably not be determined till we know definitely what part each organ plays in contributing to the diastases of the body fluids. We know that the peptones act on the liver cells, but it is not certain that the greater amount of the diastase in thoracic lymph usually following peptone injection is due to an increased rate of liberation of the liver diastases into the lymph. In no case did the diastatic power of the lymph exceed that of the serum simultaneously collected, although the peptone thoracic lymph may exceed in diastatic the normal serum.

3. *The relative concentration of the diastases in the portal and the hepatic blood.*—The conclusion of Borchardt that the diastases of the blood and lymph are identical with those of the liver suggests that the blood and lymph diastases may come mainly from the liver. On the other hand, if Schlesinger is correct, we ought to find the greatest concentration of the diastases in the portal or the mesenteric veins, for it is reasonable to suppose that these ferments would follow the course of the proteins and the carbohydrates rather than the fats in absorption. If the liver is the main source of the diastases, we ought to find a greater concentration of them in the hepatic than in the portal blood, provided the liver diastases are not liberated into the lymph and reach the blood by way of the thoracic duct. The usual effect of peptone on the concentration of the diastases in the thoracic lymph also suggests the liver as an important source of the ferments.

Our own results on this point were negative. We made parallel tests on hepatic and portal (superior mesenteric) serum on 3 dogs and 2 cats. In two cases the hepatic serum was a little stronger, in the remaining three cases the portal serum had the stronger action. But the differences were not marked in either case. Our results suggest that the liver may under certain conditions take up diastases from the blood.

It is plain, however, that these negative experiments do not eliminate the liver as an important source of the blood and lymph diastases, as the ferments from the liver may reach the blood by the way of the thoracic duct. It is also possible that they may reach the blood directly under certain conditions of the blood and lymph capillaries.

The concentration of the diastases in the blood and the lymph from different organs require further study.

4. *The effect of stimulating the central end of the vagi on the concentration of the blood diastases.*—On this point we have only

the results of two experiments to report. It is well known that stimulation of sensory nerves, particularly the central end of the vagi, leads to the liberation of the glycogen of the liver into the blood in the form of sugar. It seemed possible that if the liver is an important source of the blood and lymph diastases such increased glycolytic activity of the liver might involve an increased output of the liver diastases into the blood or lymph. In one experiment the vagi were stimulated intermittently for fifteen minutes, in the other for thirty minutes. In both cases the serum secured after the stimulation had a slightly greater diastatic action than the normal serum. But the difference is very slight and by no means proportional to the hyperglycemia produced by such stimulation. These results do not justify any definite conclusion regarding the relation of the liver to the blood and lymph diastases. They merely suggest further work.

5. *The effect of extirpation of the pancreas on the concentration of the diastases in the blood.*—The meagre literature on this point is conflicting, as we have seen. Bainbridge and Beddard (Experiment 1) conclude that the pancreatectomy does not eliminate the blood diastases, while Schlesinger reports the very opposite results.

Our own results on 2 cats are given in the following protocols:

- I. Cat, pancreas removed April 26, 1908.
 - April 27. Animal in good condition; diabetic.
 - April 30. Serum of diabetic cat slightly less active than normal serum (1 animal).
 - May 2. Serum of diabetic cat slightly less active than normal (2 animals).
 - May 4. Serum of diabetic cat = slightly greater action than normal (1 animal).
 - May 5. Serum of diabetic cat same as normal serum (1 animal).
(Experiment discontinued because of secondary infection. Post-mortem examination showed all of pancreas removed.)
- II. Cat, pancreas removed May 12, 1908.
 - May 13. Animal in good condition. Diabetic.
 - May 14. Diabetic serum = normal serum (1 animal).
 - May 19. Diabetic serum = normal serum (2 animals).
 - May 22. Diabetic serum = normal serum (2 animals).
 - May 23. Cat died. (Post-mortem showed all pancreas tissue removed).

Our results are confirmatory of those of Bainbridge and Beddard. We are at loss to account for the contrary results reported by

Schlesinger. That the pancreatic ferment should be resorbed into the blood on ligation of the duct of Wirsung is not surprising. But this proves nothing as to the source of the blood diastases under normal conditions, with the pancreatic duct patent. Even an absence of the blood diastases after pancreatectomy would not prove that the pancreas is their chief or only source, as such an absence might be due to the changes in the activities in the tissues in general following the loss of the pancreas.

The work of Carlson and Ryan on cats' salivary glands renders it highly probable that the salivary glands in that animal do not "manufacture" ptyalin. With the pancreas removed, therefore, the only explanation of the continued presence of the ferments in the blood is that they come from some other organ or from the tissues in general. This conclusion is in harmony with the fact that the tissue diastases persist in pancreatectomized animals. Unless those ferments have other sources besides the pancreas, they could not persist in the blood in normal concentration for at least nine days after removal of the pancreas, inasmuch as they are constantly being eliminated in the urine.

The resorption of either ptyalin or amylopsin from any part of the alimentary canal under normal conditions remains to be demonstrated.

6. *The effect of anæsthesia on the concentration of the blood diastases.* — These experiments were made in seeking an explanation for the fact reported by Carlson and Ryan that cats' saliva secreted under general anæsthesia contains more diastase than does the normal saliva. Inasmuch as the diastase in the cat's saliva is simply eliminated from the blood, and as the greater the concentration of the diastases in the blood the greater their concentration in the saliva, the inference is obvious that the anæsthesia may in some way increase the concentration of the blood diastases and thus cause the greater concentration of them in the saliva. The other alternative is that the anæsthesia alters in some way the activity of the salivary glands themselves, and brings about the increased concentration. If there is an actual increase in the concentration of the blood and lymph diastases during anæsthesia, that may be caused either by increased production or by a diminished rate of elimination or destruction of the ferments.

In cats ether and chloroform anæsthesia invariably produces hyperglycemia, presumably by acting on the liver directly or indirectly.

Bearing in mind the liver as a possibly main source of the diastases, an increase in the blood diastases during anæsthesia in the cat might, then, be due to the same cause that leads to the liberation of the liver glycogen.

Our experiments were made on 4 cats and 1 rabbit. The blood was drawn from the ear or the tail after twenty to one hundred and twenty minutes of ether anæsthesia, and its diastatic power compared with that of a sample of blood drawn from the same animal prior to the anæsthesia. In every case we found the serum after a period of ether anæsthesia slightly less active than the serum drawn just before the anæsthesia. The difference was constant, but not any greater than the individual variations exhibited by sera from normal animals.

It occurred to us that an actual increase in the diastases during the period of anæsthesia might be obscured by the retarding action on the ferment action by the anæsthetic in the serum. Accordingly we added ether in varying quantities to pigeon serum *in vitro* to test this point. Ether has no retarding action on the diastatic ferments of the pigeon's blood, at least in concentration up to 2 drops of ether to 2 c.c. of the serum (+ 5 c.c. starch solution). Greater concentrations were not tried.

We are therefore forced to the conclusion that there is a slight diminution in the concentration of the blood diastases during ether anæsthesia. Whether this is brought about by a decreased production of, or an increased destruction or elimination of, the ferments, remains an open question. In the only test made we found that the urine after the period of anæsthesia had a stronger diastatic action than that collected prior to the anæsthesia. It is possible that both factors are involved, or we may actually have an increased elimination of diastases into the blood in one organ (*e. g.*, the liver) coupled with a greater destruction or elimination of the ferments in other organs.

A further conclusion is this, that the increase in the concentration in the salivary diastases during anæsthesia is not due to this increased concentration in the blood. It must therefore be due to a change in the activity of the gland itself, — a change leading to an increased elimination of the ferments *pari passu* with a diminution of them in the blood and serum.

IV. THE RELATION OF DIET TO THE CONCENTRATION OF THE
DIASTASES IN BLOOD AND LYMPH

The answer to this question was sought by two lines of inquiry, namely, (1) by comparing the diastatic power of a number of carnivorous and herbivorous animals; (2) by studying the effects on the blood diastases of varying the diet of the same animal. If the blood diastase is pancreatic amylopsin resorbed from the alimentary tract, it would seem natural to look for more of the ferment in the blood of herbivora than in the blood of carnivora, and again we might reasonably look for an increase in a carnivorous animal when kept on a vegetable diet. This does not necessarily follow from our premises, however, as there may be regulative mechanisms in the body by means of which the blood diastases are maintained at a fairly constant concentration, despite variations in rate of production. But we have just shown that the pancreas bears no relation to the blood diastases under normal conditions, and that in all probability neither ptyalin nor amylopsin is under normal conditions resorbed from the alimentary tract. This aspect of the question may therefore be dismissed without further discussion.

If the blood and lymph diastases are of any significance in connection with the metabolism of the carbohydrates, we might reasonably look for a greater amount of them in animals who by nature or under experimental conditions handle a greater quantity of carbohydrate food, although, even in this case, regulative mechanisms may obscure actual quantitative differences in the rate of production and liberation of the diastases by the tissues.

1. *The concentration of the diastases in the blood and lymph of different animals.* — Schlesinger states that the concentration of the diastases in the blood of the same species remains constant, and that different species exhibit constant differences. The blood of the animals investigated by him showed the following descending series: dog, ox, rabbit, man.

We cannot confirm Schlesinger's first point. Any one who makes a sufficiently large number of tests can readily convince himself that animals of the same species kept so far as possible under identical conditions will exhibit variation in the diastatic power of the blood and lymph. The variations are usually not very great, but they may occasionally be considerable. In the majority of cases, however,

the blood and lymph of different individuals of the same species exhibit nearly the same power of diastatic action. And we are therefore justified in speaking of this degree of diastatic power as typical of the species.

Our own investigations comprised comparative tests on samples of blood from 20 cats, 6 kittens, 10 dogs, 8 rabbits, 6 pigeons, 10 chickens, 3 goats, 7 pigs, 4 sheep, and 4 oxen. In all 15 series of tests were made. The results may be illustrated by the following summary of the records. The number of animals of each species used in the series is indicated by the number within the parenthesis after the name of the species. For example, in Series XII the blood of 4 cats was checked against that of 4 pigs. Five identical test-tube mixtures were made for each sample, so that in this series the digestion in 20 tubes containing dog's serum was checked against 20 tubes containing the same quantity of pig serum.

- Series I. Cat (1) > rabbit (1).
- Series II. Cat (2) > rabbit (2).
- Series III. Pigeon (2) (much) > cat (2) = dog (1) > rabbit (1).
- Series IV. Pigeon (2) (much) > rabbit (2) (slightly) > cat (1) > dog.
- Series V. Cat (1) (slightly) > kitten (1).
- Series VI. Cat (2) (slightly) > kitten (1).
- Series VII. Pigeon (2) (much) > dog (4) (slightly) > rabbit (2).
- Series VIII. Dog (3) > chicken (4).
- Series IX. Cat (1) (slightly) > dog (1) (much) > chicken (6).
- Series X. Cat (1) > kitten (2).
- Series XI. Cat (1) = kitten (1).
- Series XII. Cat (4) = pig (4) (much) > goat (1).
- Series XIII. Cat (2) = pig (3) (much) > sheep (4) slightly < ox (4).
- Series XIV. Cat (2) (very much) > goat (2) (no action in goat serum after 24 hours).
- Series XV. Cat (1) > kitten (1).

Of the animals investigated the pigeon and the goat sera occupy the opposite extremes in diastatic power, the sera of the other animals occupying intermediate positions, without any relation to the nature of the food of the species. The diastatic power of the pigeon serum approaches that of the parotid saliva in some of the herbivora. The goat serum, on the other hand, has practically no action on boiled starch. Mixed with boiled starch in the proportion of 1 c.c. to 5 c.c. of starch and kept at 38° C., all the starch is not

changed into the soluble form even in fifteen to twenty-four hours. The diastatic power of sheep serum is not much greater than that of the goat serum. The carnivorous species (dog, cat) have a greater concentration of the blood and lymph diastases than the herbivorous species (goat, sheep, ox, rabbit). The greatest concentration is reached, however, in the pigeon, a grain-feeder.

It occurred to us that the great concentration of the diastases in the pigeon blood might be correlated with the high temperature and great rate of oxidation of birds in general, assuming that these ferments are of importance in metabolism. But the fact that the chick serum has a relatively small quantity of these ferments seems to render this correlation untenable. The rate of oxidation is also greater in the kitten than in the adult cat, yet there is less diastase in the blood of the former than in that of the latter. We had planned to extend our observations to species of birds of small size, in which the rate of oxidation is much greater than in the pigeon, but sufficient material was not available.

While it is necessary to obtain data on a greater number of species than that included in the present work, all the facts so far at hand go to show that *there is no correlation between the concentration of the diastases in the serum and the lymph, and the character of the natural diet of a species.* While this fact does not disprove the theory that pancreatic amylopsin enters the blood and then becomes one of the blood diastases, it seems to us to constitute a serious objection to that view. There is certainly as much amylopsin secreted in twenty-four hours by the goat pancreas as by the cat pancreas, yet the serum of the cat has strong diastatic power, while the serum of the goat has practically none.

This great variation in the diastatic power of the sera and lymph of different species, and the practical absence of the ferments in some species also suggest that these ferments are by-products, as it were, of organ metabolism, and when they reach the blood and lymph they are on the road to destruction and elimination and play no further essential part in the body economy. How else are we to account for the fact that the goat without the ferment lives its normal life, to all appearances, just as perfectly as the dog or the pigeon? The urine constitutes, in all probability, one channel of elimination of these diastases. It would be interesting to know whether there is a direct parallel between the concentration of the blood and the urine diastases in different species.

2. *Can the concentration of the blood and lymph diastases be effected by experimental variations in the nature of the diet?*—The preceding section leads us to look for negative results in this series of experiments. Such was the case. Placing a flesh-eating animal on a diet of bread or an herbivorous animal on a diet of meat does not alter the concentration of the diastases in their sera or lymph.

Our experiments included three series, — one with cats, one with dogs, and one with chickens.

SERIES I. CATS.

Three animals fed exclusively on meat and three other specimens fed exclusively on bread moistened with milk. The bread-feeding cats were in addition fed once every day for four consecutive days with a mixture of boiled starch and glucose (20 per cent), introduced by means of a stomach tube. On the second, third, and fourth days of this forced carbohydrate feeding the diastatic power of their sera was compared to that of the sera of the meat-fed cats. The differences in the diastatic power exhibited by these sera were those of the normal individual variations of the species. As an illustrative example, we may cite the results of the comparisons between that of the cat A (meat) and D (bread). First test day: A (slightly) > D; second day, A = D; third day, A (slightly) > D. On the third day, while one of the bread-feeding cats (D) exhibited a slightly less digestion than that of one of the meat-fed cats (A), the digestive power of the same bread-fed specimens was on that day the same as that of the two other meat-fed cats. These are individual variations and have no reference to the nature of the diet.

It may be objected that the bread diet was not persisted in long enough to produce results. That is possible. But at our hands the cats did not do well on a purely vegetable diet, and the forced feeding of starch and sugar through the stomach tube upset their digestion. We therefore abandoned the cat and turned our attention to the dog and the chicken.

SERIES II. DOGS.

Two dogs fed on meat exclusively for four weeks, and 2 other dogs of approximately the same size and age fed on bread, fat, and

a little milk for the same length of time. Comparative tests of the diastatic power of the sera were made as follows: first week, one test; second week, two tests; third week, four tests; fourth week, four tests. The results of these eleven tests were the same as those on the cats. The sera of the "bread dog" showed no greater and no less diastatic power than the sera from the "meat dog." The variations were slight and shifted equally on either side of the mean. *Hence, after four weeks of greatly increased carbohydrate consumption in the case of the dog, there is no increase in the concentration of the blood and lymph diastases.*

SERIES III. CHICKENS.

Six chickens were selected of approximately the same size and age. Three of these were put on an exclusive grain and bread diet, the other three on a meat diet. The meat was for the most part boiled and ground up. Sand and water in abundance were supplied to both groups. This diet was kept up for three weeks. During this time several parallel tests were made of the diastatic power of the sera, the animals being bled from the comb. The results showed no diminution of the diastases in the sera of the meat-fed chickens. The concentration of the blood diastases remained practically identical in the two series.

It is therefore evident that in birds (chicken) and mammals (cat, dog) the change from a meat diet to a mainly carbohydrate diet does not alter the concentration of the blood diastases for at least four weeks. This is additional evidence that the blood and lymph diastases have no direct relation to the pancreas, and take no essential part in the metabolism of the carbohydrates in the body.

Following the views of Hamburger, Borchardt, and Ascoli and Bonfanti, we have spoken of the blood diastase in the plural. We have observed incidentally, in comparing different sera, that the rate of clearing the starch solution may not be proportional to the rate of complete disappearance of the erythrodextrin. This indicates that in some sera there are diastatic substances whose action is stopped or checked when the starch is rendered soluble. Indeed, all the data so far at hand point to the conclusion that there are a number of substances in the blood and the other body fluids capable of initiating or augmenting the hydrolysis of starch.

V. SUMMARY.

1. The body fluids exhibit normally the following descending series in the concentration of the diastases: serum, thoracic lymph, neck lymph = lymph from limb, pericardial fluid, cerebro-spinal fluid.

2. Intravenous injection of commercial peptone usually increases the concentration of the diastases in the thoracic lymph. It does not affect the concentration of the diastases in neck lymph. The peptone injections may or may not be followed by an increase in the diastases in the serum.

3. There is no constant difference in the concentration of the diastases in the portal and the hepatic serum.

4. Stimulation of the central end of the vagi may cause a slight increase in the concentration of the blood diastases.

5. Pancreatectomy does not affect the concentration of the blood and lymph diastases.

6. Anæsthesia appears to cause a slight decrease in the blood diastases.

7. There is no correlation between the concentration of the blood diastases and the relative abundance of the carbohydrates in the natural diet of a species.

8. Experimental transfer from a meat diet to a diet composed mainly of starchy food stuffs, or *vice versa*, does not alter the concentration of the blood and lymph diastases.

9. There appears to be no correlation between the concentration of the blood diastases and rate of oxidation in the body.

10. All the facts so far at hand seem to point to the conclusion that the blood and lymph diastases are "discards" of the tissues in general. When once in the body fluids, these substances are in all probability on the road to destruction or elimination, and serve no further essential end in the body economy, at least so far as regards their starch-splitting power.

THE COURSE OF THE CONTRACTION WAVE IN THE STOMACH OF THE RABBIT.

By JOHN AUER.

[From the Department of Physiology and Pharmacology of the Rockefeller Institute.]

IT seems to be generally accepted that the entire course of a contraction wave of the stomach is simply peristaltic, and the tacit assumption is made that its character differs in no essential point from a peristaltic wave in any other part of the gut. For example, Rossbach,¹ Cannon,² Roux and Balthazard,³ all state that the gastric wave of contraction sweeps peristaltically from the middle of the stomach to the pylorus; according to them a gastric wave is peristaltic from start to finish. On the other hand, earlier investigators, Beaumont,⁴ Hofmeister and Schütz,⁵ describe a markedly different contraction course of a gastric wave. As the description of the gastric motor phenomena given by Hofmeister and Schütz tallies in all essentials with that of Beaumont, I shall quote only the results of the former. Hofmeister and Schütz observed the excised, bloodless stomach of dogs in a moist chamber. They found that the first sign of a wave shows itself a few centimetres from the cardia, either as a flattening of the greater curvature or as a more or less marked groove. This contraction progresses peristaltically, increasing in strength until a point about two centimetres from the antrum is reached, and here a deep, preantral constriction is formed. After the formation of this preantral constriction the sphincter antri contracts, giving an hourglass shape to the stomach for a short time. Now the preantral constriction relaxes, while the sphincter antri contracts maximally, separating the antrum from the rest of the stomach cavity. At

¹ ROSSBACH: Deutsches Archiv für klinische Medizin, 1890, xlvi, p. 316.

² CANNON: This journal, 1898, i, p. 367.

³ ROUX et BALTHAZARD: Archives de physiologie, 5 serie, 1898, x, p. 88.

⁴ BEAUMONT: Physiology of digestion, 1847.

⁵ HOFMEISTER and SCHÜTZ: Archiv für experimentelle Pathologie und Pharmakologie, 1886, xx, p. 8.

the height of the antral sphincter contraction the musculature of the antrum contracts as a whole, or first a strong shortening occurs, followed by a contraction of the circular fibres.

From the foregoing description it will be seen that the gastric wave is peristaltic only to the antrum; as soon as the antrum is reached, this structure contracts, not peristaltically, but usually as a whole. To emphasize this combination of peristalsis and non-peristalsis in a gastric wave, Hofmeister and Schütz proposed the term "peristole."⁶

According to the earlier investigators, therefore, a gastric wave is an orderly succession of peristalsis and non-peristalsis, — peristalsis over the middle portion of the stomach, and a non-peristaltic, general contraction of the antrum. According to the later observers, a gastric wave is entirely peristaltic. In order to explain this difference, Cannon⁷ suggests that abnormal conditions in the experiments of Beaumont and of Hofmeister and Schütz might be responsible for the discrepancy: Beaumont inserted a thermometer bulb into the stomach of Alexis St. Martin, and this might well have irritated the sensitive gastric mucosa, producing abnormal motor activities of the viscus; Hofmeister and Schütz used the excised, bloodless stomachs of dogs, working thus under obviously pathological conditions. On the other hand, Cannon and Roux and Balthazard worked under much more normal conditions, for they examined the stomach by means of Röntgen rays. But this method also has its drawbacks; in order to observe the stomach, food mixed with bismuth must be ingested, and when the stomach is now examined with the fluoroscope the stomach content is seen as a shadow on the screen. From the change in shape and density of this shadow the movements of the stomach are inferred, for, as Cannon has emphatically pointed out,⁸ this method does not show the stomach musculature, but only the shadow of its contents. On the whole, however, there can be no question that Cannon's method is more normal than that employed by Beaumont and by Hofmeister and Schütz, and his results, therefore, are entitled to more weight.

From the preceding short *résumé* it will be seen that the evidence, critically considered, was in favor of the view that the contraction

⁶ HOFMEISTER and SCHÜTZ: *Loc. cit.*, pp. 9-10.

⁷ CANNON: *Loc. cit.*, pp. 367-368, 374-375.

⁸ CANNON: *Loc. cit.*, p. 364.

wave of the stomach was entirely peristaltic, especially as Cannon⁹ found the same type of gastric movement in cat, dog, rabbit, guinea-pig and rat, and Balthazard and Roux¹⁰ in man, dog, and frog. It is true that Moritz,¹¹ experimenting on human subjects, and Ducceschi,¹² working with dogs, came to conclusions similar to those reached by Beaumont and by Hofmeister and Schütz, but both of these investigators used balloon-tipped sounds to study gastric motility. Moritz introduced the sound through the mouth and œsophagus into various parts of the stomach, and Ducceschi introduced registering balloons into the stomach of dogs through fistulæ which had been established at different levels. The results obtained by these methods are, however, difficult to interpret, and cannot be compared with the comparatively straightforward evidence furnished by procedures which permit, without any operative interference, direct observation of the stomach or its contents. From these considerations it will be seen that the evidence brought forward by Moritz and Ducceschi in favor of the view of Beaumont and of Hofmeister and Schütz is not sufficient to modify the statement of Cannon, Roux and Balthazard. Such evidence will, however, be submitted in this paper; it will be shown that gastric movements in the rabbit under normal conditions are similar in all essentials to those of the dog as described by Hofmeister and Schütz.

Methods. — The first method employed to study gastric movement was mere inspection of the rabbit's abdomen, after clipping the hair of this area. No operative measures whatsoever are necessary, for the rabbit's stomach can easily be observed through its thin abdominal walls. Full-grown animals should be used, for in them the stomach is larger and lies lower in the peritoneal cavity than in young rabbits; moreover, only in full-grown animals will the beginning of the antrum be visible, and if this is not visible, an erroneous impression regarding the course of a gastric wave will be received. The entire antrum is visible only exceptionally with this method. The animals used for observation should be fed for one or two hours before the experiment in order that the stomach be well distended. By means of this method, as I have pointed out in an earlier

⁹ CANNON: This journal, 1902-1903, viii, p. xxii.

¹⁰ ROUX et BALTHAZARD: *Loc. cit.*, p. 89.

¹¹ MORITZ: *Zeitschrift für Biologie*, 1895, xxxii, p. 362.

¹² DUCCESCHI: *Archivio per le scienze medicale*, 1897, No. 2 (quoted from Roux et Balthazard, *Loc. cit.*, p. 90).

paper,¹³ a large part of a gastric wave may be observed under practically normal conditions of the animal. The antrum, however, is not visible usually with this inspection method, and the functions of this important structure are shown only indirectly by the effect which an antral contraction exerts upon the preantrum. In order to observe the antrum directly, another method must be employed.

Opening of the abdominal cavity (ether narcosis) in order to observe the antrum in action would be useless, for laparotomy stops gastric movements.¹⁴ But if the stomach of a rabbit under ether is

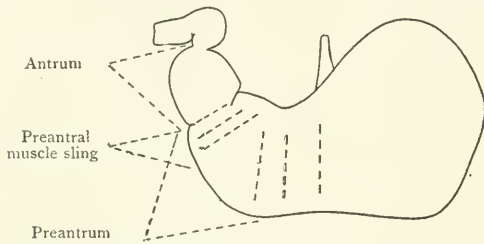


FIGURE 1.—The stomach is slightly tilted. The broken lines over the surface of the stomach show the passage of a wave, but do not show the extent of constriction.

exposed, whose vagi or splanchnics, or both sets of nerves, have been resected in the abdomen one or two weeks before, then the stomach continues to show apparently normal movements for some time after the abdomen has been opened. Laparotomy now exercises no immediate inhibitory effect upon the stomach, and the action of the antrum may be observed with ease. Though this method of observing the antrum is evidently abnormal, and though the stomach itself has been deprived partly of its extrinsic innervation, I wish to state again definitely that such a stomach shows for many minutes movements which are apparently identical with those observed through the intact belly wall of a normal rabbit. For this reason it is permissible to use this operative method to complete the information gained by inspection of a normal rabbit's stomach as described previously.

Observations on normal rabbits.— Inspection of a normal rabbit, stretched out on its back and prepared as described above, shows the stomach as a large mass occupying the upper part of the abdomen. The portion to the right¹⁵ of the median line in the right hypochondrium is largely the preantrum (see Fig. 1); just beneath the right costal margin the beginning of the antrum is usually

¹³ AUER: This journal, 1907, xviii, p. 349.

¹⁴ AUER: *Loc. cit.*, p. 359.

¹⁵ The terms "right" and "left" always refer to the animal.

located. If the animal is not full-grown, the preantrum may extend up to the costal margin, and under such conditions a false impression regarding the course of a gastric wave may be received by the observer. If the animal remains quiet, — which is an indispensable condition, for struggles inhibit gastric movements, — the observer will see after a few minutes a shallow depression beneath the right costal margin which travels peristaltically towards the right and disappears beneath the costal arch apparently. Immediately after this disappearance a moderate bulging of the entire preantrum (the mass which partly fills the right hypochondrium) occurs, and the bulging then gradually sinks away without any sign of peristalsis. This is repeated a few times, and during this interval there is no visible sign of peristalsis over the rest of the stomach. Then a shallow linear depression parallel to the long axis of the body may be observed to the left of the median line, arising apparently at the junction of the fundic and middle thirds of the stomach. This groove travels slowly to the right, passes the median line, increasing moderately in depth. This travelling constriction is preceded and followed by a bulging; the preceding bulging increases, while the one following gradually passes away. When this constriction reaches the preantrum, this part of the stomach bulges only moderately. The further course of the wave is peculiar. Instead of travelling peristaltically over the preantrum, it skips a large part of it, and appears at the same point where peristalsis was seen first, that is, just beneath the right costal margin; from now on the wave is like that described at the beginning, except that the pre-antral bulging which follows upon the disappearance of the wave beneath the costal margin is stronger. This preantral bulging is maintained a short time and then gradually sinks away, usually without any sign of peristalsis, but occasionally the constriction at the beginning of the preantrum, which has been maintained since it first was formed, may travel partly over the preantrum. The course described is the rule, and the only change noted, as the gastric movements become fully established, is an increase in strength. Occasionally a gastric wave is seen which does not pass beyond the beginning of the preantrum, and now and then the preantrum may bulge strongly when no visible wave of constriction has passed over the stomach.

It was stated above that if the preantrum was not fully visible the observer would gain an erroneous impression regarding the

course of a gastric wave, for only if the preantrum is fully seen can it be noticed that the wave skips a good part of the preantrum and disappears beneath the costal arch, and only then will the observer realize that the strong preantral bulging which follows at once after this disappearance of the wave is not due to an increase in the depth of the constriction at the beginning of the preantrum, but is apparently due to a contraction of the antrum and consequent expulsion of its contents into the preantrum. This error was committed in my earlier work on gastric peristalsis.

Observations on the exposed stomach.—The statements made so far regarding the course of a gastric wave were obtained by mere inspection of normal animals, and the chief disadvantage of this method is that the antrum is not observed directly, as explained before. In order to observe the antrum in action, the antrum had to be exposed by operation. As already described, it was found that the stomach of rabbits whose vagi or splanchnics or both sets had been resected in the abdomen some time previously, continues to move after laparotomy; in other words, laparotomy no longer inhibited gastric movements after the innervation of the stomach had been modified. Moreover, these rabbits when examined by inspection two to three days after the operation showed apparently normal gastric movements. If such a rabbit is etherized and the abdomen opened, the picture presented by the active stomach is striking. The course of a gastric cycle is the same as already described. In addition the antrum is visible and details may be observed: a shallow, linear depression at right angles to the long axis of the stomach appears to the right of the insertion of the œsophagus. This shallow groove involves slightly the greater curvature, but not noticeably the lesser one. The constriction slowly travels to the right, gradually increasing in depth and being preceded and followed by a bulging. The preceding bulging gradually increases in depth, but only causes moderate bulging when the preantrum is reached; the succeeding bulging gradually passes away. This preantral bulging is maintained by the constriction, and the wave continues to move on, but skips a large part of the preantrum and is seen next travelling peristaltically over about 0.5 centimetre of the preantrum which immediately adjoins the antrum. During this time the antrum, which appears as a pale, pink, cone-shaped cap closing the preantrum, has slowly dilated and become larger. As soon as the advancing peristaltic wave reaches the sphincter antri, which forms the rim of

the antral "cap," this sphincter contracts powerfully, so that it seems as if the antrum were separated from the rest of the stomach cavity. During this antral sphincter contraction the rest of the antrum shortens, and moves swiftly *in toto* towards the preantrum. The antral contraction is so powerful that it appears as a small gray knob, and the blood vessels which radiate over it are occluded in their peripheral portion. This strong contraction which the antrum undergoes, drives its contents largely into the preantrum, which bellies out markedly, especially on the lesser and greater curvature sides. After this total contraction the antrum slowly relaxes and again becomes pink; accompanying the antral relaxation, the bulging preantrum sinks away, apparently contracting more or less as a whole about its contents, so that part of it again enters the relaxing antrum. Now and then the constriction at the beginning of the preantrum travels a short distance over the preantrum while the antrum is relaxing. While the preantral bulging is sinking away, aided perhaps by a peristaltic wave, another wave has appeared on the anterior surface of the stomach and travels towards the preantrum; it reaches this structure shortly after it has assumed a position of rest; now the same play as above recorded is repeated.

That portion of the preantrum which is apparently not traversed by the peristaltic wave forms a triangular area, whose apex is in the lesser curvature side and whose base is formed by the greater curvature. Near the apex of this area lies the preantral sphincter, a muscular thickening which is only definitely noticeable on the lesser curvature and part of the sides. As soon as the advancing peristaltic wave has reached the apex described, the wave appears at once in that area of the preantrum which immediately adjoins the antrum, and which I shall call the preantral muscle sling, to differentiate this actively peristaltic portion from the rest of the preantrum. As the lesser curvature of the preantrum is narrower than the greater curvature, the peristaltic wave in the preantral muscle sling appears at a different angle from that over the body of the stomach. A glance at Fig. 1 will show the different positions of a gastric wave.

The gastric wave of contraction I have described is the one usually observable at the height of digestion. Now and then the same exceptions may be observed in the exposed stomach that have already been noted in the section dealing with gastric movements when observed under normal conditions (abdomen not opened); occasionally a wave passes over the body of the stomach, but causes

no contraction of the preantral muscle sling nor of the antrum; it fades away at the preantrum. At other times the preantral muscle sling and then the antrum contract when no wave is visible over the body of the stomach, or when such a wave has just appeared near the insertion of the œsophagus. Such exceptions to the general rule have also been noted by Hofmeister and Schütz¹⁶ and by Roux and Balthazard.¹⁷

Point of origin of the gastric wave.— All observers, as far as I know, state that a gastric wave begins in the middle part of the stomach, to the right of the œsophagus. This I have also found to be true in the normal rabbit at the height of digestion. If, however, the stomach is inhibited reflexedly by a struggle, the earliest sign of returning movement has always been noted in the pyloric third: first the preantral muscle sling contracted peristaltically, and shortly after this the preantrum bulged, due undoubtedly to a contraction of the antrum. This was repeated several times before an advancing wave could be distinguished over the body of the stomach. The same observation (method of inspection) was made in rabbits whose stomach innervation had been modified by a previous operation; the first sign of movement was seen in the pyloric third, but not all of these animals were suitable for observation of the preantral muscle sling through the abdominal walls. Moreover, when the stomach of these operated rabbits was exposed by laparotomy, under ether narcosis, the part of the stomach which showed motility longest was the preantral muscle sling and the antrum. On the other hand, it has already been described how a wave starting in the middle of the stomach now and then fades away at the preantrum without causing an antral contraction. From the facts just described it seems clear that a complete gastric wave is composed of two more or less independent parts, whose orderly interaction produces the normal type of gastric contraction wave seen at the height of digestion. It seems equally clear that the preantral muscle sling and antrum is the most rhythmic section of the rabbit's stomach.

The observations recorded above, made on rabbits under normal conditions and supplemented by information gained by operative measures, support the essential contention of Hofmeister and Schütz that a gastric wave of contraction is peristaltic only up to the be-

¹⁶ HOFMEISTER and SCHÜTZ: *Loc. cit.*, pp. 10-11.

¹⁷ ROUX and BALTHAZARD: *Loc. cit.*, p. 89.

ginning of the antrum and that the antrum itself contracts not peristaltically but *in toto*. There are minor points of difference between their description and mine, but they do not affect the main point at issue. It is remarkable that the gastric contraction wave of an herbivorous animal like the rabbit should show such a striking resemblance to that of a carnivore like the dog.

It gives me pleasure to acknowledge the kind criticism of Dr. S. J. Meltzer during this work.

SUMMARY.

From observations made on normal rabbits by inspection and by the amplification of these data by operative exposure of the stomach after the innervation of this viscus had been modified, it was found that a gastric wave of contraction is usually composed of two well-defined phases which, at the height of digestion, succeed each other in an orderly fashion. During the *first phase* a constriction appears on the stomach near the œsophageal insertion and travels peristaltically to the sphincter antri, apparently skipping in its course an angular section of the preantrum, the constriction at the beginning of the preantrum being maintained. During the *second phase* the sphincter antri contracts strongly, and during this contraction the rest of the antrum contracts *in toto*, moving towards the preantrum, and expelling the antral contents largely or entirely into the preantrum, causing the latter to bulge markedly. The antrum then relaxes slowly and with this relaxation the preantrum sinks away usually without any sign of peristalsis.

To emphasize this orderly succession of peristalsis and non-peristalsis in a gastric wave of contraction, it would seem advisable to adopt the term "peristole," as suggested by Hofmeister and Schütz.

The observations made on rabbits recorded in this paper are in essential agreement with the gastric wave of contraction described by Beaumont in man, and by Hofmeister and Schütz in the dog.

A complete gastric wave of contraction in the rabbit seems to be produced by the orderly interaction of two more or less independent parts, one of which originates in the middle of the stomach near the œsophagus, and the other in the neighborhood of the preantral muscle sling. Either part may occur without the other.

NOTE UPON THE EFFECT OF STIMULATION OF
THE ACCELERATOR NERVE UPON THE CAL-
CIUM, POTASSIUM, AND NITROGEN METABO-
LISM OF THE ISOLATED HEART.

BY W. H. HOWELL AND W. W. DUKE.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

IN a previous paper¹ the authors have shown, from experiments made upon the isolated heart, that there is an output of potassium from the heart substance during vagus inhibition. In the same paper we have suggested that during stimulation of the accelerator nerve there may be an output of calcium sufficient to account for the augmentation and, perhaps, for the acceleration of the beat.² During the past year we have attempted to test this last hypothesis by direct experiments made upon the perfused heart. It may be stated at once that the results were negative, but since the experiments were successful from a technical standpoint and were apparently conclusive in regard to the point in question, it seems desirable to place them upon record. Most of the experiments were made upon cats. The animals, as a rule, were heavily etherized and then bled by cutting the femorals. While bleeding, the heart was rapidly isolated by the method described in our previous paper. It was kept beating upon a supply of Locke's solution, fed to the coronary arteries at a temperature of 35° to 37° C. and under a relatively small pressure, 40 to 60 mm. Hg. The solution used had been previously thoroughly saturated with oxygen and was fed to the heart under oxygen pressure. In all of our later experiments we made use of the ingenious device described by Locke and Rosenheim³ for maintaining a continuous circulation of a small bulk of liquid in an atmosphere of oxygen.

¹ HOWELL and DUKE: This journal, 1908, xxi, p. 51.

² HOWELL and DUKE: Journal of physiology, 1906, xxxv, p. 131.

³ LOCKE and ROSENHEIM: Journal of physiology, 1907, xxxvi, p. 205.

The method of procedure was as follows: After the heart had been isolated it was thoroughly washed out for half an hour or longer by warm Locke's solution supplied under oxygen pressure from a stock flask containing about 8 litres. The outflow from the heart during this process was allowed to waste, and the washing was continued until the outflow was perfectly clear and free from traces of blood. During this period the accelerator nerves were prepared for stimulation. In most cases we used the nerve on the left side. The stellate ganglion was exposed, and ligatures were placed round the conspicuous branch, usually quite distinct, which has been designated as the *nervus accelerans* by Boehm. After the heart had been thoroughly washed out from the stock flask the irrigation was transferred to a small special supply of the same solution, which was thereafter kept in continuous circulation through the heart. From 75 c.c. to 150 c.c. of Locke's solution were used for this purpose. The liquid as it emptied into the right auricle was received through a suitable cannula into a closed receiver immersed in warm water, and was then forced back into the supply flask by bubbles of oxygen from an oxygen tank, after the manner described by Locke. The stream of oxygen was first saturated with water vapor by passing through a long tube filled with moistened beads, and as the system was entirely closed there was no concentration from evaporation. By means of this device the heart can be kept beating with great vigor and regularity for many hours (five) upon a supply of 100 c.c. or less. In isolating the heart it was necessary to close off the pulmonary circuit, and this was effected by laying two stout ligatures round the roots of the lungs.

During the continuous circulation of the small supply of liquid the heart was repeatedly stimulated through the accelerator nerves, and at the end of the experiment samples were taken of the original solution, and of the solution which had been repeatedly perfused through the heart while under stimulation from the accelerator nerves. These samples were analyzed for their content in calcium and potassium. The samples were first slightly acidified with acetic acid and brought to a boil. The sample that had been circulated through the heart gave usually a slight opalescence or precipitate due to the presence, probably, of a small amount of coagulable protein. For analysis, 10 c.c. of the clear liquid was taken, evaporated to dryness, and ashed. The residue was treated with a few drops of hydrochloric acid and was then dissolved in

water and diluted to 25 c.c. Of this solution 5 c.c., representing 2 c.c. of the original solution, were taken for a determination of the potassium, and 20 c.c., representing 8 c.c. of the original solution, for a determination of the calcium. For the potassium the delicate and satisfactory colorimetric method described in our previous paper was employed. For the calcium the solution was precipitated while warm with ammonia and ammonium oxalate. The precipitate of calcium oxalate after settling was washed repeatedly by centrifugalization, and was then dissolved in dilute sulphuric acid and titrated with a dilute standardized solution of potassium permanganate.⁴

In quite a number of our experiments the accelerator nerve failed to affect the heart beat, or at the best gave only one or two satisfactory accelerations and augmentations. In some of this group of experiments the perfusion was maintained for a number of hours and the liquids were analyzed as controls. In other cases stimulation of the accelerator caused very marked augmentation and acceleration of the beat, and the stimulation could be repeated successfully at intervals of several minutes for an hour or two. Why the nerve affected the heart in some cases and not in others was difficult to explain. In our successful experiments, all made during the spring and early summer, the following precautions were observed: (1) The accelerator nerve was kept warm during the intervals between stimulations by the application of warm cloths. (2) Care was taken in tying off the lungs not to lay the ligatures too close to the roots, since otherwise, especially on the left side, the accelerator nerve or some of its branches were caught in the ligature. Lastly, care was taken to use a solution which while somewhat strong in calcium (0.03 per cent) did not produce an independent beat of the ventricles. We had some reason for believing that in those hearts in which the ventricles beat independently of the auricles, the accelerator nerve failed to affect the heart. As this idea did not occur to us until our work was nearing completion, decisive experiments to test its correctness were not made in sufficient numbers to warrant a positive statement.

The results of our experiments seemed to prove conclusively that neither the calcium nor the potassium in the circulating liquid

⁴ The titrations for the calcium were all made for us by Mr. Burge, Fellow in Physiology, who was using the method in connection with another research. We desire to thank him for his kindness in making these determinations for us.

showed any variation in amount, after a perfusion lasting for hours, and after long-continued excitation of the heart through its accelerator nerve.

As an indication of the character of the results obtained, the protocols of two experiments may be given in detail, one of them being a control in which the accelerator was not stimulated, and one an experiment in which the accelerator was effective and was stimulated a great number of times.

Experiment, May 1, 1908.—Cat, etherized and bled. Heart isolated.

11 A. M., beat well upon the stock solution, but accelerator nerves on stimulation gave no effect. At 11.45 A. M. placed on a special supply of 150 c.c. which was maintained in continuous circulation. At 12.45 P. M. the heart was excised and suspended in a covered funnel so that all the drip could be secured. A little new solution was added to the supply flask and the circulation was continued until 4.45 P. M. The temperature of the circulating liquid varied from 35° to 37° C. At the end of the experiment the heart was still beating well. The circulating liquid was slightly turbid. On acidulation and boiling very little increase in opalescence could be detected. 10 c.c. were taken from the stock liquid and from the special supply of 150 c.c. which had been circulated through the heart for five hours. The solutions were evaporated to dryness, ashed, moistened with a few drops of HCl, again evaporated to dryness, dissolved in 25 c.c. water, of which 5 c.c. were used for determination of the potassium and 20 c.c. for the calcium.

Calcium.—8 c.c. of original Locke's solution contained 0.955 mgm. Ca. 8 c.c. of the circulated solution contained 0.966 mgm. Ca.

Potassium.—In colorimeter the stock solution (B) compared with the standard solution of potassium chlorplatinate containing in each cubic centimetre 0.00265 mgm. K, gave the following reading:

$$\text{Standard} = 40; \text{B} = 49$$

Hence B contained 0.00216 mgm. K to each c.c. Since the solution had been diluted 50 times, the original solution contained $(0.00216 \times 50 \div 52.3)$ 0.21 mgm. KCl to each cubic centimetre, or 0.021 per cent KCl.

Compared with (B), the solution which had been perfused through the heart (A) gave the following reading:

$$\text{A} = 40; \text{B} = 39$$

Hence A contained 0.0105 per cent potassium compared with 0.0108 per cent in B, the liquid before circulation.

Experiment, May 21, 1908. — Young cat, etherized, bled. Heart isolated at 10.30 P. M., beat very well upon the stock solution. Accelerator nerve prepared on left side. Stimulus applied to the ganglion gave a very pronounced acceleration and augmentation of the heart beat. The heart was placed on the special supply (100 c.c.) at 11.15 A. M., and during the continuous circulation of this solution the accelerator nerve was stimulated successfully 38 times at intervals of from two to seven minutes, the stimulus being increased in strength at intervals, as follows:

- First 18 stimulations, induction coil (GaiFFE's) at 50.
- 5 stimulations, induction coil (GaiFFE's) at 75.
- 10 stimulations, induction coil (GaiFFE's) at 100.
- 2 stimulations, induction coil (GaiFFE's) at 125.
- 3 stimulations, induction coil (GaiFFE's) at 150.

After more than two hours of the stimulation the heart was still beating very well, and was turned over to another observer for a different experiment. In spite of much cutting and handling it continued to beat well for five hours.

Ten c.c. were taken from the stock liquid and from the perfused liquid and treated as usual (see above) for the analyses of calcium and potassium. The calcium analyses were made in duplicate by Mr. Burge, and the potassium analyses in duplicate by Mr. Burge and by one of the authors (H.).

Calcium. —

- 8 c.c. of original stock solution contained I. 0.855 mgm. Ca.
- II. 0.855 mgm. Ca.
- 8 c.c. of the circulated liquid contained I. 0.855 mgm. Ca.
- II. 0.855 mgm. Ca.

Potassium. —

- Analysis by H. Stock solution = 0.137 mgm. K per c.c.
- Circulated solution = 0.132 mgm. K per c.c.
- Analysis by B. Stock solution = 0.138 mgm. K per c.c.
- Circulated solution = 0.136 mgm. K per c.c.

Nitrogen excretion in the isolated heart. — Burian⁵ has shown that in skeletal muscle there is apparently a continuous formation and elimination of hypoxanthin, the amount being increased when the muscle is made to contract. This result was obtained by perfusing the muscle with a circulating liquid composed of Ringer's solution and defibrinated blood. It seemed probable that in our

⁵ BURIAN: Zeitschrift für physiologische Chemie, 1904-1905, xliii, p. 532.

experiments in which the contracting heart was perfused for hours with a limited supply of Locke's solution decisive evidence might be obtained in regard to the production of purin bases or of other forms of nitrogenous waste. It was not possible at the time the experiments were made to examine separately the liquid that had been perfused through each heart, but the specimens of perfused liquid, after they had been acidified and boiled to remove protein, were brought together and kept, with the addition of a little toluene, until it was convenient to make an examination.

Dr. W. Jones was kind enough to examine this liquid for the purin bases, and he reports that no evidence could be obtained of a trace of such bodies. In this respect, therefore, the heart muscle seems to differ from the skeletal muscle, although possibly the difference may be due to the fact that in our experiments the hearts were supplied with a circulating liquid containing an abundance of dextrose. On the other hand, the perfused liquid, after being boiled with dilute sulphuric acid, gave very distinct evidence of the presence of creatinin when tested by Weyl's and Jaffe's reaction. The heart, therefore, must give off creatinin (or creatin) to the circulating liquid, and it will be interesting to determine how far this elimination is associated with functional activity.

THE DIFFERENT FORMS OF NITROGEN IN PROTEINS.¹

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C. A. BRAUTLECHT.

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

IN studying the chemistry of the many forms of protein that are found in nature it is of great importance to be able to determine with accuracy the proportion of the various decomposition products which these proteins yield on hydrolysis. If this could be done even for two or three of these decomposition products, it would be a great help in differentiating the several forms of protein now described. It is evident that identity of two forms of protein cannot be shown by this means, but it is equally evident that differences can be thus established which will contribute to a distinct advance in our present knowledge of these substances.

With the means now available it is not yet possible to accurately determine most of the mono-amino-acids, for the results obtained by the best methods and most careful work unquestionably fall considerably below the actual amount of these amino-acids which are yielded by hydrolysis.

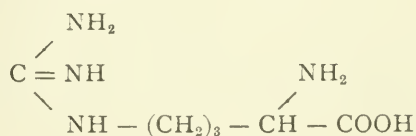
In this paper we give the results of an extended study of the determination of basic products of decomposition which have led us to believe that under suitable conditions a very considerable degree of accuracy can be obtained in determining ammonia, histidine, arginine, and lysine. We also believe that these determinations afford the best means now available for differentiating the many forms of proteins. Unfortunately we cannot test by direct experiment the actual degree of accuracy of these determinations, but we can prove the constancy of the results, and also obtain indirect evidence that makes it highly probable that they closely

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

agree with the quantity of these substances actually yielded by the protein.

Ammonia can apparently be determined with very great accuracy, for repeated estimations on the same protein even under widely different conditions of hydrolysis give results which are practically identical. The liberation of ammonia from the protein by acid hydrolysis is so similar to that from asparagine as to make it highly probable that a union of NH_2 with a carboxyl group exists in the protein molecule, as has long been assumed. This assumption is further supported by the close parallel shown by the amount of ammonia and that of the dibasic glutaminic and aspartic acids which most of the different proteins yield. The fact, however, must not be overlooked that several of the proteins examined do not conform to this rule, and further study is required to discover the cause of this divergence.

The amount of ammonia evolved by boiling with a solution of sodium hydroxide corresponds closely, for the proteins thus far studied, to the sum of the nitrogen produced by acid hydrolysis in the form of ammonia and one half of the nitrogen contained in the arginine yielded by these proteins. Nitrogen in the group CONH_2 and one half of that in arginine



is the only nitrogen among the known decomposition products of the proteins which is converted into ammonia by alkaline hydrolysis. Our results, therefore, which are in accord with the facts at present known, make it improbable that the proteins contain any other nitrogen easily converted into ammonia, such, for instance, as in the form of R-CO-NH-CO-R , as recently suggested by Bergell and Feigl.²

Taken together, the data obtained respecting the determination of ammonia make it highly probable that the results are very accurate, and that this ammonia originates from an amide union in the protein molecule.

Osborne and Harris³ showed that after complete hydrolysis with

² BERGELL and FEIGL: *Zeitschrift für physiologische Chemie*, 1908, liv, p. 258.

³ OSBORNE and HARRIS: *Journal of the American Chemical Society*, 1903, xxv,

hydrochloric acid uniform results could be obtained for the nitrogen precipitable by phosphotungstic acid by Hausmann's method if slightly modified and applied under uniform conditions. We have now determined the actual quantity of histidine, arginine, and lysine in most of the proteins examined by Osborne and Harris, and have found that the amount of nitrogen contained in these bases corresponds closely with that precipitated by phosphotungstic acid. Hausmann's method is, therefore, most useful in comparing different proteins as well as for controlling their separation from one another.

In view of the close agreement between the amount of nitrogen precipitated by phosphotungstic acid and the sum of the nitrogen contained in the arginine, histidine, and lysine which the large number of different proteins from many sources yield on hydrolysis, it seems improbable that other *basic* products than those just named will be found in the future among the decomposition products of the proteins. In respect to accuracy, the determinations of histidine, arginine, and lysine appear to leave little to be desired if the methods of analysis are carefully and properly carried out.

It was found that for many proteins a much longer hydrolysis was necessary to liberate all of the bases than has been heretofore supposed. Apparently a considerable part of these bases is present in some of the proteins in very difficultly hydrolyzable combinations which require twenty-four hours, or more, continued boiling with 25 per cent sulphuric acid for their complete dissolution. This condition was found to hold especially for the proteins of leguminous seeds.

The wide differences between seed proteins in the proportion of nitrogen precipitated by phosphotungstic acid is chiefly caused by differences in the amount of arginine which was obtained from all of them. This forms about 1 per cent of the protein containing the least, and over 14 per cent of those containing the most, basic nitrogen. The amount of histidine which was also obtained from all of the proteins was nearly the same in the majority of the proteins analyzed, that is, about 2.5 per cent. The amounts of lysine found in the several proteins differed considerably, none being present in any of the alcohol soluble proteins, 4 to 5 per cent in most of the leguminous seed proteins, and over 6 per cent in conalbumin from hen's egg.

The proteins, when arranged in the order of their yield of arginine, fall into three groups: first the oil seeds, then the leguminous seeds, and finally the cereal grains,—the only exception being the glutelin of maize, which is one of the least well characterized and studied of all the proteins in the list, and may be a mixture of several different proteins. It is interesting to note that a similar relationship of these proteins was apparently recognized by Ritthausen as a result of his studies of their external properties, for he designated his book on the plant proteins as “Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Oelsamen.”

We have in the chemical constitution of these seed proteins an apparent relationship not only to the biological relations of the plants which produced them, but also to the chemical constitution of the seeds themselves.

EXPERIMENTAL PART.

I. Nitrogen as ammonia. *a. Constancy of the amount of ammonia formed by hydrolysis.*—The following results of determinations of the amount of ammonia obtained according to the method described by Osborne and Harris⁴ were taken at random from our notebooks and serve to illustrate how closely repeated determinations may be expected to agree. The figures marked with an asterisk are those published in the paper referred to, and are introduced to show that different analysts working with different preparations of the proteins can obtain substantially identical results.

TABLE I.

NITROGEN AS AMMONIA IN PER CENT OF THE PROTEIN.

Gliadin	4.40†, 4.44†, 4.35, 4.30, 4.33, 4.33, 4.34*, 4.36*.
Hordein	4.10†, 4.06*.
Zein	2.99†, 2.97*.
Edestin	1.83†, 1.86, 1.86*, 1.86*, 1.93*, 1.86, 1.81, 1.80, 1.87.
Squash-seed, globulin	1.35†, 1.28*.
Glycinin	2.14†, 2.11*, 2.12*.
Vignin	1.89, 1.86, 1.91*, 1.91*.
Cotton-seed, globulin	1.94, 1.96, 1.92*.
Legumin, pea	1.68, 1.66*, 1.72*.
Vicilin, pea	1.64, 1.78, 1.67*.
Ovovitellin, hen's egg	1.24, 1.29, 1.28*, 1.24*.
Conalbumin, hen's egg	1.13, 1.23*, 1.18*.

⁴ OSBORNE and HARRIS: *Journal of the American Chemical Society*, 1903, xxv,

That this agreement is not the result of uniform methods of work peculiar to this laboratory is indicated by the results recently published by Wood⁵ for the nitrogen as ammonia obtained from four different preparations of wheat gliadin, namely, 4.40, 4.51, 4.31, and 4.55 per cent.

b. Effect of temperature employed for distilling with magnesia.—As our former determinations were all made by distilling the products of hydrolysis with magnesia at 100°, they are open to the criticism that more or less nitrogen, other than that originally present in amide combination, may have been converted into ammonia by the action of the magnesia at the high temperature employed in the distillation. We have therefore repeated our determinations in several proteins by distilling with magnesia in a vacuum at 40°. That the results were the same as at 100° is shown by the figures given in the preceding table which are marked with a dagger.

Since by distilling in a vacuum the fine spray caused by the sudden bursting of the bubbles of foam is very easily carried over into the distillate, especial care was taken to construct a safety tube which rendered this impossible, and to confirm its efficiency by evaporating the distillates to dryness and testing the residues for traces of magnesia.

c. Is the nitrogen yielded as ammonia amide nitrogen?—None of the amino-acids which are known products of protein hydrolysis yield any ammonia by long boiling with strong hydrochloric acid, and cannot therefore be considered as contributing any part of the ammonia formed from proteins by acid hydrolysis. That the nitrogen that is converted into ammonia is contained in the protein in amide union seems probable from the following experiments:

Five portions of gliadin, each weighing 1 gm., were dissolved in 50 c.c. of 20 per cent hydrochloric acid, and the solutions were boiled for the times indicated, with the following results:

TABLE II.

Boiled for 30 min., nitrogen as NH ₃ = 4.30 per cent.
Boiled for 1 hour, nitrogen as NH ₃ = 4.35 per cent.
Boiled for 2 hours, nitrogen as NH ₃ = 4.33 per cent.
Boiled for 3 hours, nitrogen as NH ₃ = 4.33 per cent.
Boiled for 4 hours, nitrogen as NH ₃ = 4.33 per cent.
Boiled for 6 hours, nitrogen as NH ₃ = 4.40 per cent.

⁵ T. B. WOOD: The journal of agricultural science, 1907, ii, part 2, p. 139.

Without boiling, but after the solution had stood at 20° for two hours, only 0.22 per cent of nitrogen as ammonia was obtained, while after seventeen hours at the same temperature 1.67 per cent was found. Under similar conditions asparagine behaved in the same way, yielding 1.4 per cent of nitrogen as ammonia after seventeen hours at 20°, and exactly one half of its nitrogen as ammonia after boiling for half an hour.

d. Relation of the quantity of nitrogen obtained as ammonia to that of the dibasic amino-acids. — If it is assumed that the amino-acids are united in the protein in polypeptide union, as has been made almost certain by the work of Emil Fischer, it would be only natural to look to the dibasic acids as affording the greater part of the carboxyl groups required for an amide union of a part of the nitrogen in the protein molecule. Fischer⁶ has already suggested this, and Osborne and Gilbert⁷ pointed out that the presence of a large proportion of glutaminic acid was accompanied by a similarly large proportion of ammonia among the decomposition products of several proteins. They did not find, however, a strict numerical relation for the proteins then under consideration, and they stated that definite conclusions could not be drawn until the proportion of aspartic acid was also known. As the amount of aspartic acid has now been determined in most of these proteins, we have calculated the amount of ammonia which corresponds to one molecule of ammonia for each molecule of the glutaminic and aspartic acids found in those proteins in which these substances have been most carefully determined. It will be seen from the following table that for most of them the result agrees closely with that actually found by distilling with magnesia. Marked exceptions, however, are shown by the proteins of the cereals, for which the amount calculated falls very much below that found analytically, and also by those of the pea, for which it falls much above.

It is probable that the excess of ammonia as calculated over that found is, in fact, somewhat greater than these figures indicate, for while the ammonia determinations are unquestionably quite accurate, the quantities of glutaminic and aspartic acids that were found are doubtless somewhat smaller than those which are actually present. *Small* minus differences may therefore be due to the uncertainties

⁶ Cf. EMIL FISCHER: *Berichte der deutschen chemischen Gesellschaft*, 1904, xxxvii, p. 485.

⁷ OSBORNE and GILBERT: *This journal*, 1906, xv, p. 333.

TABLE III.
RATIO OF AMMONIA TO GLUTAMINIC AND ASPARTIC ACIDS.

Protein.	Percentage of NH_3 calculated for 1 mol. of NH_3 to 1 mol. of glutaminic and aspartic acids.	Percentage of NH_3 found by distillation.	Difference between amount calculated and that found.
Squash-seed, globulin	1.84	1.64	+0.20
Edestin	2.19	2.28	-0.09
Cotton-seed, globulin	2.40	2.33	+0.07
Sunflower-seed, globulin . .	2.93	3.13	-0.20
Excelsin	1.99	1.80	+0.19
Amandin	3.36	3.70	-0.34
Conglutin-a	2.63	2.55	+0.08
Legumin, vetch	2.27	2.16	+0.11
Legumin, pea	2.64	2.05	+0.59
Vicilin, pea	3.15	2.03	+1.12
Legumelin, pea	2.02	1.23	+0.79
Vignin	2.46	2.34	+0.14
Phaseolin	2.35	2.06	+0.29
Glycinin	2.75	2.56	+0.19
Leucosin	1.23	1.41	-0.18
Glutenin	2.83	4.01	-1.18
Maize, glutenin	1.54	2.12	-0.58
Gliadin	4.39	5.11	-0.72
Hordein	4.20	4.87	-0.67
Zein	2.29	3.61	-1.32
Casein	1.38	1.61	-0.23
Ovalbumin	1.34	1.63	-0.29

attending the isolation of these dibasic acids, but in the case of the cereal proteins these differences are so large that it does not seem possible to explain them in this way.

In gliadin we have found 5.11 per cent of the protein as ammonia, whereas the amount calculated for union with the glutaminic acid and aspartic acid is only 4.39 per cent. The difference, 0.72 per cent, corresponds to 6.1 per cent more glutaminic acid than that found, or to 5.6 per cent more aspartic acid. If this difference were divided between the two acids, it might be possible to consider that these quantities had escaped separation, but this seems hardly probable, for the accuracy of the ammonia determination has been confirmed by a large number of closely agreeing determinations made in this and other laboratories, and the determinations of glutaminic acid have been confirmed by the results obtained⁸ with 1000 gm. of gliadin from which 30 per cent was separated by direct crystallization and, from the esters of the amino-acids, 6 per cent more. If it is assumed that by esterification 80 per cent of the glutaminic acid can be separated, the 6 per cent found would correspond to 7.5 per cent, making the total 37.5 per cent, which agrees closely with the 37.3 per cent obtained from 100 gm. by direct determination. This last result has been confirmed by Abderhalden and Samuely,⁹ who found 36.5 per cent. The amount of aspartic acid, 1.24 per cent, which Abderhalden and Samuely¹⁰ found was also nearly the same as that found in this laboratory, namely, 0.58 per cent.

For glutenin the difference between the ammonia as calculated and that actually found is still greater than for gliadin, namely, 1.18 per cent of the protein. As the proportion of glutaminic and aspartic acids obtained from glutenin in this laboratory agrees closely with that given by Abderhalden and Malengreau,¹¹ being 23.42 and 0.91 per cent and 24.0 and 0.64 per cent respectively, there is no reason to suppose them to be very far from correct. It is therefore probable that the cereal proteins in some way differ in structure from all the others which have been examined, and

⁸ OSBORNE and CLAPP: This journal, 1906, xvii, p. 231.

⁹ ABDERHALDEN and SAMUELY: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 194.

¹⁰ ABDERHALDEN and SAMUELY: *Ibid.*, 1905, xlv, p. 276.

¹¹ ABDERHALDEN and MALENGREAU: *Zeitschrift für physiologische Chemie*, 1906, xlvi, p. 513.

that they may possibly contain some other dibasic acid not yet isolated from their decomposition products.

In the case of the proteins of the pea the difference, which is in the opposite direction, might indicate that a part of the nitrogen yielded as ammonia was in the combination $R-CO-NH-CO-R$, as recently suggested by Bergell and Feigl,¹² for in this case two molecules of the dibasic acid would be united to only one NH. This, however, is probably not the case, for distillation of these proteins with sodium hydroxide solution (page 190), has given no evidence of the presence of this grouping, which Bergell and Feigl have shown to be stable in acid solutions but to yield ammonia on boiling with alkalis. None of the other proteins, when distilled with alkali, yielded any indication of this diamide binding, and we have, as yet, no reason to suppose that it occurs in the protein molecule.

This marked agreement between the ammonia as determined and that calculated for the proteins of seeds, other than those of the cereals and the pea, indicates that this ammonia exists in the protein as amide nitrogen in combination with one of the carboxyl groups of the dibasic acids.

c. Nitrogen converted into ammonia by alkaline hydrolysis. — All of the known decomposition products of the proteins are stable in alkaline solutions with the exception of arginine, from which, theoretically, one half of the nitrogen should be split off as ammonia on boiling with fixed caustic alkalis. If the proteins yield no other products sensitive to alkalis, the amount of nitrogen which they should yield as ammonia when distilled with a strong solution of sodium hydroxide ought to be equal to the sum of their amide nitrogen and one half of the nitrogen of the arginine which they contain.

Before trying experiments in this line it is important to know how arginine behaves under the conditions of the experiments planned. Two tenths of a gram of pure arginine copper nitrate were therefore distilled with 300 c.c. of decinormal sodium hydroxide solution, and when 200 c.c. had distilled over the distillate was titrated. The residual solution was then made up to 300 c.c. with decinormal sodium hydroxide solution and the distillation was repeated. The solution was then again made up to 300 c.c. with

¹² BERGELL and FEIGL: *Zeitschrift für physiologische Chemie*, 1908, liv, p. 258.

water and distilled, and this process was repeated until no more ammonia came over.

The results were as follows:

TABLE IV.
Calc. for $\frac{1}{2}$ Arginine, N = 19.0

No.	Mgr. N.	Mgr. N.	No.	Mgr. N.	Mgr. N.
1	1.2	1.3	16	0.5	0.4
2	1.2	1.0	17	0.5	0.3
3	1.4	1.2	18	0.3	0.4
4	0.8	0.8	19	0.2	0.6
5	0.8	1.0	20	0.2	0.2
6	0.6	1.0	21	0.4	0.4
7	1.3	0.7	22	0.0	0.4
8	0.6	0.8	23	0.0	0.2
9	0.4	0.6	24	0.0	0.3
10	0.9	0.9	25	0.2
11	0.4	0.8	26	0.2
12	0.8	0.6	27	0.4
13	0.7	0.6	28	0.2
14	0.5	0.6	29	0.0
15	0.5	0.4	30	0.0
Total				14.2	16.5

These results show that nearly one half of the arginine nitrogen is thus very slowly eliminated as ammonia.

One air-dry gram of each of the following proteins, corresponding to the quantities of moisture and ash-free substance indicated under each, was then distilled with sodium hydroxide in the same way as described for arginine.

TABLE V.

Distillation.	Gliadin, wheat, 0.9198 gm. Mgr. N.	Legumin, pea, 0.8979 gm. Mgr. N.		Vicilin, pea, 0.9264 gm. Mgr. N.		Excelsin, para-nut, 0.9024 gm. Mgr. N.
1	27.2	15.4	17.6	17.8	15.4	11.4
2	12.0	4.4	5.8	3.6	5.2	4.5
3	1.6	2.2	2.6	2.2	3.0	3.5
4	2.6	1.8	1.6	1.6	1.2	2.4
5	0.4	1.8	1.6	1.2	1.6	1.4
6	1.0	1.0	1.0	0.8	1.8
7	1.3	1.2	1.0	1.0	1.6
8	1.0	0.8	0.8	1.0	1.6
9	0.6	0.8	0.4	0.6	1.6
10	0.8	0.4	0.4	0.4	1.6
11	1.4	1.0	1.2	0.8	1.6
12	0.8	0.6	0.2	0.4	0.8
13	0.6	0.8	0.4
14	0.2	0.4	0.9
15	00.0
Total	43.8	33.3	36.2	31.4	31.4	35.1
Per cent of dry and ash-free protein	4.76	3.71	4.04	3.39	3.39	3.72
Amide N.	4.30	1.69	...	1.70	...	1.48
$\frac{1}{2}$ Arginine N.	0.51	1.88	...	1.42	...	2.25
Sum	4.81	3.57	...	3.12	...	3.73

The results obtained for gliadin, which contains a relatively large proportion of amide nitrogen and a relatively small proportion of arginine nitrogen, and those for excelsin, in which the proportion of amide nitrogen is small and of arginine nitrogen is large, are in very close agreement with the calculation. Those obtained for legumin and vicilin are slightly higher than the calculated.

The greater part of the nitrogen was evolved by all these proteins in the first two distillations, corresponding to the ease with which amide nitrogen is converted into ammonia by caustic alkalis. The nitrogen subsequently coming off as ammonia was evolved slowly in much the same way as from arginine, although a little more quickly. Possibly the nascent arginine set free from the protein by alkaline hydrolysis is somewhat more easily affected by alkalis than the free arginine itself. The close agreement between the results thus obtained and those calculated shows that these proteins contain little or no nitrogen which can be thus converted into ammonia, except the amide and arginine nitrogen.

II. Nitrogen in the basic amino-acids. *a. Agreement between the nitrogen precipitated by phosphotungstic acid and that contained in the basic amino-acids.*— In the former paper from this laboratory, already referred to,¹³ it was shown that the nitrogen precipitated by phosphotungstic acid under the conditions there laid down agreed closely with that contained in the basic amino-acids of the four proteins in which these bases had then been determined. Since then we have determined these bases in a large number of the other proteins discussed in the paper above referred to.

In making these determinations we have followed the method of Kossel and Patten¹⁴ with the following slight changes:

1. The solution filtered from the first barium sulphate precipitate, while still slightly acid, was concentrated under strongly reduced pressure at about 70°. The ammonia was then removed by continuing the concentration on a water bath in an open dish after adding an excess of barium carbonate.

2. The first silver precipitate was obtained with silver nitrate instead of silver sulphate, as the solubility of the latter salt is too small to permit a sufficient addition within a reasonable time.

¹³ OSBORNE and HARRIS: *Journal of the American Chemical Society*, 1903, xxv, p. 323.

¹⁴ KOSSEL and PATTEN: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 39.

3. The solution obtained from this first silver precipitate, which contains the arginine and histidine, was brought to about 250 c.c. volume and made to contain 5 per cent of sulphuric acid. The histidine was then precipitated by mercuric sulphate solution, converted again into the silver compound, and nitrogen determined in the solution obtained from the latter in the usual way. The filtrate from the mercury precipitate was freed from mercury with hydrogen sulphide, and from sulphuric acid by neutralizing to litmus with baryta and adding barium nitrate as long as a precipitate formed. From this solution the small quantity of histidine which was not precipitated by the mercuric sulphate was separated by adding an excess of silver nitrate, as shown by the brownish precipitate produced on adding a drop of the solution to baryta water. Barium hydroxide was then added to the solution until it was *neutral* to litmus. A small quantity of histidine silver always separated at this point, and the precipitation was made complete by the addition of 5 c.c. of cold saturated solution of barium hydroxide. The filtered solution was then tested, and if a permanent precipitate formed on the further addition of a drop of barium hydroxide solution to 10 c.c. of the solution, filtered clear, 2 c.c. more barium hydroxide was added to the main solution and the test repeated. When the 10 c.c. of test solution remained clear after adding a drop of barium hydroxide solution, the small precipitate of histidine silver was filtered out and added to the main portion. By thus transposing Kossel's precipitations of histidine with silver and mercury we found it much easier to make a complete separation of histidine from arginine, since, after making the solution of the arginine exactly neutral to litmus, only a small and tolerably uniform further quantity of barium hydroxide was required to separate the little histidine which it still contained. The arginine and histidine were estimated, as Kossel and Patten direct, by determining the nitrogen in an aliquot part of their solutions, since much more accurate results can be thus obtained than by weighing definite compounds of these substances. In every case the histidine was identified as the dichloride and the arginine as the copper nitrate double salt, and the purity of the products determined by the character of their crystallization. By weighing the arginine copper nitrate we found that about 85 to 90 per cent of the amount calculated from the nitrogen content of the solution could be obtained in a pure crystalline condition. As the mother

liquor from which these crystals separated still contained much arginine, we are convinced that the method of calculating the arginine from the nitrogen content of its solution gives a very close measure of the quantity. Owing to the great difficulty encountered in separating histidine dichloride in a pure, crystalline condition, the amount recovered from the solution usually falls considerably below that calculated from the nitrogen. We have, however, convinced ourselves, by the character of the crystallization and by recovering from 75 to 80 per cent of the calculated quantity of histidine in a pure condition, that the results obtained by calculation from the nitrogen content of the solution agree closely with the amount of histidine actually present.

The lysine was weighed as the recrystallized picrate, which was in all cases very pure.

The results of these determinations are given in Table VI, page 194, which shows the percentage of the total protein formed by the histidine, arginine, and lysine found, the percentage of the protein formed by the nitrogen contained in these bases as determined by calculation, the percentage of the protein which the nitrogen precipitated by phosphotungstic acid forms, and the difference in per cent of the protein between the nitrogen calculated from the proportion of bases found and that precipitated by phosphotungstic acid.

Of these twenty-six different proteins, seventeen yielded an amount of bases the nitrogen of which differs from the amount of that precipitated by phosphotungstic acid by less than 10 per cent of the latter. For three others the calculated nitrogen exceeds the quantity precipitated by phosphotungstic acid by a relatively considerable, but absolutely small, amount. These latter, glutenin, hordein, and zein, all contain very little base, and the differences are unquestionably due to unavoidable errors of analysis, for it is well known that the phosphotungstates of these bases are somewhat soluble, and consequently when the amount of base is small the quantity of nitrogen precipitated by phosphotungstic acid is less than that actually contained in the bases present. When the amount of bases is large, the bulky precipitate of the phosphotungstates carries with it some of the mono-amino-acids, which compensates for the error caused by solubility. For the six remaining proteins the calculated nitrogen was less than the quantity precipitated by a little more than 10 per cent. Of these phaseolin and legumelin

TABLE VI.

	Histidine.	Arginine.	Lysine.	Basic N. Calculated.	Basic N. Precipitated.	Dif- ference.	Basic N. in p. c. of that precipitated.
	per cent	per cent	per cent	per cent	per cent	per cent	
Globulin, squash-seed . . .	2.42	14.44	1.99	5.69	5.99	-0.30	95.00
Excelsin, para-nut.	2.50	14.29 ¹	1.64	5.57	5.76	-0.19	96.70
Edestin, hemp-seed	2.19 ²	14.17 ²	1.65 ²	5.48	5.91	-0.43	92.70
Globulin, cotton-seed	3.46	13.51	2.06	5.69	5.71	-0.02	99.65
Globulin, castor-bean	2.74	13.19	1.54	5.29	5.64	-0.35	93.80
Amandin, almond	1.87	12.16	0.72	4.56	4.15	+0.41	109.90
Legumin, pea	1.69	11.73	4.98	5.20	5.11	+0.09	101.50
Legumin, vetch	2.94	11.06	3.70	5.07	5.17	-0.10	98.07
Conglutin-a, yellow lupine	2.51	10.93	2.74	4.77	5.16	-0.39	92.50
Vicilin, pea	2.17	8.91	5.40	4.50	4.92	-0.42	91.46
Glycinin, soy-bean	2.10	7.69	3.39	3.59	3.95	-0.36	90.90
Ovovitellin	1.90	7.46	4.81	3.82	4.36	-0.54	87.62
Vignin, cow-pea	3.08	7.20	4.31	3.98	4.28	-0.30	92.90
Glutelin, maize	3.00	7.06	2.93	3.63	3.52	+0.11	103.10
Ovalbumin, hen's egg	1.71	4.91	3.76	2.76	3.20	-0.44	86.25
Leucosin, wheat	2.83	5.94	2.75	3.25	3.50	-0.25	92.87
Conalbumin, hen's egg	2.17	5.07	6.43	3.45	4.16	-0.71	83.00
Legumelin, pea	2.27	5.45	3.03	2.95	3.45	-0.50	85.50
Legumelin, soy-bean	2.04	5.35	4.91	3.21	3.08	+0.13	104.22
Phaseolin, kidney-bean	2.62	4.87	4.58	3.15	3.62	-0.47	87.00
Glutenin, wheat	1.76	4.72	1.92	2.37	2.05	+0.32	115.60
Casein, cow	2.46	3.39	5.95	2.99	3.49	-0.50	85.70
Gliadin, wheat	0.58	3.16	0.00	1.18	1.09	+0.09	108.30
Gliadin, rye	0.39	2.22	0.00	0.83	0.90	-0.07	90.20
Hordein, barley	1.28	2.16	0.00	1.05	0.77	+0.28	136.40
Zein, maize	0.82	1.35	0.00	0.65	0.49	+0.16	132.60

¹ An error in the calculation of the result for arginine previously given for excelsin (This journal, 1908, xix, p. 53) has since been discovered; the correct result being 14.14 per cent instead of 16.06 per cent.

² KOSSEL and PATTEN: Zeitschrift für physiologische Chemie, 1903, xxxviii, p. 39.

from the pea were found to yield a larger quantity of bases when hydrolyzed for twenty-four hours than when hydrolyzed for twelve hours, and it is therefore not improbable that a longer hydrolysis than the twenty-four hours employed would have yielded somewhat more bases than were found. Of the four still remaining proteins, casein has been shown by Emil Fischer to contain diaminotrioxododecanic acid which is precipitated by phosphotungstic acid. As three separate determinations of the bases in casein gave us results in good agreement, it is probable that the difference indicated for casein in the table is caused by the presence of this other acid.

In view of these facts it would seem that determination of the nitrogen precipitated by phosphotungstic acid furnishes a valuable means for controlling the results of base determinations in proteins and should always be employed where any doubt exists as to the amount of bases present.

b. The probable accuracy of the determinations of the basic amino-acids. — What has just been said of the agreement between the nitrogen calculated from the bases found by Kossel's method and that precipitated by phosphotungstic acid according to Hausmann's modified method, indicates that the total quantity of the bases can be determined with considerable accuracy.

As to the completeness of the separation of arginine and histidine, the evidence already furnished by Kossel shows that, when properly conducted, satisfactory separations can be made. We think that by transposing the precipitations of histidine with mercury and silver the degree of accuracy of the histidine determinations is increased, for the mercury precipitate is slightly soluble and if this is made last a corresponding quantity of histidine is lost. If, on the other hand, the greater part of the histidine is first separated from the arginine with mercuric sulphate, the small quantity remaining with the arginine is subsequently easily separated from the latter and is thus saved. The evident purity of the histidine and arginine which their respective final solutions contain is strong evidence of the completeness of the separation of these two substances, not only from one another but also from all other protein decomposition products. The results of alkaline hydrolysis which have already been discussed give strong additional evidence of the accuracy of the arginine determinations. Further evidence is also afforded by the agreement between duplicate determinations

on one and the same protein. This is illustrated by the following table:

TABLE VII.

	Histidine	Arginine	Lysine
	per cent.	per cent.	per cent.
Legumin, vetch . .	2.04	10.98	3.86
	2.06	10.97	3.99
	2.94	11.06	3.70
Globulin, squash-seed	2.42	14.44	1.99
	2.62	13.91	1.42
Excelsin, para-nut.	14.14	1.64
	2.47	14.29	1.47
	2.59	13.82	2.28
Edestin, hemp-seed .	2.36 ¹	13.97 ¹	1.67 ¹
	2.10 ¹	14.36 ¹	1.63 ¹
	1.93	1.52
Globulin, castor-bean	2.04	13.20	...
	2.74	13.19	1.54
Casein, cow	2.50	3.42	5.55
	1.48	3.81	5.45
	2.46	3.39	5.95
Zein, maize.	0.43	1.16	0.00
	0.82	1.35	0.00

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

The agreement between these figures is as close as could be expected with such a difficult and complicated method of analysis. Taking all the evidence together, it is highly probable that, when properly conducted, very satisfactory results can be obtained in determining these three products of protein hydrolysis. These determinations together with that of ammonia afford the best means at present available for characterizing the different proteins. It must not be forgotten, however, that good determinations can only be obtained by exercising great care, and that experience with the methods is also necessary.

c. Length of time required for hydrolysis in order to set all the bases free. — During the course of this work the unexpected, but important, discovery was made that many proteins require for complete decomposition a much longer hydrolysis with 25 per cent sulphuric acid than had heretofore been employed. In determining

the bases in several proteins, especially those from leguminous seeds, a very considerable difference was found between the quantity of nitrogen in the bases isolated and that precipitated by phosphotungstic acid. This we naturally assumed to be caused by the presence of some other base which had escaped detection with Kossel's method. On repeating some of our determinations the results differed so much from those first obtained that we became convinced that these proteins had not been completely hydrolyzed. The determinations were therefore repeated in all cases where there was a wide difference between the calculated nitrogen and that precipitated by phosphotungstic acid, and it was at once found that this difference either disappeared or was much reduced.

Table VIII on page 198 gives the results of these determinations, and plainly shows that a long-continued boiling is necessary for complete decomposition of many proteins. The figures marked with a star were obtained by treating the protein with a mixture of three times its weight of sulphuric acid and six times its weight of water, and digesting for several hours on a water bath until the protein was dissolved. The solution was then boiled in an oil bath continuously for eight hours on three successive days, making twenty-four hours of boiling in all. The other figures were obtained after similar treatment, but the boiling lasted for only twelve hours.

These figures show that while in every case more nitrogen was separated in the bases after the longer hydrolysis, from some proteins, as, for instance, excelsin and vetch legumin, the increase was slight. From most of the other proteins a very considerably larger amount of bases was obtained, and in nearly all cases this increase extended to each one of the bases. It is thus plain that all proteins are not equally easily decomposed by boiling with sulphuric acid, and that complete decomposition requires very long boiling. It is also probable that some of the proteins, for example, phaseolin and legumelin, were not completely decomposed even after twenty-four hours' boiling, for not inconsiderable differences are still shown between the nitrogen calculated from the bases and that precipitated by phosphotungstic acid. It might be thought that the higher result by Hausmann's method is due to a loss of lysine during hydrolysis, such as Hart¹⁵ has described as taking place,

¹⁵ HART: *Zeitschrift für physiologische Chemie*, 1901, xxxiii, p. 347.

TABLE VIII.

Protein.	Histidine.	Arginine.	Lysine.	Basic N. calculated.	Basic N. precipitated.	Difference.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Excelsin, para-nut . . .	1.47	14.14	1.64	5.26	5.76	-0.50
	2.47	14.29	1.47	5.55	...	-0.21
	2.59*	13.82*	2.28*	5.59	...	-0.17
Conglutin-a	0.79	11.56	2.17	4.35	5.16	-0.81
	2.51*	10.93*	2.74*	4.77	...	-0.39
Legumin, vetch . . .	2.04	10.98	3.86	4.82	5.17	-0.35
	2.06	10.97	3.99	4.86	...	-0.31
	2.94*	11.06*	3.70*	5.07	...	-0.10
Globulin, cotton-seed	2.69	...	2.27
	2.74	10.91	2.24	4.68	5.71	-1.03
	2.11	10.49	1.58	4.25	...	-1.46
	3.46*	13.51*	2.06*	5.69	...	-0.02
Legumin, pea	2.42	10.12	4.29	4.73	5.11	-0.38
	1.80	8.01	4.17	3.86	...	-1.25
	1.99	8.36
	1.69*	11.73*	4.98*	5.20	...	+0.09
Vicilin, pea	1.93	8.40	4.28	4.04	4.92	-0.88
	1.61	6.88	4.52	3.52	...	-1.40
	2.28	7.42	3.65	3.71	...	-1.21
	2.17*	8.91*	5.40*	4.60	...	-0.32
Vignin, cow-pea	6.44	3.46
	2.18	6.38	3.95	3.40	4.28	-0.88
	3.08*	7.20*	4.31*	3.98	...	-0.30
Phaseolin, kidney-bean	1.97	4.89	3.57	2.78	3.62	-0.84
	1.74	...	3.92	2.87	...	-0.75
	...	5.13
	2.62*	4.87*	4.58*	3.15	...	-0.47
Glycinin, soy-bean . .	1.39	5.12	2.71	2.54	3.95	-1.41
	1.78	...	3.03
	2.10*	7.69*	3.39*	3.69	...	-0.26
Casein, cow	2.50	3.42	5.55	2.90	3.49	-0.59
	1.48	3.81	5.45	2.68	...	-0.81
	2.46*	3.39*	5.95*	2.91	...	-0.58
Legumelin, soy-bean .	1.55	4.40	2.79	2.38	3.71	-1.33
	2.04*	5.35*	4.91*	3.21	...	-0.59

unless a certain amount of sodium chloride is added to the sulphuric acid used in hydrolyzing the protein. We were not able, however, to confirm Hart's observation, for the amount of lysine which we obtained from a number of proteins is practically the same or even greater when no sodium chloride was used, as shown in Table IX.

TABLE IX.

Protein.	Without NaCl.		With NaCl.	
	No. of hours hydrolyzed.	Lysine.	No. of hours hydrolyzed.	Lysine.
		per cent.		per cent.
Casein	12	5.55	12	5.45
	24	5.95		
Cotton-seed, globulin . .	12	2.27	12	1.58
	24	2.06		
Legumin, vetch	12	3.99	12	3.86
	24	3.70		
Vignin	12	3.46	12	3.95
	24	4.31		
Amandin	12	0.72	12	0.47
	24			
Glycinin	12	2.71	12	3.03
	24	3.38		

It is evident from these figures that where higher results were obtained after adding sodium chloride and boiling for only twelve hours, the cause is to be found in the more energetic hydrolysis produced by the hydrochloric acid set free rather than in a destruction of the lysine.

d. The undetermined nitrogen of protein hydrolysis.—The nitrogen of the protein other than the amide and the basic nitrogen largely exists, so far as is now known, in the form of *a*-amino-acids. It is not probable that all of the decomposition products of the protein are yet known, for attempts to determine the amount of each of the known substances has in no case given a result which did not fall far short of the total of the protein. A considerable part of this deficit is doubtless made up of the known substances that are determined, for losses necessarily occur in the processes of isolating and separating them.¹⁶ This, however, probably does

¹⁶ Cf. OSBORNE and HEYL: This journal, 1908, xxii, p. 362.

not account for all of the unknown residue. If we assume that the amino-acids that are found in a well-conducted analysis of the decomposition products of a protein are united in the molecule with the elimination of water, and that the dibasic acids are united to an amide group which replaces one hydroxyl, and then subtract the sum of these radicals from 100, we can calculate the percentage of the unknown residue. If the nitrogen unaccounted for in the same analysis is calculated as per cent of this unknown residue, we find that in the case of gliadin this unknown part contains 13.3. in excelsin 14.0, and in legumin 14.3 per cent of nitrogen. Among the known decomposition products of the proteins this percentage of nitrogen is equalled only by glycocoll, alanine, tryptophane, and serine, even if the calculation is made for the radicals of the mono-amino-acids in polypeptide union, that is, after subtracting one molecule of water from their molecular weights. In making this calculation no account is taken of the fact that the amount of the amino-acids isolated in a condition fit for weighing is distinctly less than the quantities actually yielded by hydrolysis. As this loss falls mostly on substances which contain less than 13 per cent of nitrogen, the actual proportion of nitrogen in the unknown residue of the protein must be even higher than that indicated by the above calculation. As it is improbable that this unknown residue is wholly made up of undetermined quantities of the four amino-acids¹⁷ above mentioned, it is fair to presume that the proteins contain a considerable amount of some still unknown substance or substances relatively rich in nitrogen.

¹⁷ Cf. EMIL FISCHER: Sitzungsberichte der königliche preussischen Akademie der Wissenschaften, 1907, p. 35.

THE EFFECT OF SALT SOLUTIONS ON THE RESPIRATION, HEART BEAT, AND BLOOD PRESSURE IN THE SKATE.

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THE experiments recorded in this article were made upon the skates *Raia erinacea* and *Raia binoculata* in the summers of 1902, 1903, 1906, and 1907.

According to Garry² and Sumner,³ the percentage of salts in the skate and selachian's blood does not differ from that in sea water, the lowering of the freezing-point being -1.8° C. and the osmotic pressure 22 atmospheres. The sea water is therefore isotonic with the skate's blood. Baglioni⁴ estimated it isotonic with an $\frac{m}{8}$ NaCl plus 2.5 per cent urea. Although the osmotic pressure of the selachian's blood is the same as sea water, its salt content is less, but the lowering of the freezing-point is compensated by the urea in the blood.

Employing Welcker's method, I ascertained that a small skate weighing from 550 to 700 gm. possessed 25 to 30 gm. of blood, or about one twentieth of the body weight. Harris⁵ states that the weight of the blood in the skate is only one fortieth of the body weight, but he does not describe the method employed for ascertaining his data. Although there are wide variations in blood pressure, I found that the mean pressure for an average-sized skate was about 20 mm. of mercury.

During the operation the skate was placed ventral side up on an inclined board, partly submerged in water, and held in place by

¹ I desire to thank very heartily the Directors of the Marine Laboratories for the privilege of working in these institutions and for the courtesies I received there.

² GARRY, W. E.: Biological bulletin, 1905, viii, no. 4, p. 257.

³ SUMNER, F. B.: Bulletin Bureau of Fisheries, 1906, p. 596.

⁴ BAGLIONI: Zentralblatt für Physiologie, 1903, xix.

⁵ HARRIS, D. F.: Journal of physiology, 1903, xxx, p. 319.

fish netting; a constant stream of fresh sea water of a definite volume, velocity, and temperature entered the mouth through a forked glass tube covered with rubber tubing.

As soon as the water enters the mouth and flows out of the gill slits over the body, thus keeping the skate practically submerged, and the netting comes in contact with the body, the fish is perfectly quiet and remains so for hours, apparently contented and comfortable. A cannula filled with 20 per cent glucose or glucose and 1 per cent ammonium oxalate is inserted in one of the side branches of the aorta and joined to a delicate mercury manometer placed quite on a level with the blood vessel. To the open end of the manometer is attached a Hürthle manometer which records the heart action, while the pressure is read off directly from the manometer.

By means of a silk loop one of the gill arches is attached to a light lever which records the respiratory movements. In control experiments the tip of the ventricle was exposed, and by means of a silk loop joined to a light lever, so that, in addition to the above records, separate ones were obtained of the rate and force of the heart's activity.

The salts employed in the experiments were all chemically pure, either from Kahlbaum or specially prepared in the laboratory, and they were all standardized. The water employed in making up the solutions was twice distilled in glass.

To avoid mechanical stimulation a cannula to which was connected a rubber tube was inserted either in the median or lateral caudal vein and tied in place. Subsequently 4 c.c. of the solution per kilo weight of fish was slowly pressed from a graduated glass tube into the cannula by means of a rubber bulb attached to its upper end.

During the inspiratory phase the mouth and spiracles are opened, the floor of the mouth is lowered, the gill clefts or external branchial apertures are closed, and water enters the mouth and spiracles. In the expiratory phase the mouth and spiracles close, the floor of the mouth returns to normal position or is elevated, and the gill clefts open to allow the water to escape. Occasionally a peculiar spouting takes place, during which water is thrown from the mouth and spiracles. This is observed in the aquarium also, and may be due to irritation, touch, or the stimulation of the mucous lining of the mouth by slime particles or vitiated water.

I first investigated the effect of different concentrations of the same solution, seeking the weakest solution that produced any change in the three functions under consideration, also which concentration proved toxic, and whether the injurious dose could be modified or antagonized by a definite dose of another salt.

Observations were made, and curves secured before, during, and after the injection of the salt solutions. From one to five minutes after the injection a change in one or more of the functions under consideration usually occurred, and this was followed by a second change that lasted from five to twenty minutes, before the records again assumed their normal character. The first effect may be the reaction of the salt upon the constituents of the blood and indirectly upon muscle and nerve cells; the second is due to osmotic pressure changes. That injection of hypertonic salt solutions in the blood of the dog increases the osmotic pressure, which may become normal within twenty minutes, was proved by Magnus.⁶ Moreover, those salts that set free O, CO₂, H, or OH ions, in their reactions, exert their influence more often through these ions, and give results that differ from those produced by salts having the same cations, but do not react with the same resulting ion products; for example, NaCl is different in its effects from Na₂CO₃, probably because of the OH ions set free by the Na₂CO₃ dissociation.

It is possible that the blood pressure, respiratory, and cardiac activities depend upon the number of molecules and ions in the blood, and upon the dissociation tension of the salts. The prepotent anions or cations may stimulate or inhibit enzyme action, and thus aid in producing the inner stimulus that affects alike the force of cardiac and respiratory activity and directly or indirectly the blood pressure.

A careful study of very many experiments with the same salts was made, the average results summed up, and the most typical of a series of experiments put in tabular form.

Sodium chloride solutions. — In Table I it is seen that $m/32$, $m/16$, $m/8$ NaCl solutions produce, as a rule, practically no change in the heart or respiratory activity or blood pressure. An $\frac{m}{3}$ NaCl solution increases the force and blood pressure, but leaves the rate of the heart and respiration unaltered (Fig. 1).

An $m/1$ solution causes a rise in blood pressure and force of

⁶ MAGNUS: *Archiv für experimentelle Pathologie.*, 1900, xliv, p. 99.

TABLE I.

THE EFFECT OF VARIOUS SOLUTIONS ON THE RESPIRATION, HEART ACTION, AND BLOOD PRESSURE OF THE SKATE.¹

Mol. solution of substance.	Heart.			Respiration.			Blood pressure.	
	Rate.	Force.	Duration.	Rate.	Force.	Duration.	Before injection.	After injection.
NaCl <i>m</i> /32	unchanged	unchanged	min.	unchanged	unchanged		24	24
NaCl <i>m</i> /16	unchanged	unchanged	..	unchanged	unchanged	..	20	20
NaCl <i>m</i> /8	unchanged	unchanged	..	unchanged	unchanged	..	14	14
NaCl <i>m</i> <small>very</small>	unchanged	increased	..	unchanged	increased	..	26	28
NaCl <i>m</i> /1	decreased in 3 min. normal	increased	..	unchanged or decreased	unchanged or decreased	..	17	20
NaCl 2 <i>m</i>	decreased	increased then decreased	..	decreased inspiration long	decreased	..	20	23
KCl <i>m</i> /100	neutral	unchanged	..	unchanged	unchanged	..	18	18
KCl <i>m</i> /32	slower	unchanged	1	increase then less	decreased or unchanged	1	18 20	19
KCl <i>m</i> /8	decreased	unchanged	1	decreased	unchanged	..	20	20
KCl <i>m</i> <small>very</small>	decreased then ceased	increased for 30 sec. then decreased	5	increased then stopped or decreased	decreased	7	19	22
CaCl ₂ <i>m</i> /32	unchanged or decreased	unchanged or increased	1	unchanged or increased	unchanged or increased	3	20	20
CaCl ₂ <i>m</i> /8	unchanged or decreased	unchanged or increased	1-3	unchanged or decreased	unchanged or increased	1-3	17	18
CaCl ₂ <i>m</i> <small>very</small>	decreased or irregular	increased	1-3	decreased or irregular	increased	1-3	22	24 22
MgSO ₄ <i>m</i> /64	decreased or unchanged	increased or unchanged	15	decreased or unchanged	decreased	15	20	20
MgSO ₄ <i>m</i> /8	decreased or unchanged	increased or unchanged	5	decreased or unchanged	decreased	5	18	19

¹ The solutions were intravenously injected in the proportion of 4 c.c. to each kilo weight of fish.

TABLE I (continued)

Mol. solution of substance.	Heart.			Respiration.			Blood pressure.	
	Rate.	Force.	Duration.	Rate.	Force.	Duration.	Before injection.	After injection.
MgSO ₄ $\frac{m}{8}$	decreased or unchanged	decreased after increase	min. 2	decreased	decreased	3	22	22
or $m/1$ Na ₂ CO ₃ $m/32$	unchanged	increased	15	unchanged	unchanged	..	22	24
Na ₂ CO ₃ $m/8$	unchanged or less	increased	15	unchanged	unchanged	..	20	23
Na ₂ CO ₃ $\frac{m}{8}$	unchanged or less	increased	2	unchanged or decreased	increased	..	26	28
Na ₂ HPO ₄ $m/64$	less or unchanged	rise or unchanged	5	decreased or unchanged	decreased	5	23	24
Na ₂ HPO ₄ $m/8$	less or unchanged	rise or unchanged	5	increased or decreased	decreased	5	24	22
Na ₂ HPO ₄ $m/4$	less or stopped unchanged	less	5	unchanged less then rapid	decreased	3	23	19
NaOH $m/64$	unchanged or decreased	increased	5	unchanged	unchanged	..	25	26
NaOH $m/32$	unchanged or decreased	increased	15	decreased or unchanged	decreased or unchanged	..	24	29
NaOH $m/8$	unchanged	increased	10	increased	decreased	..	24	27
NaOH $m/4$	decreased or unchanged	increased	10	unchanged or increased	decreased	..	26	29
Urea $m/50$	unchanged	unchanged	15	unchanged	unchanged	15	20	20
Urea $m/1$	unchanged	unchanged	15	unchanged or decreased	unchanged or increased	1	21	21
Urea 2 m	slight rise	slight rise	2	unchanged	unchanged	15	20	20
Urea 4 m	unchanged	increased	..	unchanged	increased	..	22	21
HCl $m/64$	decreased	increased	2	unchanged	increased or unchanged	2	19	20
HCl $m/32$	decreased	increased	1	decreased	increased or unchanged	1	24	26

TABLE I (continued)

Mol. solution of substance.	Heart.			Respiration.			Blood pressure.	
	Rate.	Force.	Duration.	Rate.	Force.	Duration.	Before injection.	After injection.
			min.			min.	mm. Hg	mm. Hg
HCl <i>m</i> /8	increased	weak	1	increased	increased or unchanged	1	21	10
NH ₄ Cl <i>m</i> /64	unchanged or less	unchanged	3	unchanged or increased	unchanged or less	5	26	26
NH ₄ Cl <i>m</i> /8	unchanged or less	unchanged	15	unchanged	expiration increased	15	26	28
NH ₄ Cl $\frac{m}{\frac{1}{8}}$	much less	much less	15	long expiratory irregular	first increased then regular or lessened	15	26	22
Na ₂ SO ₄ <i>m</i> /64	unchanged	unchanged	15	unchanged	unchanged	15	26	26
Na ₂ SO ₄ <i>m</i> /2	unchanged	slight increase	15	unchanged	slight increase	15	26	27
Na ₂ SO ₄ <i>m</i> /1	unchanged	increase	5	unchanged	increased	5	27	29
BaCl ₂ <i>m</i> /64	unchanged	diastole increased	15	unchanged or less	expiration increased	2	18	20
BaCl ₂ <i>m</i> /32	unchanged	unchanged or increased	1-8	unchanged	increased	8	16	18
BaCl ₂ <i>m</i> /8	unchanged or spasmodic	increased irritability	2-5	stops or spasms	..	10-20	23	25
BaCl ₂ <i>m</i> /4	toxic	toxic
Distilled water 3-4 c.c.	decreased	slight increase	2	decreased	unchanged	2	24	26
NaH ₂ PO ₄ <i>m</i> /64	rapid in 2 min. normal	weak increased	1	increased or normal	decreased then increased	2
NaH ₂ PO ₄ <i>m</i> /16	increased	increased decreased to normal	10	unchanged	increased	10
NaH ₂ PO ₄ <i>m</i> /8	decreased then normal	increased then normal	5	increased	increased	10

heart's rhythm at once which usually continues for about twenty minutes. But the force and respiration are either unchanged for one or two minutes and then decrease, or may, as does the heart rate, first decrease and then return to normal.

Following the injection of a 2 m NaCl solution, the blood pressure increased for about five minutes and then became normal, the

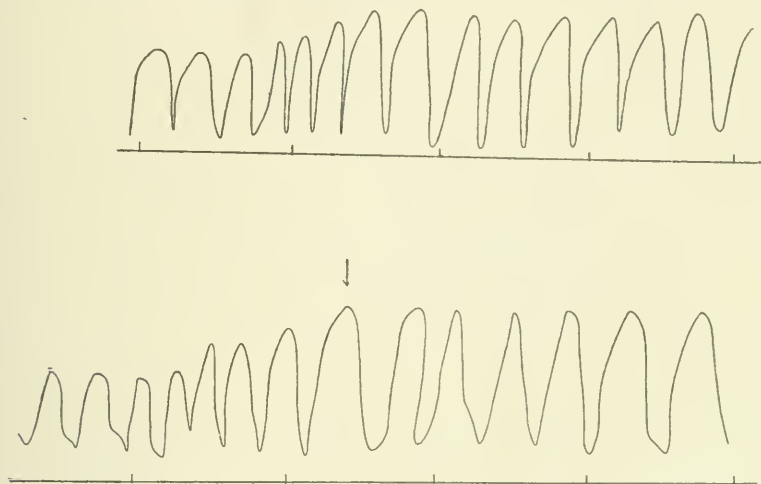


FIGURE 1.—The lower curve was drawn by a lever attached to the heart of the skate, the upper curve by a lever attached to the gill arch (respiration). The arrow marks the injection of $m/8$ solution of sodium chloride. The force of the respiration and the heart beat was increased, but the rate was unchanged.

rate and force of the heart and respiratory movements decreased for one to twenty-five minutes, but in many cases the force of the heart's activity first decreased for one to two minutes and then increased, or increased as the blood pressure rose. In every instance the systole and the inspiratory phases were prolonged above normal with a 2 m NaCl solution.

It might be concluded that the blood salts can be slightly increased by 4 c.c. per kilo $m/64$ to $m/8$ without stimulating or inhibiting any of the three functions that are considered, and that equilibrium of the osmotic pressure is rapidly established. On the other hand, $\bar{1}$ stimulates the force of both heart and respiration. I infer that its effect is directly upon the nerve centres for one to five minutes, before the attraction of water to the blood establishes equilibrium. An increase to $m/1$ has a stimulating effect

only upon the force of the cardiac centre, thereby increasing also the blood pressure, but strangely enough does not affect the respiratory centre in the same direction but rather depresses its force and rate as well as the rate of the heart's activity. But a $2m$ solution has again a like depressing influence upon both force and rate of respiratory and cardiac rhythm and at first causes a rise in blood pressure.

Potassium chloride. — An $m/32$ KCl solution produces a first effect of from twenty seconds to one minute upon the heart and respiratory activities. More often during this time the heart rate is slower, due to prolonged diastole, and the force remains unchanged. The inspiratory phase is, however, lessened, while the expiratory is relatively prolonged. In one experiment the blood pressure remained unchanged. The heart rate decreased from 24 to 22 contractions per minute, due to lengthened diastole, and the force was unchanged. The respiratory rate decreased from 36 to 30 per minute, the inspiratory phase being prolonged but the force unaltered.

After an $m/8$ KCl solution was injected, for from twenty to sixty seconds the heart activity greatly decreased and diastole was much prolonged; occasionally the action increased above the normal rate for one to two minutes, and in ten to fifteen minutes was normal. The force remained unchanged. The respiratory rate, as a rule, decreased for fifteen to thirty seconds, with prolonged inspiratory phase, then either suddenly increased above normal or gradually returned to the normal rhythm. The force was usually unchanged. The blood pressure either fell or increased slightly, about 2 mm. Hg for from one to two minutes.

Following the injection of $\frac{m}{8}$ KCl, the pressure fell either very suddenly to zero, or in rapid leaps from 20 to 16 in one minute and zero in two minutes, or often 19 to 22 in one minute, then zero within three minutes. The heart decreased in rate, for example, from 30 to 18 with increased force in one and zero in two minutes. Respiration slowed from 39 to 30 in five seconds, to 18 and very shallow and often irregular and spasmodic in ten minutes, or rapid 46 to 60 in five seconds, and gradually to zero in seven minutes, but in some cases continued actively as long as twenty minutes after the heart had ceased contracting.

The toxic influence of potassium chloride solutions is counteracted by sodium chloride and calcium chloride. The rate of the

heart's action was 36, the respiration 26. At 10.05, $\frac{m}{8}$ KCl was injected; within a few seconds the heart action became irregular and respiration rapid, then irregular with prolonged respiratory phases. The blood pressure fell. At 10.15 an injection of $\frac{m}{8}$ NaCl was followed in a few seconds by regular and strong cardiac and respiratory contractions, which in ten minutes were quite normal, but the force of the heart beat was stronger. In other experiments the toxic effect of a $\frac{m}{8}$ KCl was quickly counteracted by an $m/8$ NaCl solution.

It was also shown that a dose of $m/4$ CaCl_2 that preceded or followed an $\frac{m}{8}$ KCl or $m/1$ KCl solution, counteracted their toxic effects. For example, after CaCl_2 $\frac{m}{8}$ the blood pressure stood at 14 mm., heart action at 24, respiratory action at 36. When $\frac{m}{8}$ KCl was injected, the blood pressure fell to 2 mm., heart to four contractions, and respiration increased to 42. At the end of two minutes pressure was again 14, heart and respiratory rate had increased, though shallow. At the end of five minutes pressure and action of heart and respiration were quite normal, but respiratory rate more rapid. This shows that when a stronger solution of Ca is injected, and in the blood is in excess of the normal, it counteracts the toxic effect of $\frac{m}{8}$ solution of KCl quicker and the toxicity is less marked on the respiration than on the heart beat. In some experiments the toxic effect of KCl $\frac{m}{8}$ was overcome if CaCl_2 was injected soon after the KCl solution.

Calcium chloride. — It was observed that after an injection of the usual amount of $m/32$ CaCl_2 solution the blood pressure remained unchanged. Respiratory activity was usually increased for one to three minutes, and the cardiac contractions continued unchanged or returned to normal after about one minute of decreased rate and increased force. Following an injection of an $m/8$ CaCl_2 solution, the rate of cardiac and respiratory activity is usually decreased and the force of both increased. The blood pressure rises for about one minute. Often systole and inspiratory phases predominated, and furthermore the solution proved antitoxic to many toxic salt solutions. The effect of an $\frac{m}{8}$ CaCl_2 was first a decrease in rate of cardiac and respiratory activity, often irregular with prolonged systole and inspiratory phase. The force of both activities was usually increased, as was also the blood pressure for from one to three minutes. If the force of the heart beat was not increased, the blood pressure did not rise above normal.

Magnesium sulphate.—An $m/64$ or $m/8$ $MgSO_4$ solution has a more depressing influence on respiration than on cardiac activity. The rate was either unchanged or less, but force and blood pressure usually slightly greater for the heart, while the rate and force of respiration were generally decreased.

An $\frac{m}{100}$ or $m/1$ solution produces for about two minutes an increase in blood pressure followed by a decrease. Respiratory and cardiac rate and force all decrease, but occasionally the force of the heart's activity is first increased. Sensory reflexes are all reduced. With every strength of the solution the force and rate of respiratory rhythm and the cardiac rate were decreased, but blood pressure and cardiac force were increased or decreased after a previous increase. With strong solutions, for example, $m/1$, the diastole and the expiratory phase predominated, and the activities may become regular, but an $m/8$ $CaCl_2$ overcomes the toxic effect and increases the force of the respiratory phase and blood pressure.

Sodium carbonate.—These solutions are most effective in strengthening the force of the cardiac rhythm and thus raising the blood pressure. They do not stimulate respiratory activity except in strong doses, when they may increase the force. Their pronounced influence upon the heart may be due to OH ions that are set free by their dissociation.

Disodium hydrogen phosphate.— Na_2HPO_4 solutions are not so stimulating to cardiac rhythm as are Na_2CO_3 solutions, but for the same strength are more toxic. Like Na_2CO_3 the solutions act chiefly on the force of the cardiac action and are indifferent in weak solutions on respiratory movements.

Sodium hydroxide.—Solutions of NaOH augment in all concentrations the force of the heart beat, but they have a rather depressing effect on the respiratory centre. The OH ions here, as in the Na_2CO_3 , are the stimulating factors, but act more powerfully than in Na_2CO_3 solutions. The respiratory force decreases with increased strength of the solution, while the blood pressure rises in all concentrations employed, showing that increase in blood pressure is not necessarily associated with rise of respiratory activity but rather with force of heart beat.

Urea.—From the series of experiments with different concentrations of urea solutions, the conclusion is drawn that injection of urea solutions of high concentrations, 2 to 4 m., namely, increase force of respiratory and cardiac activity. The rate is practically

unchanged, and weaker solutions are quite indifferent to blood pressure, cardiac, and respiratory activity. It has been found by Schröder⁷ that selachian's blood normally contains 2.6 per cent of urea, and Baglioni⁸ found that the selachian's heart beats longer in urea than in NaCl. According to Loeb this is due to the OH ions which are present when the dissociation of the urea occurs. A weak solution $m/20$ of Na_2CO_3 to which a 2 m. urea solution has been added greatly increased the force of cardiac action and blood pressure.

Hydrochloric acid.— For the first minute or two weaker solutions, ranging from $m/100$ to $m/64$, increased the force of the heart beat and the respiration and raised the blood pressure, but decreased or left unchanged the rate of heart and respiration. A stronger solution $m/32$ for the first minute or two decreased the rate, but usually increased the force of both the respiratory and heart action, and the blood pressure usually rose for the first minute. $m/8$ is toxic, but the respiratory rhythm continues several minutes after the heart ceases, or if it continues, it is rapid and faint, and the respiratory movements are continued with less force and rate. It is supposed that the stimulating action is due to the H ions. In all concentrations between $m/100$ and $m/16$ there is an initial increase in pressure and force followed by a decrease in all activities before a return to either normal or less.

Ammonium chloride.— Different strengths of NH_4Cl solutions show that weak solutions are quite indifferent. An $m/8$ solution increases blood pressure and force of respiratory movements, but an $\frac{m}{4}$ depresses cardiac and blood pressure activities and greatly stimulates the expiratory phase of respiration, causing an irregular spasmodic rhythm for fifteen minutes, and this is replaced by a weak but regular activity.

Sodium sulphate.— The effects of dehydrated chemically pure Na_2SO_4 solutions are most pronounced in the strong concentration of $m/2$ and $m/1$, both of which increase the force of cardiac, respiratory, and blood pressure activities, leaving the rate unchanged. The effect lasts longer with the weaker solution, and the after effects are not, as with the saturated $m/1$ solution, a weakening of the rhythm and fall in pressure.

⁷ SCHRÖDER, V.: *Zeitschrift für physiologische Chemie*, 1890, xiv, p. 596.

⁸ BAGLIONI: *Zentralblatt für Physiologie*, 1905, xxx, No. 12.

Barium chloride. — The effects of intravenous injections of BaCl_2 solutions are increased irritability and increased force and blood pressure, especially with weak solutions. Like most of the other salt solutions, barium chloride does not increase the rate of either heart or respiration. In strong solutions it is toxic, producing at first irregular spasmodic cardiac and respiratory phases with strong diastolic and expiratory phases, and with rise of blood pressure, or cessation of activity. The toxic effects can be avoided if small doses are injected with gradually increasing strengths and the toxic effect is overcome by injections of CaCl_2 solutions.

Distilled water. — The effect of distilled water passes off quite rapidly. For the first two minutes the rate of cardiac and respiratory activity is lessened and the force of the heart beat and blood pressure slightly increased. The blood is rapidly affected and clots easily; soon the equilibrium of salts is again established and the normal action resumed.

Acid sodium phosphate. — Acid sodium phosphate solutions cause an increase in force of respiratory and cardiac activity, and for the weaker solutions at first also an increase in rate. Diastolic and inspiratory phases are often prolonged, and the toxic effect is overcome by an alkali solution of sodium phosphate or one of CaCl_2 .

CONCLUSION.

A consideration of the results tabulated in the foregoing pages discloses the facts that with the salts employed the increased force of cardiac activity is usually accompanied by increased force of respiratory action and blood pressure. But NH_4Cl *m/8*, Urea, and Na_2HPO_4 are exceptions. NH_4Cl increases blood pressure without increasing cardiac force. Urea produced increased force of respiratory and cardiac activity without raising blood pressure, and Na_2HPO_4 *m/8* increases force of cardiac but decreases force of respiratory action. Moreover, most of the salt solutions of a definite concentration that augmented the force of cardiac also increased the force of the respiratory rhythm, and proved either indifferent or depressing in their influence upon the rate of both of these activities.

The exceptions are MgSO_4 *m/8*, which stimulates force of cardiac but not that of respiratory activities and is depressing to rate,

and NaOH $m/8$, that stimulates cardiac force and rate but not the respiratory rhythm.

The toxic effect of many solutions at certain concentrations was counteracted by definite strengths of other salts. Of these antagonistic solutions CaCl_2 $m/32$ or $m/8$ proved most extensively favorable. It was interesting to learn that certain concentrations of some salt solutions prolong the cardiac systole and the inspiratory phases, while other solutions favor diastolic and expiratory phases. $m/2$ NaCl and also $\frac{m}{8}$ CaCl_2 prolong systole and inspiration, while KCl $m/8$ NaH_2PO_4 $m/8$ prolongs inspiratory activity and diastole. Moreover, strong solutions of MgSO_4 , NH_4Cl , and BaCl_2 prolong diastole and expiratory activity.

The salts that were especially depressing upon the cardiac and respiratory phases were KCl and MgSO_4 . There are certain optimum or neutral per cents of salts and acid solutions. Less concentrated solutions stimulate or temporarily increase activity, while greater concentrations inhibit or decrease activity or prove toxic. Moreover, increased blood pressure, as a rule, is accompanied by a decrease in rate of cardiac and respiratory activity.

THE INHIBITORY EFFECT OF MAGNESIUM UPON SOME OF THE TOXIC EFFECTS OF ESERIN.

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INTRODUCTION.

IN their studies on the inhibitory effect of magnesium salts upon peristalsis, Meltzer and Auer¹ experimented with peristalsis produced by eserin, and remarked that "magnesium sulphate completely inhibited the general muscular tremor caused by eserin." On the other hand, Matthews and Jackson² in a later study failed to obtain this result. In both papers the statements concerning the inhibitory effect of magnesium upon the eserin tremor were only incidental and were very brief. At the suggestion of Dr. Meltzer, in order to clear up the contradiction and to throw more light upon the point in question, I made it the subject of a special investigation. At the same time the eserin tremor was studied by the graphic method, and, among other things, the possible value of magnesium salts as an antidote for eserin poisoning was investigated.

THE GRAPHIC PRESENTATION OF ESERIN TREMOR.

I am not aware that the literature contains any account of an attempt to obtain a graphic record of the muscular tremor caused by eserin. In the present investigation such a record was especially desirable, since it could serve as objective evidence in the decision of what takes place upon the injection of magnesium.

Various methods were tried (chiefly on dogs), but the most satisfactory was the following: The etherized animal was fastened in the supine position, lying partly on one side with both hind legs

¹ MELTZER and AUER: This journal, 1906, xvii, p. 317.

² MATTHEWS, S. A., and JACKSON, D. E.: This journal, 1907, xix, p. 12.

tied to the same side of the board. This brought uppermost the external surface of the thigh, to which the tambour was to be attached. An ordinary receiving tambour with a collar button contact was fastened to the skin by passing two bands of tape at right angles over the back of the tambour and stitching the tape to the skin at the margin of the tambour. With the leg flexed as described, the skin over the outside of the thigh is under considerable tension, and stitching to the skin holds the tambour in good apposition to the underlying muscles. Moreover, in this position there is a minimum of transmission of respiratory movements, — which interfered greatly with the tambour in any other position, especially when the respiration was increased in depth after small doses of eserin.

Examination of the tracings obtained shows that there is a rather marked regularity in the rate of fibrillary contraction. The lowest number counted for a period of five seconds was 30, the highest 38, that is, an average of about 34 for five seconds, or 400 per minute. Apparently the *rate* does not vary greatly as the effect of an individual dose of eserin progresses. The *strength* of the muscular contractions does vary, however, quite markedly, being much stronger soon after the injection than at a later time. There are two types of movements to be distinguished. After an injection of eserin one of the first evidences of the toxic effect is a sort of writhing convulsive movement caused by the contraction of whole muscles, or groups of muscles. These will be referred to in this paper as convulsive movements. The second type of movement is a fibrillary contraction. It is this movement which appears as a tremor. Typical fibrillary contractions did not develop usually till after the convulsive movements were quite marked. As a rule, the convulsive movements disappeared by the time the fibrillary contractions were pronounced, — at least they were much more transitory in character than the fibrillary contractions.

In a few animals the convulsive movements seemed to predominate throughout, while in others the fibrillary contractions were the more prominent of the two.

The following tracing (Fig. 1) was obtained as already described. It shows the muscular tremor and convulsive movements caused by eserin.

THE ACTION OF MAGNESIUM ON THE MUSCULAR TREMOR
CAUSED BY ESERIN.

In this series of experiments the animals were etherized, tracheotomy performed, and cannulas inserted in both right and left anterior jugular veins. One cannula was used to inject magnesium sulphate, the other for injecting eserine sulphate. The magnesium sulphate was used in $m/1$ solution and injected from a burette. The eserine in one-half per cent solution was injected from a 1 c.c.

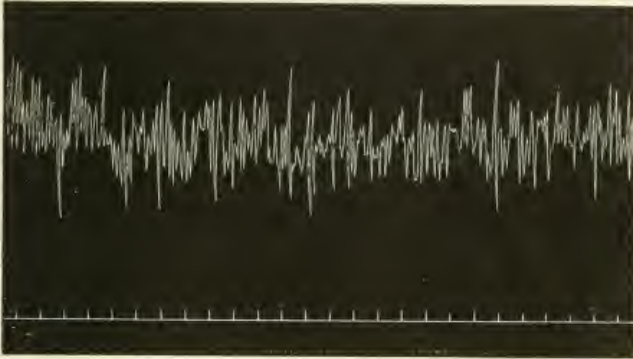


FIGURE 1.—The individual oscillations of the writing-point represent fibrillary contractions, while the irregular wavelike variations in level, each composed of several fibrillary contractions, represent convulsive movements.

hypodermic syringe, the needle of which was thrust through the wall of a short piece of rubber tubing on the distal end of the cannula. Two cubic centimetres of normal saline were injected slowly immediately after the eserine to make sure that all the eserine had entered the circulation. In most cases arrangements were made to take tracings of the tremor by the graphic method, as described above. The animal was always allowed to recover from the effects of the ether before proceeding with the experiment.

The method of procedure was varied somewhat in different experiments. In the majority of cases the dosage of eserine was from 1 to 3 mgm., and in several animals this dose was repeated several times at short intervals. Sometimes the magnesium sulphate was started before the height of the eserine effect had developed, at other times a little later. To test this point there were twelve experiments performed on dogs and four on rabbits.

Results. — No matter what the variation made in method or time of injection the result was an invariable stoppage of all convulsive movements and tremor. This stoppage was brought about in from one-half to one and one-half minutes, according to the rapidity of injection of magnesium. If the dosage of magnesium was sufficient to thoroughly overcome the eserin tremor, there was never a recurrence of the fibrillary contraction or convulsive movements. When doses of magnesium sulphate insufficient to completely overcome the eserin effect were employed, the eserin effect usually returned some-

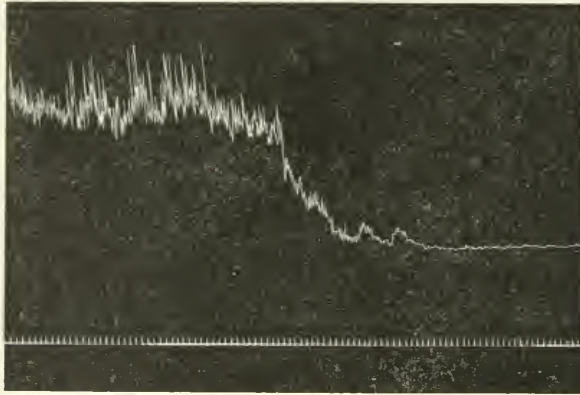


FIGURE 2. — To be read from left to right; 5.25 c.c. $MgSO_4$ were slowly injected. Even the very fine movement shown at the right of the tracing disappeared before the injection was finished. There was no return of the eserin effect during the following eighty minutes.

what after the action of magnesium had worn off. The effect which returned, however, was very easily controlled by an additional small amount of magnesium. It was found that an animal recovered not only more quickly, but with less danger to life, if the magnesium were given in repeated small doses with several minutes' intermission rather than in a single large dose.

Fig. 2 is taken from an experiment in which all movements stopped after slowly injecting m/l magnesium sulphate. In this tracing 2 mgm. of eserin were followed in two and one-half minutes by a slow injection of $5\frac{1}{4}$ c.c. of m/l magnesium sulphate. The tremor stopped in about thirty seconds, and during the next eighty minutes (the length of time the experiment was continued) there was no return of fibrillary contractions or convulsive movements.

The following protocol in which a moderate dose of eserin was given will serve to show in detail the points above mentioned.

- Experiment 11, February 27, 1908.* — Bitch, 5450 gm., good condition, young.
- 10.39. Ether, tracheotomy, cannula in jugular.
 - 11.39. Animal quiet, lid reflex active, respiration 92, heart 116.
 - 11.39. 2 mgm. *eserin* and 4 c.c. saline.
 - 11.41. Respiration much faster and deeper, pupils wide, convulsive movements marked.
 - 11.42½. Respiration 140, lid reflex active, fibrillary contractions becoming strong.
 - 11.45. Convulsive movements somewhat reduced, but fibrillary contractions very strong.
 - 11.48. Respiration 156, strong fibrillary contractions, *start magnesium sulphate* (m/1) jugular.
 - 11.54. *Stop magnesium sulphate* — 4½ c.c. in. All fibrillary contractions and quivering gone. Respiration good, regular, 56 per min. Heart 160, regular, fairly strong.
 - 12.06. There are some slight fibrillary contractions over areas which are quite muscular. Lies quietly. Heart 96. Respiration 88, shallow.
 - 12.12. *Start magnesium sulphate*. There are very slight muscular movements.
 - 12.14. *Stop magnesium sulphate* — ¾ c.c. in.
 - 12.19. No fibrillary contractions or convulsive movements.
 - 12.23. By very close observation a fine fibrillary contraction can be made out occasionally on shoulder. None anywhere else.
 - 1.30. Animal still, quiet. Heart 148, regular, strong. Respiration 32.

MAGNESIUM SULPHATE AS AN ANTIDOTE FOR ESERIN.

In the foregoing experiments it was apparent that animals which received magnesium were benefited and indeed in some cases were prevented from dying. This suggested then a systematic study to find how far magnesium could be used as an antidote for eserin, and the most effective dose.

This point was tested on rabbits only, and in almost every experiment two animals were used, — one animal used as a control received eserin only, while the other received both eserin and magnesium sulphate. The eserin was always injected intramuscularly in the lumbar region, in doses which were usually sufficient to kill. The magnesium was injected intravenously through the ear vein.

These animals were not operated at all, and consequently no etherization was necessary. In some cases the injection of magnesium was not begun until the eserine effect was marked or the animal seemed in danger of dying. In other cases the injection of magnesium sulphate followed immediately the injection of eserine. In a total of twenty-two trials the animals survived heavy toxic and fatal doses. The general tremor and convulsive movements stopped after the injection of from 2 to 4 c.c. of $m/1$ magnesium sulphate. The depression of respiration due to such large doses of magnesium was always rather marked and was sometimes sufficient to cause danger of death from asphyxia. In such cases the intravenous injection (ear vein) of 1 c.c. of $m/1$ calcium chloride was usually sufficient to counteract the depression. It was interesting to note that if too much calcium chloride (2 to 4 c.c.) was given, some of the eserine effect almost always returned in a short time.³

Another method of giving the magnesium which was tried was to inject just enough intravenously to prevent immediate death from eserine, and then to inject the remainder of the magnesium — part intramuscularly and part subcutaneously in the back. In this way the magnesium, except what was immediately necessary, was only slowly absorbed. The favorable effects resulting from this slow absorption of the magnesium were twofold: The action of eserine was kept down for a much longer period, and the depression of respiration was not so marked as it would have been had all the magnesium been given intravenously.

The action of eserine develops so swiftly that, if the dose is heavy, it is difficult to do anything to prevent death. The average time which elapsed between the injection of eserine and death in six of the *control* rabbits was slightly less than eight minutes, with doses of eserine varying between 2 and $3\frac{1}{2}$ mgm.

In seven experiments the intravenous injection of magnesium sulphate was begun immediately or within one minute after the injection of eserine — that is, before there was any visible effect of eserine — and continued very slowly till 2 to 4 c.c. were given.

No visible tremor could be made out in these experiments during four to seven minutes after the injection of eserine, and typical muscular tremor and convulsive movements either never developed or were only feeble in strength and transitory. The longer the

³ Here we have an instance where calcium chloride brings out the fibrillary contractions instead of inhibiting them.

time which elapsed between the injection of eserine and the injection of magnesium sulphate, the greater the danger to the life of the animal. Moreover, under these circumstances larger doses of magnesium sulphate were necessary, and recovery was delayed.

The following protocols — one in which the animal received eserine only, the other in which both eserine and magnesium sulphate were given — will serve to illustrate the antidotal effect of magnesium :

Experiment (A). Control. Eserine only. April 2, 1908. — White female rabbit

1470 gm. Good condition, young adult.

10.51. 2 mgm. eserine — intramuscularly, right lumbar region.

10.54. Fibrillary contractions fairly strong. Strong convulsive movements.

10.56. Getting worse very rapidly — lies prostrate. Strong convulsive movements. Fibrillary contractions very strong.

10.58. Only an occasional respiration. Heart almost stopped. Lid reflex gone. Fibrillary contractions and convulsive movements reduced somewhat.

10.59. Dead.

Experiment (B). Eserine and Magnesium. March 24, 1908. — Male rabbit,

1485 gm., good condition, young adult.

2.29. 2 mgm. eserine — intramuscularly, lumbar region.

2.32. Strong quivering of whole body; convulsive movements have begun.

2.34. Weakness developing very swiftly; convulsive movements exceedingly strong, fibrillary contractions becoming marked.

2.35. $1\frac{1}{2}$ c.c. $m/1$ magnesium sulphate — ear vein.

2.37. Fibrillary contractions practically gone. Convulsive movements all gone. Considerable muscular weakness. Lies at full length on belly. Can hold head up.

3.00. Still quite weak. Fibrillary contractions have returned slightly; much improved. Got up when touched. Respiration has been excellent since giving magnesium. Heart in good condition also.

3.10. Very much better. Placed on floor, walks about with ease, but prefers to be quiet. A few feeble fibrillary contractions.

3.20. Seems perfectly recovered.

In the two following protocols one animal (the control) received eserin only, while the other received eserin, magnesium, and calcium. In the latter case the magnesium was given part intravenously and the remainder subcutaneously and intramuscularly instead of giving all of it intravenously.

Experiment 49 (A). Control. Received eserin only. April 13, 1908. — Female rabbit, 2130 gm., fine condition.

10.53. 3 mgm. eserin, intramuscularly, right lumbar region.

10.56. Powerful convulsive movements, and fibrillary contractions.

10.58. Gaspings, lies at full length on belly. Fibrillary contractions and convulsive movements still strong.

11.00. Dead. Convulsive movements gone. Fibrillary contractions persist strongly.

11.10. Fibrillary contractions still fairly strong.

Experiment 49 (B). Received eserin and magnesium. April 13, 1908. — Male rabbit, 2400 gm. Fine condition.

10.53. 3 mgm. eserin, intramuscularly, right lumbar region.

10.55. Began slowly injecting magnesium sulphate, ear vein.

11.00. (2½ c.c. magnesium sulphate are in) no sign of eserin effect.

11.03. Stop magnesium sulphate — 4 c.c. injected altogether. Some awkwardness in moving about. There have been no signs of fibrillary contractions or convulsive movements. Animal appears remarkably well.

11.06. No fibrillary contractions, but there are some movements of hind legs as if attempting to get up.

11.06. 2 c.c. magnesium sulphate. Intramuscular, lumbar region.

11.15. No fibrillary contractions at any time so far. Respiration good. Heart excellent. Lid reflex active. Pupils about normal. There has been some defecation during the past few minutes. Cannot sit up.

11.20. Beginning to come out from magnesium effect. No fibrillary contractions. But there are some movements of hind legs, chiefly flexion and extension.

11.25. Eserin effect gradually increasing; also respiration poor, due to magnesium. 2 c.c. calcium (m/1) ear vein. After the calcium the heart very poor, probably injected too rapidly.

11.30. 1 c.c. magnesium sulphate intramuscularly.

11.35. There have been no fibrillary contractions at any time. Respiration is much improved now. Lies quietly.

11.44. Improving rapidly. No fibrillary contractions. No convulsive movements. Heart regular. Lid reflex excellent. Raised himself up on fore feet, hind legs drawn up under him.

- 11.50. Sits on haunches continuously now.
- 12.00. Placed on floor; walks about, but is a little awkward.
- 12.30. Perfectly recovered.

THE EFFECT OF MAGNESIUM SULPHATE ON ESERIN MYOSIS.

One of the best known effects of eserin is the production of myosis. It became of interest to know whether magnesium would inhibit this action of eserin on the pupil. Seven experiments were performed on rabbits to test this point. In all except two of these, controls were used. One or two drops of eserin in one-half per cent solution were dropped into each conjunctival sac. The effect of eserin, both local and general, began to appear in from eight to sixteen minutes after instillation. The pupil gradually became constricted.

The general effects were the same as those resulting from intramuscular or intravenous injections of small doses of eserin, and if untreated they lasted from one to one and a quarter hours. The myosis in untreated animals lasted usually about two to two and a half hours or more.

The action of magnesium upon this myosis, and also on the general effects caused by instillations of eserin, was first tried by injecting small doses of magnesium by the ear vein. One to 2 c.c. of *m/1* magnesium sulphate removed the fibrillary contractions and hyperesthesia and apparently the excessive peristalsis, and left the animal in a very much improved condition. This dose of magnesium, however, had no effect on the myosis. Two experiments were then performed in which the animals were etherized, tracheotomy performed, and a cannula inserted in one anterior jugular vein. After recovery from the ether, conjunctival instillations of eserin were made, and when the myosis was well developed, magnesium sulphate (*m/1*) was slowly injected from a burette connected with the jugular cannula. As soon as the respiration became shallow, artificial respiration was begun and continued during the remainder of the experiment. The magnesium was injected at the rate of 1 c.c. or less per minute. It was continued until the heart had stopped. The myosis was not at all affected by the magnesium in either experiment.

DISCUSSION.

As stated above, the stimulus which prompted this investigation was the contradictory statements with regard to the inhibitory effect of magnesium salts upon eserine tremor. The experiments here noted are overwhelming evidence for the fact that magnesium sulphate can abolish the muscular tremor of eserine. Large doses of magnesium inhibit the tremor completely and permanently. Smaller doses of magnesium salt will abolish or reduce the tremor only temporarily. This temporary effect of the smaller dose is even stronger evidence of the inhibitory action of the magnesium, since it cannot be claimed that the stoppage of the tremor was simply due to the wearing off of the eserine effect. The inhibitory effect is in direct proportion to the dose of magnesium and in inverse proportion to the dose of eserine; that is, the inhibition is the more complete the larger the dose of magnesium and the smaller the dose of eserine.

Without entering into a discussion as to the nature of this inhibition, it may be stated that the point of attack of both substances is probably not the same, and that magnesium suppresses the tremor only symptomatically, not neutralizing the poisonous effect of eserine at the point of attack.

As to the reason for the failure of Matthews and Jackson to notice the inhibitory effect of the magnesium salt, it is difficult to form a positive opinion on account of the brevity and indefiniteness of their report. There is, however, one point in their statement which might account for their failure. They used $2\text{ m}/1$ solutions of magnesium sulphate in intravenous injections. Such a concentrated solution, even if injected very slowly, is capable of stopping the heart as soon as it reaches it, and thus would prevent the circulation from carrying the magnesium to the periphery.

The present investigation has also demonstrated that magnesium sulphate is capable of mitigating other dangerous symptoms produced by eserine, and that thus magnesium may come into practical consideration as an antidote for eserine poisoning. The dose of magnesium which may be required might in some cases be dangerously large. Here, however, a timely administration of a calcium salt would readily overcome the ill effects of magnesium.

Finally, the experiments with instillation of eserine demonstrate that while magnesium sulphate can overcome certain general effects

produced by instillation of eserin, the myosis cannot be overcome by any amount of the salt.

SUMMARY.

The following points have been demonstrated by the experiments reported on in this paper: that the eserin tremor can be registered successfully; that magnesium unquestionably abolishes eserin tremor; that magnesium has a certain value as an antidote for eserin poisoning, and that magnesium has no influence whatever upon eserin myosis.

I wish to express my indebtedness to Dr. S. J. Meltzer, at whose suggestion and under whose direction this investigation was carried on.

THE PROLONGED EXISTENCE OF ADRENALINE IN THE BLOOD.

BY D. E. JACKSON.

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I. EARLIER EXPERIMENTS.

IN a series of experiments performed in 1904 it was shown by Weiss and Harris¹ that if a solution of the active principle of the suprarenal glands be injected into a cat and blood from this cat be transfused into a second cat, after the vaso-constriction had disappeared in the first, a rise in blood pressure would be produced in the second cat. These investigators were able to demonstrate the vaso-constrictor action in the second cat when the transfusions were made at intervals of five, seven, and thirty minutes after the adrenaline solution had been injected into the first cat. By a different method they also confirmed these observations upon frogs. In these animals they clamped the vessels of one of the hind legs and then injected adrenaline into the aorta or into the heart directly. After the general vaso-constriction, as seen by microscopical observation of the circulation in the web of the normal hind leg, had disappeared, they unclamped the vessels of the other leg and allowed the blood to circulate freely through it. Following the return of the circulation to this limb they were able to observe a marked vaso-constriction in the vessels of that web also.

R. Ehrmann,² following the suggestion of Meltzer,³ obtained similar results. He used the frog's eye as an indicator, and determined the presence of adrenaline in the blood by the mydriasis which it produced. By this means he also showed that the blood of the suprarenal vein contains adrenaline.

¹ WEISS and HARRIS: *Archiv für die gesammte Physiologie*, 1904, ciii, p. 510.

² EHRMANN: *Archiv für experimentelle Pathologie*, 1905, liii, 2, p. 97.

³ MELTZER: *This journal*, 1904, xi, p. 454.

De Vos and Kochmann,⁴ using rabbits, repeated the experiments of Weiss and Harris. They withdrew blood from the carotid artery of the first rabbit and reinjected it into the jugular vein of the second, and arrived at the conclusion that no special rise in pressure would be produced in the second rabbit by blood withdrawn from the first after ten minutes had elapsed from the time of the last injection into the first rabbit.

The condition revealed by these experiments is very peculiar. An active and extremely potent drug is carried in the circulation directly to the smaller blood vessels and arterioles at a time when these structures are apparently perfectly normal and are remarkably sensitive to freshly added quantities of the same drug, and yet the adrenaline remaining in the blood from the former injection ceases to have any perceptible action whatever on the structures which it at first stimulated.

II. THE INJECTION OF ADRENALINE BLOOD INTO THE SAME ANIMAL FROM WHICH THE BLOOD HAD BEEN TAKEN.

Methods. — Most observers have experimented upon frogs, cats, or rabbits in investigating the continued existence of adrenaline in the blood of an animal after the disappearance of the visible effects produced by an intravenous injection of the drug. In the work described in this article dogs have been used in all but a few cases in which experiments were performed upon cats. The adrenaline chloride (1:1000) of Parke, Davis, and Company has been employed throughout. The animals were anæsthetized with ether.

The first experiments were performed in order to determine whether an animal will again show a reaction to injections of its own blood withdrawn after the visible effects of a previous injection of adrenaline have disappeared. In each case the animals were arranged in the ordinary manner for taking blood-pressure tracings, and cannulas were inserted into the femoral artery and femoral vein. Blood was withdrawn from the artery and reinjected into the vein. The blood pressure was recorded from the carotid artery. Immediately after its withdrawal the blood was whipped and the clots carefully removed by filtration through cotton. The tempera-

⁴ DE VOS and KOCHMANN: Archives internationales de pharmacodynamie et therapie, 1905, xiv, p. 83.

ture was kept at about 38° C. by immersing the beaker in a basin of water whose temperature was regulated by means of a gas flame and a thermometer. Blood was withdrawn at various intervals after the injection of adrenaline, and the quantity of adrenaline injected each time varied from 1/20 c.c. of a 1:10,000 solution up to 2 or 3 c.c. of the 1:1000 solution, the object being to test the reactions under as many different conditions as possible.

Discussion of results. — If a dog receive an intravenous injection of 1/2 c.c. to 2 c.c. of the 1:1000 solution of adrenaline (this amount is sufficient to produce a great rise in blood pressure), and a quantity of blood varying from 15 c.c. to 40 c.c. be withdrawn before the blood pressure has entirely regained its normal equilibrium, and then this blood (after having been whipped and carefully filtered free from clots) be reinjected into the same animal, there will result a rise in blood pressure. This rise may depend very largely, if not entirely, upon the increased volume of blood which results from the injection, and in many cases may be almost exactly reproduced by the injection of an equal volume of salt solution. The following experiment will illustrate this point:

Dog: weight, 5.5 kilos. — Anaesthetized with ether. Bled 25 c.c. while dog was normal. Blood whipped and kept at 38° C. Injected 2 c.c. 1:10,000 adrenaline, and when the pressure returned to normal, bled 40 c.c., whipped and kept at 38° C.

1st injection, 15 c.c. of blood withdrawn after adrenaline. Pressure rose from 106 mm. to 112 mm. Difference, 6 mm. Pressure lowered a little by further bleeding.

2d injection, 15 c.c. of blood withdrawn before adrenaline. Pressure rose from 75 mm. to 81 mm. Difference, 6 mm.

3d injection, 15 c.c. salt solution. Pressure rose from 76 mm. to 81 mm. Difference, 5 mm.

It was also observed that in many cases the fall which was produced by the withdrawal would almost exactly equal (often exceed) the rise which was produced by the reinjection of the given volume of blood. This is perhaps mainly accounted for by the fact that when blood is withdrawn from a large artery, as the femoral, there is an immediate lowering of the pressure in the main arterial system. This is at once recorded by the manometer. When, however, blood is reinjected into a vein, a certain period of time will elapse before the results are shown in the aorta. During this time the in-

jected blood is distributed to the lungs, and a slight readjustment of the vessels may take place before the rise is recorded from the carotid. So that the withdrawal of a certain amount of blood may actually cause a fall greater than the rise produced by the reinjection of an equal volume of blood. The part which adrenaline plays in producing the rise when blood is reinjected depends upon the variation from the normal which the blood pressure possessed at the time of the withdrawal of the blood. If the withdrawal be made when the arterial pressure is greatest, then the secondary rise produced by this blood will bear all the marks of a typical adrenaline rise. This effect decreases with great rapidity as the blood pressure returns to normal. The results are exactly the same, no matter whether the blood be reinjected into the same or a different dog, as will presently be shown.

If, upon reinjection, the blood which has been withdrawn after adrenaline will produce in any dog a rise characteristic of the drug, it will generally do so in the animal from which it was taken. There, however, appears to be considerable individual peculiarity in this respect, and the variation seems to depend largely upon the dog as well as upon the adrenaline content of the blood injected. It should also be mentioned in this connection that after a number of injections an animal becomes less and less sensitive to adrenaline.

In conclusion it may be stated that if the circulation has entirely regained its normal equilibrium before the withdrawal of blood, then the rise produced by the reinjection of this blood will not bear the characteristics typical of the adrenaline rise, and the results may be exactly paralleled by the injection of an equal volume of blood withdrawn from the animal before the injection of adrenaline.

III. THE INJECTION OF BLOOD FROM AN ADRENALINE ANIMAL INTO A NORMAL ANIMAL.

Methods. — In the second series of experiments transfusions of blood were made from one dog into another. The animals were arranged as previously described, and blood pressure tracings from each were obtained simultaneously upon the same kymograph. In the earlier experiments transfusions of blood from one dog to the other were made by withdrawing the blood from one dog, whipping it, and reinjecting it into the other dog. In the later experiments

this method was abandoned, and transfusions were made directly from one dog to the other by means of glass cannulas inserted into the femoral artery of one dog and connecting with the femoral vein of the other dog. In many cases two sets of cannulas were used, these being arranged so that transfusions could be made from the first dog into the second, or, *vice versa*, from the second dog into the first. In this manner it was possible to check the results obtained from each animal. Ordinary arterial cannulas of the T-pattern were used for these transfusions, the short arms of two cannulas being connected by a short piece of rubber tubing. A burette was connected to the long (or straight) arm of one cannula, and a short piece of rubber tubing with a clip was placed on the long arm of the other cannula. In this manner both cannulas could be readily filled with salt solution and the air expelled. The arrangement possessed the disadvantage that a small amount of salt solution would be carried over with each injection, and, also, that the exact quantity of blood transfused could not be measured. The first objection, however, is not significant. In regard to the second it may be said that a close approximation to the amount transfused could usually be made by measuring the amount of salt solution which would pass through the injecting cannula in a given time, and then comparing the rise produced in this time with the time normally required to produce a similar rise by transfusion from the other dog. Variations in blood pressure in the dog furnishing the blood would, of course, affect the results somewhat. With a large number of experiments, however, in which transfusions varying in quantity through a wide range were made, the measurement of the exact amount of blood transfused at each time became less important.

Time of transfusion. — In these experiments emphasis has been laid not so much upon the time which elapsed between the primary injection and the transfusion as upon the relation which the rise in arterial tension produced in the second dog bore to the variation from the normal which the blood pressure of the first dog possessed at the time of the transfusion or withdrawal of blood. It seems evident that the rapidity with which adrenaline is destroyed in the tissues varies considerably in the different species, and to some extent in individuals of the same species. It is probable that this has had an important bearing upon the variation in results which have been obtained by previous observers.

Results of transfusion. — If a dog receive intravenously an in-

jection of from 1 c.c. to 2 c.c. of 1:1000 adrenaline, and if, while the pressure is at a considerable elevation above the normal, a transfusion of from 15 c.c. to 50 c.c. of blood be made into another dog, then a rise in pressure will be produced in the latter animal. The extent and character of this rise will depend upon the condition and reaction of both animals, and the quantity and adrenaline content of the blood transfused. It sometimes occurs that blood from one dog will have a tendency to produce a fall when injected into another. This may directly affect the tracing. Also, if the first dog be greatly fatigued, the results in the second dog may be somewhat influenced. If the transfusion be made during the time while the pressure in the first dog is at a very considerable height above the normal, then the rise in the second dog will in almost every case bear the typical characteristics of an adrenaline rise (Fig. 1). This is exactly what one would expect from the nature of the case. The second dog would simply receive a very much diluted portion of the original drug, which possibly had remained entirely unaffected by its passage through the first dog. I have, however, observed in a large number of cases that a rather noticeable secondary effect usually takes place in the second dog.

Secondary fall. — This consists in a very decided fall in pressure following upon the rise (Fig. 2). This fall generally goes for some 10 mm. to 25 mm. below the normal, but the pressure soon rises again to near (usually a little above) its original level. I was at first inclined to believe that this fall might be due to small quantities of the original drug which had undergone a partial decomposition or transformation in the first dog. Later, however, I was led to believe that the fall was largely produced by depressor substances which resulted from the increased metabolism of the tissues themselves in the first dog.

IV. THE LOSS OF POWER OF ADRENALINE IN THE BLOOD.

It was stated on page 229 that if a dog be given an intravenous injection of adrenaline, and if, after its circulation has entirely regained its normal equilibrium, blood be withdrawn from the animal, the reinjection of this blood intravenously into the same dog will not produce an adrenaline rise in its blood pressure. And this result also holds good if the blood be reinjected into another dog, or if transfusions be made directly from the first dog into the

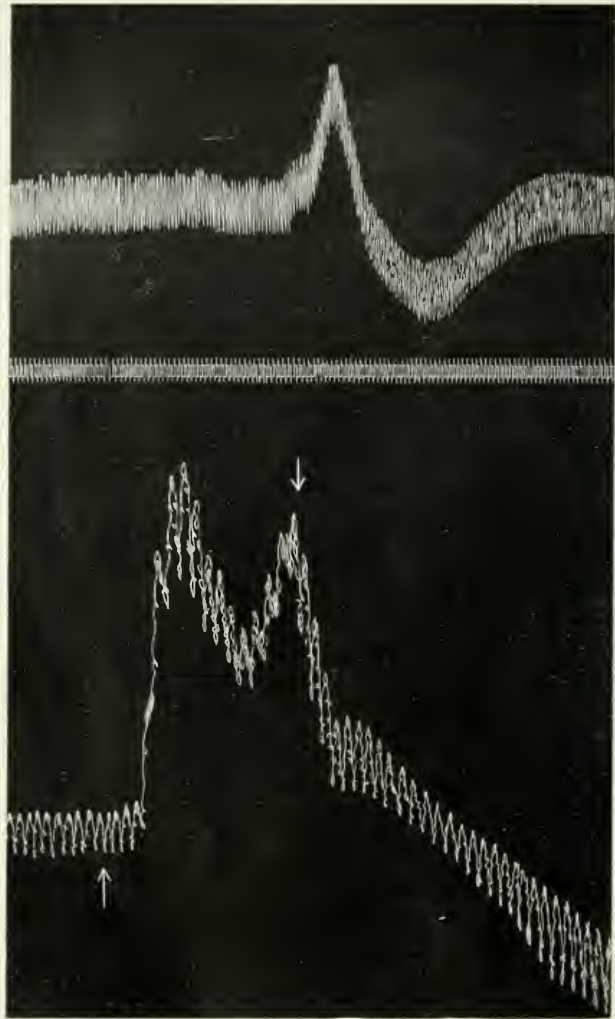


FIGURE 1. — Curves of the carotid blood pressure in two dogs. Normal pressure of lower tracing (Dog I) 95 mm. Hg; of upper tracing (Dog II) 142 mm. Hg. Time in second intervals. At the first arrow in the lower tracing, Dog I received intravenously 3 c.c. of 1:5000 adrenaline chloride. The pressure rose to 182 mm. Hg. One minute after the injection of the drug the pressure had fallen to 160 mm., and blood was transfused (second arrow) for fifteen seconds directly into Dog II (upper tracing). This tracing probably shows the effects of adrenaline transfused into the second dog.

second. There are a number of conceivable ways in which a loss of power on the part of the drug might be accounted for. Among the most probable of these may be mentioned fatigue, the alkalinity

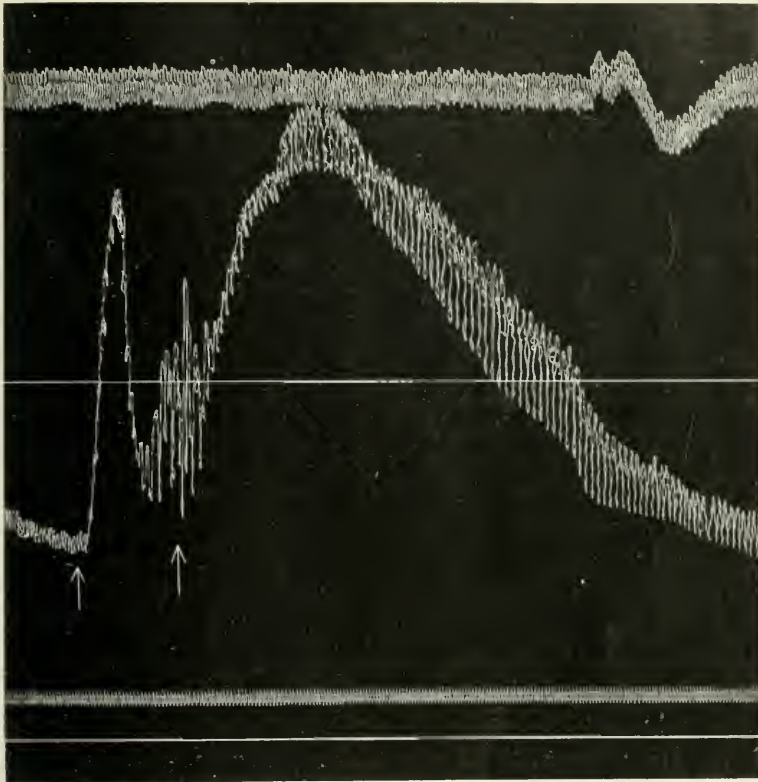


FIGURE 2. — About four fifths the original size. Carotid blood pressure in Dog I (lower curve) and Dog II (upper curve). The lower horizontal line is atmospheric pressure for lower carotid curve, the upper horizontal line is atmospheric pressure for the upper curve. Time is written every second. At the first arrow in the lower tracing Dog I received 5 c.c. of 1:5000 adrenaline chloride. Two minutes later Dog I was bled for fifteen seconds into Dog II (upper tracing). At the second arrow in the lower tracing the vagi nerves were cut.

of the blood, the formation of an anti-body, excretion, and oxidation. Concerning each of these a few words may be said, and other features bearing on this action of the drug will be discussed later in this article.

Fatigue. — That the failure of the vessels and certain smooth muscles to remain contracted during prolonged periods following

the injection of adrenaline is not largely due to fatigue is readily shown by the fact that the action of the drug may be brought on many times in succession by merely continuing to inject small quantities of adrenaline into the blood. Table I will illustrate this point. The consecutive injections were made at the time when the blood pressure had fallen (after the rise produced by the preceding injection) to near its original level. This time varied considerably in the individual cases. The adrenaline solution was of the strength of 1:10,000.

Table I shows plainly that fatigue of the vascular system cannot account for the fugitive effects of the drug if it continues to exist in an active form for some time after it is injected. The period of time elapsing between any two successive injections in the above experiment was, on an average, about one and one-half to two minutes.

Alkaline solution. — It was early shown that adrenaline is unstable in an alkaline solution.⁵ It is evident, however, that this is not the cause of the failure of adrenaline to produce a prolonged action in this case; for although the blood is of a weakly alkaline reaction, it was long ago demonstrated by Oliver and Schäfer⁶ that adrenaline remained perfectly active when dissolved in blood and allowed to stand for a period of twenty-two hours, either with or without free access to the air. Since 1895 this observation has been fully corroborated.⁷ In fact, the earlier part of this work consisted in a confirmation of the statement that adrenaline remains active for a considerable time when mixed with blood outside the body. This was tried several times, and the results were perfectly uniform throughout. No perceptible loss of power was produced in the adrenaline when mixed with the blood for any length of time up to half an hour. No attempt was made to test for any destruction of the drug beyond this time, as the results would have had but little bearing upon the present experiments. For example, 1 c.c. of a solution of 1:10,000 of adrenaline chloride in water produced a rise in the blood pressure of 62 mm., and the injection of 1 c.c. of a solution of whipped blood in which the same proportion of adrena-

⁵ B. MOORE: *Journal of physiology*, 1895, xvii, Proceedings of the Physiological Society, p. xiv.

⁶ OLIVER and SCHÄFER: *Journal of physiology*, 1895, xvii, Proceedings of the Physiological Society, p. xiii.

⁷ EMDEN and VON FUERTH: *HOFMEISTER'S Beiträge*, 1903, iv p. 421

line had been dissolved and left standing at a temperature of 38° C. for a period of twenty minutes, produced a rise of the same pro-

TABLE I

THE RISE OF CAROTID BLOOD PRESSURE FOLLOWING CONSECUTIVE INJECTIONS OF ADRENALINE SOLUTION INTO THE FEMORAL VEIN OF A DOG.¹

Number of injection.	Quantity injected.	Blood pressure.		
		Before injection.	After injection.	Difference.
	c.c.	mm. Hg.	mm. Hg.	mm. Hg.
1	1.0	138	252	112
2	1.5	137	274	137
3	1.5	137	269	132
4	1.5	133	264	131
5	1.5	132	256	124
6	1.5	132	257	125
7	1.5	138	262	124
8	1.5	137	268	131
9	1.5	137	274	137
10	1.5	138	250	112
11	1.5	140	253	113
12 ²	1.5	144	249	105
13	1.5	132	239	107
14	1.5	129	231	102
15	1.5	133	241	108
16	1.5	132	238	106
17	2.0	126	241	115
18	1.5	132	239	107
19	1.5	133	231	98

¹ The dog weighed 10 kilos. Pressure taken from right carotid artery. Drug injected into right femoral vein.

² Pressure had not fallen to normal at time of injection.

portions and character as that produced by the pure adrenaline chloride solution.

Anti-body. — It was shown by Elliott and Durham⁸ in 1906 that no anti-body, in the ordinary sense of that term, was formed when an animal was given hypodermic injections of adrenaline at intervals of a day or so for a period of several weeks.

Excretion. — We may consider that not more than the merest traces of the drug, if any at all, are thrown off in the pure form in the ordinary excretions of the body; for during the brief period of increased blood pressure the kidneys almost entirely cease to excrete. And in four dogs I securely clamped the aorta, the inferior vena cava, and the azygos veins just above the diaphragm. In these animals the injection of adrenaline into the jugular vein produced a perfectly normal rise in blood pressure. But the time required for the pressure to fall again to its former level was somewhat prolonged. That, however, would be expected, since the usual influence of the visceral vessels was prevented from affecting the recorded pressure. These experiments show that the heart, lungs, and anterior half of the dog's body may give normal reactions to adrenaline, and that the effects of the drug disappear in approximately the usual length of time. If adrenaline should be excreted in any of the ways by which drugs are usually eliminated, we should expect that the greater part of such elimination would take place in the organs lying posterior to the diaphragm. Table II will show the action of the drug on the anterior half of the animal's body.

I might add that in two dogs, in addition to the vessels mentioned above, I also clamped off the vessels going to and coming from the fore limbs, part of those to the head, and most of the smaller vessels going to the thoracic wall, etc. (artificial respiration was used). These manipulations, of course, greatly influenced the extent and character of the blood pressure tracings obtained. So far as could be determined from the tracings, however, the effects of an injection of adrenaline disappeared in about the usual time. In one of these dogs about as much adrenaline was required to stop the heart (between 4 and 5 c.c. of 1:1000) as would probably have been required to kill the animal when intact. These experiments show that none of the visceral organs are necessary in order for an animal to overcome the ordinary vascular effects of an injection adrenaline.

⁸ ELLIOTT and DURHAM: *Journal of physiology*, 1906, xxxiv, p. 400.

Oxidation. — As the pressure in the first dog descends from the highest point reached after an injection of adrenaline towards the original level, the effect which the adrenaline may have upon the rise produced by transfusion into the second dog decreases with

TABLE II.

THE RISE IN BLOOD PRESSURE FOLLOWING THE INJECTION OF ADRENALINE SOLUTION IN A DOG IN WHICH THE AORTA, THE INFERIOR VENA CAVA AND THE AZYGOS VEIN WERE CLAMPED ABOVE THE DIAPHRAGM.

Number of injection.	Quantity injected.	Carotid. Blood pressure.			Time required for return to original height.
		Before injection.	After injection.	Difference.	
	c.c.	mm. Hg.	mm. Hg.	mm. Hg.	
1	1.0	144	203	64	2.0
2	0.75	150	188	38	1.5
6	1.0	100	188	88	2.5
7	1.0	138	186	48	1.5

A medium-sized, yellow dog, in good condition, was etherized and given artificial respiration. The chest was opened and the aorta, the inferior vena cava, and the azygos vein were clamped above the diaphragm. The blood pressure was recorded from the right carotid artery. Injections of adrenaline solution (1:10,000) were made from a burette into the left jugular vein. When the aorta was clamped, the pressure rose from 100 mm. to 144 mm.

great rapidity (see Figs. 1, 2, and 3). This is undoubtedly due largely to the rapid destruction (oxidation) of the adrenaline. It seems, however, that a number of other factors are concerned. There can be no question but that a certain amount of fatigue is produced in the circulatory system by the injection of a heavy dose of adrenaline. A number of other structures outside of the heart and vessels are also brought into activity. This strong muscular contraction will have two results which may have an immediate bearing upon the reaction produced in the second dog: first, the fatigue of the vascular system will help to conceal the amount of adrenaline which actually remains over for a short time in the blood of the first dog; and second, a small amount of fatigue products will be produced in the first dog, and may be transfused over to the

second, in which their presence may again help to modify the results observed (cf. Secondary fall, p. 231).

Dilator action.—It was mentioned above that fatigue in the first animal would, in a great many cases at least, have an influ-

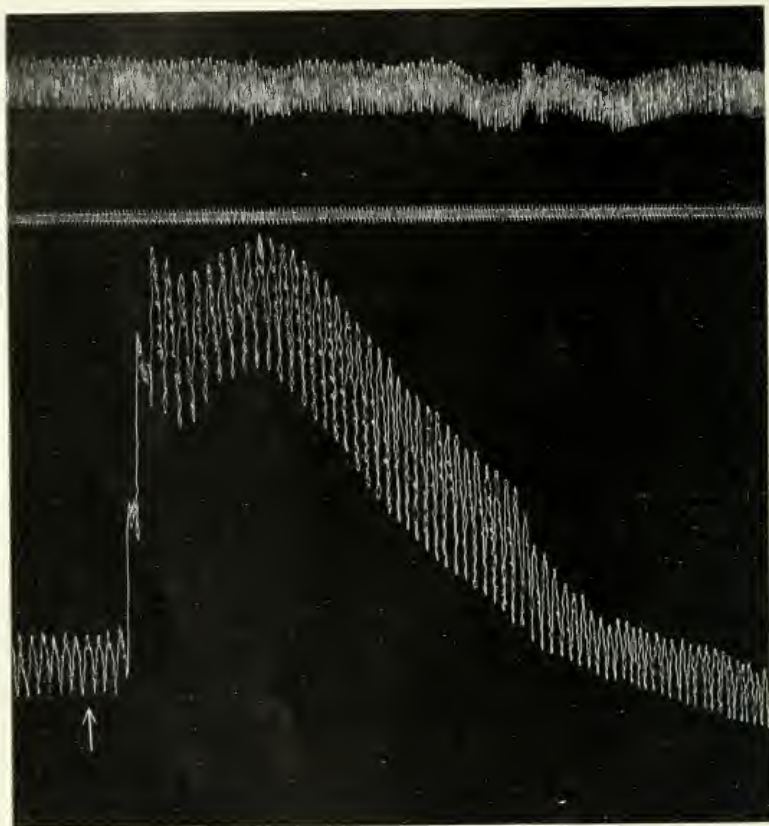


FIGURE 3. — The carotid blood pressure in Dog I (lower curve) and Dog II (upper curve). The middle line is time in second intervals. At the arrow in the lower tracing Dog I received 3 c.c. of 1:5000 adrenaline chloride. One and one-half minutes later Dog I was bled for fifteen seconds directly into Dog II (upper tracing).

ence upon the rise in the animal receiving the blood; and there is evidence that still another factor is concerned in the action.

In a long series of experiments performed upon dogs in which a normal rise and fall of blood pressure was produced by adrenaline, it was found that, following upon the rise, the pressure in most cases descended for some distance below its original level. The extent of this fall from the original height varied considerably in

the individual dogs, and also to some extent with the quantity of adrenaline injected. In regard to the latter, however, it should be stated that a small dose of the drug was often just as effective as a large dose in producing the fall. The extent of this descent below the original pressure was usually from 4 to 15 mm., and the pressure usually regained its former equilibrium in about half the time which had been occupied by the rise above the normal pressure. By many transfusions into a second dog it was determined that, in general, in those cases in which there was a marked descent of the pressure below the original level, transfusions of blood made from the first dog during the earlier part of this period of depressed pressure would usually show, to an appreciable extent, the effects of adrenaline in the second dog. But after the pressure had reached its lowest level and had actually begun to ascend, then, in practically no case whatever, was it possible to demonstrate the presence of adrenaline by a rise in pressure in the second dog.

There can be no doubt but that fatigue of the vascular system in the first dog is, in part, responsible for the descent of the pressure below the original level. Since, however, the presence of adrenaline may be demonstrated during the earlier part of the period of lowered pressure, it seems probable that the adrenaline itself may also be partly responsible for the slight vaso-dilatation. It has been repeatedly shown⁹ that under various conditions adrenaline may produce a dilatation of the arterioles. And it seems not unlikely that after a brief period of extreme activity the fatigued vaso-constrictors begin to relax, and, as the vessels approach their normal calibre, the adrenaline remaining in the blood expends its action in part, if not even entirely, upon the vaso-dilators. By such a combined reaction of the drug its presence in small quantities may be concealed for a short time. In a certain number of cases it was found that no noticeable descent of the pressure below the normal was produced after a rise. And in these dogs it seemed that the adrenaline disappeared more rapidly than in the others.

Limit of influence. — It would appear that transfusion of only a comparatively small quantity of blood containing such a dilute solution of adrenaline would not show any typical adrenaline rise in pressure in the second dog at all. And, as a matter of fact, this is the result usually obtained, the extreme limit of the period

⁹ T. SOLLMANN: This journal, 1905, xiii, p. 246; H. DALE: Journal of physiology, 1906, xxxiv, p. 175.

during which the pressure in the second dog may show the presence of adrenaline being, as a rule, the time at which the pressure in the first dog reaches its lowest level. And the limit may be reached a great deal earlier than that (Figs. 2 and 3). The following protocol will illustrate the brevity of the period during which transfusions from the first dog may show typical adrenaline effects in the blood pressure of the second dog.

Dog I; weight, 12.3 kilos. Dog II; weight, 8.6 kilos. — Both in good condition. Both etherized and placed side by side on the operating-table. Left femoral artery of Dog I connected with right femoral vein of Dog II. Transfusions made from the larger dog (I) into the smaller (II). Manometer tracings from right carotid artery of each dog taken simultaneously upon the same drum. Tracing from Dog II recorded above tracing from Dog I. Time marked in seconds. Fresh preparation of adrenaline; strength used, 1:5000. Adrenaline injected into Dog I by means of a burette connected with the right femoral vein. Normal pressure of Dog I = 141 mm., of Dog II = 150 mm. Hg.

11.43. Injected into Dog I 0.75 c.c. Pressure rose from 144 mm. to 190 mm. Hg. Difference, 46 mm. Hg.

11.45. Injected 1 c.c. Pressure rose from 120 mm. to 181 mm. Difference, 61 mm.

11.46½. Injected 2 c.c. Pressure rose from 118 mm. to 218 mm. Difference, 100 mm.

11.49. Injected 5 c.c. Pressure rose from 118 mm. to 274 mm. Difference, 156 mm.

11.51. Two minutes after injection, pressure fallen to 186 mm. Bled into Dog II for fifteen seconds. During time of transfusion pressure in Dog I fell about 12 mm.

Dog II. Pressure rose from 150 mm. to 158 mm., then fell to 141 mm., then rose to 156 mm.

12.02. Injected 4 c.c. into Dog I. Pressure rose from 112 mm. to 215 mm. Difference, 103 mm.

12.04. Pressure fallen to 125 mm. Bled into Dog II for ten seconds. During bleeding pressure in Dog I fell about 12 mm.

Dog II. Pressure rose from 150 mm. to 156 mm., then fell to 136 mm., then rose to 152 mm. This shows the secondary fall (of 14 mm.) mentioned above.

12.10. Injected 3 c.c. into Dog I. Pressure rose from 100 mm. to 218 mm. Difference, 118 mm.

12.11½. Pressure had fallen to 150 mm. Bled into Dog II for fifteen seconds. During bleeding pressure in Dog I fell 20 mm.

Dog II. Pressure rose from 150 mm. to 158 mm., then fell to 152 mm., then rose to 156 mm.

12.20. Injected salt solution into Dog II for ten seconds (15 c.c.). Pressure rose from 136 mm. to 144 mm. Difference, 8 mm.

12.33. Injected 3 c.c. into Dog I. Pressure rose from 95 mm. to 182 mm. Difference, 87 mm.

12.34. One minute after injection. Pressure had fallen to 160 mm., *i. e.*, 65 mm. above normal.

Bled into Dog II for ten seconds. During bleeding pressure in Dog I fell 20 mm.

Dog II. Pressure rose from 142 mm. to 157 mm. This tracing probably showed the effects of adrenaline in the second dog. It was made one minute after the primary injection, and the pressure in the first dog had fallen only 22 mm. from the highest point reached when the transfusion was made (Fig. 1).

12.41. Injected 25 c.c. of salt solution into Dog I. Pressure rose from 88 mm. to 106 mm. Difference, 18 mm.

1.02. Injected 5 c.c. into Dog I. Pressure rose from 112 mm. to 212 mm. Difference, 100 mm.

1.04. Pressure fallen to 112 mm. Bled into Dog II for twenty seconds. During bleeding pressure in Dog I fell 36 mm.

Dog II. Pressure rose from 142 mm. to 146 mm., then fell to 134 mm., then rose to 140 mm.

1.07. Injected 10 c.c. into Dog I. Pressure rose from 92 mm. to 224 mm. Difference, 132 mm.

1.08½. Pressure fallen to 168 mm. Bled into Dog II for twenty seconds. During bleeding pressure in Dog I fell 61 mm.

Dog II. Pressure rose from 144 mm. to 154 mm., then fell to 137 mm.

1.30. Dog I killed.

1.40. Dog II killed.

Only one transfusion (that at 12.34) in this series appeared to show the typical effects of the adrenaline on the blood pressure of the second dog. While all the others showed an elevation of pressure in the second dog when the transfusions were made, still, the character of these rises was entirely different from the typical adrenaline curve. I do not believe that this was due to a slow transfusion of the blood, for this was forced from the artery of Dog I under much greater pressure than that under which drugs are ordinarily injected.

Effusions. — In a few experiments performed upon cats it ap-

peared that the ability of these animals to destroy adrenaline was certainly less than in the case of dogs. I was not, however, able to observe a continued existence of the drug in a cat's blood for more than two or three minutes, and in most cases much less than that. It was observed by Lesage¹⁰ that in cats a large injection of adrenaline may often cause pericardial effusion and thereby greatly lessen the effectiveness of the heart's action. In many cases this would easily conceal the presence of a small quantity of adrenaline in the animal's blood.

Different species. — The results of these experiments upon dogs compared with the results which others have obtained upon different animals seem to indicate very definitely that the rise produced in a second animal by the transfusion of blood from a first after the disappearance of the visible effects of a previous injection of adrenaline varies considerably with the species. It seems that the dog can carry on a much more rapid destruction of the drug than can be effected by the cat or rabbit. Weiss and Harris¹¹ obtained a rise of 15 mm. of mercury in a second cat when the transfusion was made thirty minutes after the last injection of adrenaline into the first cat. I have been absolutely unable to obtain even the faintest indications of such a result in dogs. To be sure, the transfusion of blood into a second dog will give a rise in pressure in most cases at any interval after the first had received adrenaline, or even if no adrenaline at all had been given. But the character of a rise produced by adrenaline is considerably different from one produced by the simple injection of blood alone. In the latter case the rise is fairly permanent, while the adrenaline rise is transient, and is always followed by a fall.

Overdose. — It is conceivable that in case an animal should receive an enormous quantity of adrenaline, it might continue, for a while at least, to survive when its blood contained more of the drug than its tissues could either destroy or continue to react to. A transfusion made under such conditions could, of course, show but one result. In case the animal survived, however, the drug would have been destroyed by the time the blood pressure had finally regained its normal equilibrium.

¹⁰ LESAGE: Archives internationales de pharmacodynamie et therapie, 1904, xiii, p. 245.

¹¹ WEISS and HARRIS: Archiv für die gesammte Physiologie, 1904, ciii, p. 510

Lack of oxygen. — In a few cases it was observed that when the respiration had stopped from the administration of a slight excess of ether, but when the blood pressure was still high and the circulation was apparently perfectly effective, then the injection of adrenaline had no noticeable result for a considerable time. That is, the rise which would normally appear in a few seconds was greatly delayed. The immediate suggestion as to the cause of this would be that the vascular system in general, and the heart in particular, were so depressed, and that the lack of oxygen was so great, that a reaction at once to the adrenaline was not possible; or that the blood stream was so slow that the drug was not distributed over the body for several minutes. It is probable that all these factors contributed, in part at least, to the result. There can be no doubt, however, but that the drug can remain in the blood of an animal which has ceased breathing, for a very noticeably prolonged period of time before its general effects are produced. But when once the action has been fully established, then the destruction of the drug is effected synchronously with it, and the substance and its action both rapidly and completely disappear.

Distribution. — A consideration, which, though small, is certainly not to be overlooked in estimating the adrenaline content of blood by the transfusion method, is that a sample of blood withdrawn, for example from the femoral artery, may not be entirely representative of all the rest of the blood in the body at that particular instant. If there be considerable constriction of the arterioles in various areas, then undoubtedly there will be slight variations in the time of relaxation in different regions, not all of which could have been acted upon equally by the adrenaline injected. Consequently the adrenaline content of a sample of blood withdrawn from a particular vessel at a given time may not be perfectly representative of the blood in all the rest of the animal's body at the instant of withdrawal.

At first it may seem that this variation in the adrenaline content of the blood in different parts of the body is too small to affect the results of transfusion into a second animal. When, however, it is recalled that the drug is not mixed in equal proportions with all the blood of the body at the time of injection; that the action of the drug is only of brief duration; that the mechanical conditions of the circulation are not such as to ensure immediately perfectly thorough intermingling of a small injection of the drug with the

whole volume of the blood; that in all probability some areas of the body possess a greater power than others to destroy adrenaline; that only extremely small quantities of the drug are required to affect the blood pressure, and the great dilution in which the adrenaline must exist in any of the blood of the first dog, — then it becomes evident that the possibilities for even the slightest variations in the adrenaline content of the blood transfused are not to be disregarded.

V. CONCLUSIONS.

1. When injected intravenously into a dog, adrenaline ceases to act in approximately the usual length of time when the blood has no access to most of the excretory organs of the body.

2. If a dog receive intravenously an injection of adrenaline varying from 1 c.c. to 2 c.c. of 1:1000 adrenaline, and if, while the pressure is at a very considerable height above the normal, a transfusion of from 15 c.c. to 40 c.c. of blood be made from this dog into another, a characteristic adrenaline rise will probably be produced in the second dog.

3. Blood withdrawn from a dog, after it has received an injection of adrenaline, will, when reinjected, produce a rise of the same character, no matter whether it be injected into the same or a different dog.

4. When a dog has received an injection of adrenaline and its blood pressure has again entirely regained its former equilibrium, the rise produced by transfusion of its blood into another dog (or reinjection of the whipped blood into the same dog) will not show the presence of adrenaline (Fig. 3).

5. The presence of adrenaline was not shown by the character of the rise in pressure produced in a second dog by transfusions which were made when more than one minute's time intervened between the injection of adrenaline and the transfusion of the blood (Figs. 1 and 3).

6. So long as the blood pressure of a dog remains greatly elevated after an injection of adrenaline, a transfusion of its blood would simply carry over a small portion of the still visibly active drug. Since the length of time required for the disappearance of the effects of an injection of the drug varies greatly, it is evident that transfusions into a second dog should be made with reference

to the variation from the normal which the blood pressure of the first dog possesses at the instant of the withdrawal of blood, rather than with reference to the length of time intervening between the primary injection and the transfusion.

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THE RÔLE OF INORGANIC PHOSPHORUS IN THE NUTRITION OF ANIMALS.¹

BY E. B. HART, E. V. MCCOLLUM, AND J. G. FULLER.

[*Contribution from the Chemical Laboratory of the Wisconsin Agricultural Experiment Station.*]

THE importance of the ash constituents of various food stuffs for the maintenance of a normal condition of health in man is indicated by the disturbances which follow all attempts of various observers² to subsist any length of time on an ash-free diet. Forster's³ experiments on dogs and pigeons with a diet composed of ash-free carbohydrates and fats and of meats containing only a small amount of salt point to the same conclusion. Lunnin's⁴ experiments with mice, somewhat modified to overcome the acidity resulting from proteid sulphur metabolism by addition of carbonate of soda, but with the other essential ash constituents missing, likewise failed to sustain these animals.

The recognition by Bunge of the relationship between the ash content of milk and the time required to double the weight of newborn animals of various species is in this connection extremely interesting. For example, the required time for doubling the weight in the following species, man, horse, cow, and dog, is, respectively, one hundred and eighty, sixty, forty-seven, and nine days. The percentage of ash in the milk of these different animals in the order named is 0.22, 0.41, 0.80, and 1.31. From this he arrives at the conclusion that the more rapidly the suckling grows, the greater the needs of the organism for those food stuffs which serve for the building up of the tissues, namely, proteins and salts. These experiments and our general understanding from other lines of

¹ Published with the permission of the Director of the Wisconsin Experiment Station of the University of Wisconsin.

² TAYLOR: Studies on an ash-free diet, Publications of the University of California, Pathology, 1904, i, p. 71.

³ FORSTER: Zeitschrift für Biologie, 1873, ix, p. 207.

⁴ LUNNIN: Zeitschrift für physiologische Chemie, 1881, v, p. 31.

inquiry of the part played by the ash constituents in intestinal digestion and absorption of proteids and fats, in favoring the action of ferments in intracellular proteolysis and lipolysis and the accelerating action of various alkaline salts on the conduct of carbohydrates and various other organic complexes, to say nothing of the development of skeletal tissues rich in calcium phosphates, only emphasize the important rôle played by the mineral constituents of the body in the absorption and metabolism of food and in body growth.

The practical significance of this subject of the relation of ash constituents of feeding stuffs to animal nutrition has very lately received direct comment.⁵

It has been observed that some of the locally grown feeding materials on the Hawaiian Islands, especially the forage crops, are deficient in lime. This is attributed to the fact that they were grown on volcanic soils. Animals receiving such feeding materials failed to make good growth, and their yield of milk was unsatisfactory.

Investigations in the Transvaal⁶ on certain locally grown feeding materials have also demonstrated that these materials show a deficiency of calcium in proportion to phosphoric acid, and to this deficiency has been attributed a lack of thrift and other unfavorable symptoms with horses and mules receiving these feeding materials.

Considerable attention, both in this country⁷ and abroad,⁸ has been devoted to the problem of supplementing certain feeding materials either with complex ash mixtures, such as wood ash, or with more limited definite salts, such as the calcium phosphates. These efforts have been undertaken either in the hope of finding suitable agents for combating the pathological condition known as rickets, or in aiding the development of body tissues in young and growing animals receiving rations believed to be deficient in the necessary ash constituents.

In the case of rickets the evidence seems to support the contention that calcium phosphate is a useful supplement to the diet, but it is still an open question whether this salt subserves a useful function when added to the ration of normal food materials.

⁵ Experiment Station Record, 1908, xix, p. 803.

⁶ H. INGLE: *Journal of comparative pathology and therapeutics*, 1907, xx, p. 35.

⁷ HENRY: Sixth annual report, Wisconsin Agricultural Experiment Station, 1889, p. 15.

⁸ N. SCHENKE: *Landwirthschaftliche Versuchs-Station*, 1903, lviii, p. 19.

Again, in experiments where but a single grain has been used, such as corn, supplemented with ash materials for the nutrition of growing swine, no consideration has been given to the limiting power of the organic nutrients from a single source for the complete and normal development of the animal. This factor, as well as the limitation and relative proportions of ash constituents, in a ration must be given careful consideration by students of nutrition.

Our own problem was to determine whether inorganic phosphates, such as di- and tri-calcium phosphates, could take the place of organic forms of phosphorus in a ration for growing swine. The growing young, in its rapid cell expansion and consequent increasing nuclei formation, with an accompanying increase of nucleins and nucleo-proteids, presents admirable advantages for such studies. It is apparent that if such a fundamental process as nucleo-proteid and phosphatide formation in the animal body is a chain of syntheses, involving an inorganic form of phosphorus, then the comparative values of forms of phosphorus contained in the ration, be they lecithins, nucleins, phytates, or inorganic phosphates, in so far as they are carriers of phosphorus, become a matter of secondary importance. Whatever special influence they might exert would then more properly lie in the nature of the non-phosphorus group to which the phosphorus group is attached. It would extend the already widely established range of synthetic processes in the animal cell.

In a previous article⁹ a partial history of the subject of phosphorus metabolism experiments was given, and for lack of space no attempt will here be made to discuss the experiments of earlier investigators bearing on this subject.

A summary of definite conclusions from the evidence given in the literature would be a difficult matter. However, it appears to be well established: (1) that added ash constituents are absorbed from the digestive tract; (2) that animals receiving rations somewhat low in calcium and phosphorus, or these elements in improper proportions, with brittle bones as the result, do absorb added calcium phosphates with remedial effect; (3) that even with normal rations, added calcium phosphate is absorbed with increased bone formation. Some of the questions still in contradiction or totally uninvestigated in this matter are: (1) How far must the ash in-

⁹ JORDAN, HART, and PATTEN: Technical Bulletin No. 1, New York Agricultural Experiment Station, 1906.

redients be in organic combinations to be of nutritive value? (2) What is the relative value of the form of the ash constituents in the ingested food, for the formation of specific bodies in important secretions rich in these ash constituents? (3) What are the minimum limits of ash constituents for normal vigorous development? (4) What is the influence of the proportion of base to acid-forming constituents of the ash on metabolism? (5) Can an excess of ash constituents in the food materially influence the ash constituents of specific secretions like milk?

The problem undertaken by us concerned itself primarily with the study of the limiting power of the animal organism to supplement inorganic phosphates for organic forms of phosphorus in its growth. It was believed that such studies would throw light on the synthetic limitations of the animal cell in so far as the forms of raw material for nucleo-proteid building were concerned, and the question of how far phosphates added to a ration deficient in such ash materials could be utilized. It was believed that if it could be established that a ration so low in phosphorus as to retard normal development and induce pathological symptoms in young animals could produce normal development when fortified with calcium phosphate, or with organic forms of phosphorus such as phytin, it would aid in giving a definite solution to our problem.

Plan of the investigation. — Our studies for the last two years have been conducted with young growing pigs, and have been arranged in accordance with the following plan:

1. Feeding several lots of pigs over long periods of time, rations differing greatly in the form of phosphorus which they supplied, the quantity of phosphorus furnished to be as nearly equal in all cases as possible.

2. One lot, to be designed the basal lot, was to receive as low a phosphorus ration as it was possible to prepare.

3. Another lot, to be designed the standard lot, was to receive a mixture of normal feeds carrying a variety of forms of phosphorus.

4. The other lots were to receive a basal ration, fortified with the forms of phosphorus, whose influence it was desired to study.

5. All rations were to be maintained on the same nutritive plan as far as our knowledge would permit.

6. Composition of the feeds was to be accurately known.

7. In a limited number of cases the rations were to be carefully sampled and weighed and the excreta quantitatively collected.

8. The weight of the animals was to be taken periodically.

9. After continued feeding over a long period of time, the tissues of the animals were to be subjected to such chemical examinations as were essential to the elucidation of the problem.

EXPERIMENT I.

The rations. — It was necessary to provide a ration as low in phosphorus as possible. This was secured by the use of rice, wheat gluten, and washed bran. This gave us our basal ration, to which was added the form of inorganic phosphorus to be tested. When the washed bran was substituted directly by wheat bran, we had under experiment the organic form of phosphorus, phytin, removed by washing whole wheat bran. The removal of phytin from the bran not only lowered the phosphorus content of the bran, but also lowered the magnesium and potassium content. These losses were returned to the basal ration by the proper addition of a mixture of 200 gm. of cane sugar and 100 gm. each of sodium chloride, magnesium chloride, and potassium sulphate. The proportion of these mixtures added to the daily ration will be indicated later.

Five lots of pigs were used. Lot 1 received the basal ration; lots 2 and 3 received the basal ration, but in addition different amounts of precipitated calcium phosphate, lot 2 receiving about 40 gm. per pig, and lot 3 about 20 gm. per pig daily. The precipitated phosphate used was shown to consist of a mixture of 70 per cent di-calcium phosphate and 30 per cent tri-calcium phosphate. Lot 4 received a ration composed of rice, wheat gluten, and whole bran. This supplied phosphorus, magnesium, and potassium in the organic complex, phytin. A fifth lot was fed a standard ration for growing pigs, consisting of corn, oats, wheat middlings, and oil meal, and which furnished a variety of forms of phosphorus.

The experimental animals. — Sixteen vigorous young pigs of mixed breeding were used in the experiment. All males were castrated. The average weight of the pigs in all lots was 47 pounds. At the beginning of the experiment they were in fair flesh, apparently healthy and vigorous.

Management of the experiment. — The animals were confined within the hog barn during the entire experiment. All pens were bedded

with pine wood shavings. This the animals fortunately did not eat, thereby introducing no vitiating factors in the intake of phosphorus. Feeds were mixed weekly. This was very carefully done, in order to make the daily ration as constant as possible.

A preliminary feeding period of two weeks preceded the actual initial date of the experiment. This was done in order to gradually accustom the animals to the rather severe ration. All were allowed equal amounts daily. During the course of the experiment, as the pigs required it, the amounts of the different rations were gradually increased. The animals were weighed individually once a week at a definite time.

Methods of analyses. — Such analytical data as were required were collected on a carefully selected sample of the feeding materials, or on selected parts of the slaughtered animal at the termination of the experiment.

The preparation of the skeleton. — The bones were carefully scraped as free from meat as possible, stirred in hot water for a short time, to further facilitate complete removal of meat, and dried at 60°. After the above treatment all determinations of ash were made on the air-dried bones.

Sequence of feeding. — Feeding began on November 29, 1906. A preliminary period of about two weeks was consumed in initiating the final rations. This was reached on December 15. The final mixture of feed that appeared best adapted for this work and would furnish a proper supply of nutrients consisted of 28½ pounds of ground rice, 6 pounds of wheat gluten, and 15 pounds of bran, either washed or whole, as the case demanded. To this amount of feed mixture was added 500 gm. of a mixture of 200 gm. of sugar, 100 gm. each of sodium chloride, magnesium chloride, and potassium sulphate, respectively. It is remarkable to what a high degree of palatability the ration was brought by the salt mixture addition. It was impossible to secure a proper food consumption until this addition was made.

Where phosphates were being studied, 420 gm. and 840 gm., respectively, of precipitated calcium phosphate were added to the above quantities of the washed bran ration.

The initial consumption per pig was about 2½ pounds per day. This was increased, as the animals demanded it, until on March 1 the average consumption of all animals but those receiving the basal and standard rations was approximately 3 pounds per day.

The pigs receiving the basal ration did not increase their food consumption appreciably above the initial amount. The lot receiving the standard ration consumed at this date, March 1, $3\frac{1}{3}$ pounds daily per pig. The experiment covered ninety-five days.

The tables that follow are condensed from a considerable body of data, and were limited to those which are essential to an analysis of our conclusions.

Discussion of the data from Experiment I. — The discussion to follow can best be given by a consideration of the separate history of each lot. Throughout the experiment it was attempted to hold the amounts of digestible organic nutrients as nearly alike in all the rations as possible. Their character in the standard ration, as compared with the other rations, may have materially differed.

Lot 1, basal lot. — The intake of phosphorus in the basal ration was reduced to 1.12 gm. per pig daily, when the consumption was 2.2 pounds of feed. That was as low as it was practicable to prepare considerable quantities of feeding material. The phosphorus in this ration was wholly of an organic type, including nucleins, lecithins, and probably traces of phytates. At the beginning of the experiment, December 15, it was found that the addition of the sugar-salt mixture greatly enhanced the appetite of the animals and aided in creating a relish for this feed. After a few weeks, however, their appetites showed signs of languishing, with a decreased activity of the animals themselves, as compared with the animals of all other lots. A great deal of time was necessary for the consumption of the daily ration. These conditions manifested themselves more and more as the experiment progressed. It became the habit of the animals to confine themselves to their sleeping-pens in a lying posture from meal to meal. By January 17 one of the animals showed stiffness of the hind legs and a partial loss of their control. A few days later the other animals of the same lot manifested similar symptoms. In no other particular were the animals visibly affected. They continued to gain in weight. These attacks of weakness of limbs and stupor would disappear after a few days, to again return for a more prolonged period. The periods of attack and partial recovery alternated in this manner during the experiment. By the end of January it became necessary to assist the animals to their feeding-troughs. Their appetites were poor. They continued to lie a large share of the time in a dormant, stupefied condition. Excitability was at a low ebb. When standing, the

hind limbs assumed an oblique position, the hind feet resting far beneath the body and near the fore feet. When in this position, they would slowly lift one foot from the floor, drawing the leg toward the body to again relax the contracted limb. About February 12 one animal in this lot entirely lost the power of sustaining its hind quarters. The fact that up to this time the animals receiving the basal ration had gained in weight, showed fair flesh, and a fairly healthy external appearance, although physically weakened, is worthy of special notice. By March 12 all animals in the lot were in a low state of vitality. Loss of weight had begun, and very little feed was consumed; they were induced to move with the greatest difficulty and apparently not without pain. By March 20 the animals were so weak that further continuance of the experiment was abandoned. One animal from the lot was killed, and an effort to restore to health the two remaining animals was made. It was planned to restore the debilitated animals by supplementing the basal ration in the one case with organic phosphorus and in the other with inorganic phosphates. However, the pigs were in such a weakened condition that a more palatable ration had to be given. Milk and wheat middlings were used, with immediate results. Rapid improvement resulted, and on June 8, after a lapse of nearly three months, these two animals, 409 and 410, weighed 150 and 125 pounds, respectively, showing gains of 65 and 59½ pounds after their recovery. They now appeared strong and healthy.

It should be emphasized that, on a ration with as low an intake of phosphorus as 1.12 gm. daily, it took considerable time before pronounced weakness in these animals resulted. An experiment over a shorter duration of time where the animals are merely gaining weight is too often construed as evidence that the animal is receiving proper feeding materials.

The proportion of dry matter in various parts of the animal does not indicate that they differ materially from corresponding parts of animals receiving normal foods.

The proportion of calcium and phosphorus in important tissues of this animal likewise showed no marked variation from corresponding tissues of normally fed animals, or from animals receiving the basal ration, supplemented with inorganic phosphates.

The size and especially the texture of the bones did show a decided variation from those of animals from the other lots. They were spongy and loose in texture. When broken, they appeared

honeycombed almost to the outer surface. It appeared to be an extreme case of osteoporosis. Their breaking strength per millimetre was practically but one-third that of the corresponding bones from the animals of all other lots. The specific gravity of the bones was less than 1, while in all other lots it was greater than 1. Further, and of greatest significance, there was but 31 per cent of ash in the thigh bones, while all other lots contained from 46 to 55 per cent of ash in these bones.

Lots 2 and 3.— The animals in these lots, with one exception, showed, throughout the history of the experiment, progressive growth. No. 403 alone failed to make as vigorous development as all the other animals. This, we believe, is to be attributed at least in part to a constitutional weakness. The somewhat unpalatable ration demanded animals of initially strong and vigorous constitutions, and we feel fortunate that in this experiment our selections were generally successful. The intake of phosphorus in lot 2 was 6.57 gm. per pig daily, when the feed consumption was 2.2 pounds, while in lot 3 it was 3.84 gm. per pig for a like consumption of feed. In the first instance 29 per cent of the intake was in organic form, while but 17 per cent was in a similar form in the ration fed lot 3.

The appetite remained keen throughout the experiment. There was no manifestation of weakness of any of the animals at any time. On March 20 one animal from the lot receiving the smaller quantity of inorganic phosphate was killed.

The examination of the tissues for dry matter as well as for their calcium and phosphorus content is shown in Tables IV and V, and indicates practically similar quantities as in the tissues of animals receiving normal feeds.

The bones showed an appreciable increase in size over those of the animals from lot 1 and were somewhat larger than those receiving the unsupplemented feeds. Their texture was compact and in appearance silky and hard. The specific gravity was 1.157, and the breaking strength threefold that of the basal lot and per square millimetre equal to that of the standard fed lot. The weight of the skeleton surpassed that of all slaughtered animals from the other lots. The ash content of the thigh bones was 55 per cent as compared with but 31 per cent in the basal lot.

The two animals remaining in this lot, both females, were continued on the ration with the lower content of supplemented calcium

phosphate. Both animals were bred. They developed into strong, vigorous sows. At the time of farrowing No. 406 weighed 280 pounds, while No. 416 weighed 310 pounds. Six pigs were born in each litter. The mothers appeared to furnish a fair milk supply, as indicated by the normal growth of the young. The young were allowed to follow the mothers, feeding from their rations as they desired. When they had reached individually a weight approximating 30 pounds, they were separated from the mother, and three of the young animals continued on the inorganic phosphorus ration in the experiment of 1907-1908. They continued to make fair gains, reaching weights of about 75 pounds at four months of age. At that age one animal showed a breaking strength of its thigh bones of 1.9 pounds per square millimetre, a result not differing greatly from that of a normally fed animal.

Lots 4 and 5. — These animals received the non-supplemented normal feeds and were maintained as standards for comparison throughout the experiment. Lot 4 received its supply of phosphorus largely in the form of phytin. Probably 85 per cent of the ingested phosphorus was in this form. The remainder consisted of nucleins and lecithins. There was nothing in the history of this lot to indicate that these animals were not properly nourished. Consistent increases in weight were made by all of them.

Lot 5 received its phosphorus in the form of nucleins, lecithins, and pytates. There was not a preponderance of any one form. These animals gained in weight regularly and in every way were vigorous and strong.

The dry matter and calcium and phosphorus content of the tissues of both slaughtered animals from lots 4 and 5 were not appreciably different from those of the other lots. The bones were hard, close in texture, and of a specific gravity of 1.10 and 1.19, respectively.

The breaking strength of the thigh bones was not far from those of lots 3 and 4, and approximately three times that of lot 1. The ash content of the corresponding bones of the skeleton was respectively 53 per cent for lot 4, and 46 per cent for lot 5. This will be remembered as much higher than the ash content of lot 1 and comparable with that of lot 3.

TABLE I.
COMPOSITION OF MATERIALS USED.

	Water. Per cent.	Protein. Per cent.	Fat. Per cent.	Phosphorus. Per cent.
Rice	13.50	8.30	0.35	0.100
Wheat gluten	7.50	73.50	1.50	0.205
Washed bran	7.40	12.14	4.00	0.110
Whole bran	10.90	14.60	4.50	1.510
Corn ¹	10.60	10.30	5.00	0.313
Oats ¹	11.00	11.80	5.00	0.355
Middlings ¹	12.10	15.60	4.00	0.870
Oil meal	10.10	33.20	3.00	0.789
Calcium phosphate	15.750

¹ From average composition of American feeding stuffs, HENRY'S Feeds and feeding.

TABLE II.
DAILY RATIONS

LOT 1.			LOT 2.		
Feeds	Amount per pig daily in pounds.	Total phosphorus in grams.	Feeds.	Amount per pig daily in pounds.	Total phosphorus in grams.
Ground rice . .	1.24	0.56	Ground rice . .	1.20	0.54
Washed bran . .	0.65	0.32	Washed bran . .	0.63	0.30
Wheat gluten . .	0.26	0.24	Wheat gluten . .	0.25	0.23
Sugar-salt mixture	0.048	0.00	Sugar-salt mixture	0.046	0.00
			Calcium phosphate	0.077	5.50
Total	2.20	1.12	2.203	6.57
LOT 3.			LOT 4.		
Ground rice . .	1.22	0.55	Ground rice . .	1.26	0.57
Washed bran . .	0.64	0.30	Whole bran . . .	0.67	4.59
Wheat gluten . .	0.26	0.24	Wheat gluten . .	0.27	0.24
Sugar-salt mixture	0.047	0.00			
Calcium phosphate	0.038	2.75			
Total	2.205	3.84	2.20	5.40
LOT 5.					
Ground corn. . .	0.67	0.95	Wheat middlings .	0.67	2.64
Ground oats . . .	0.67	1.07	Oil meal	0.22	0.79
Total				2.23	5.45

TABLE III.
AVERAGE GAINS IN WEIGHT OF EACH LOT IN POUNDS.

LOT 1.				LOT 2.			
Number of animal .	409	410	411	Number of animal	402	403	404
Weight Dec. 15 . .	50	45.5	34	Weight Dec. 15 .	51.5	40.5	73.0
Weight Mar. 20 . .	85	65.5	84	Weight Mar. 20 .	130.0	61.0	132.0
Gain	35	20.0	30	Gain	78.5	21.5	59.0
Average gain 28.33				Average gain 52.6			
LOT 3.				LOT 4.			
Number of animal	406	408	416	Number of animal	401	405	412
Weight Dec. 15. . .	39	64	62	Weight Dec. 15 . .	40	47.5	47.5
Weight Mar. 20 . .	78	123	122	Weight Mar. 20 . .	114	100.0	102.0
Gain	39	59	60	Gain	70	52.5	54.5
Average gain 52.6				Average gain 59.0			
LOT 5.							
Number of animal	407		413		414		415
Weight Dec. 15	39		65		49		65
Weight Mar. 20	78		138		110		138
Gain	39		73		61		73
Average gain 61.5							

TABLE IV.

1907. PER CENT OF SOLIDS DRIED AT 100° FOR FIVE HOURS.

Part.	Basal.	Phosphate.	Whole bran.	Standard.
Number of animal	411	408	412	415
Kidney	20.38	17.5	17.3	17.0
Pancreas	37.7	40.8	33.9	32.0
Heart	20.5	23.1	21.4	22.9
Brain	20.4	21.6	18.2	22.1
Blood	21.1	18.7	22.1	14.2
Liver	27.2	29.6	27.9	31.6
Ovaries	16.2	16.7	17.0	15.2
Spleen	21.4	21.8	24.5	21.1
Leg muscle	23.8	25.0	28.3	27.1
Tenderloin	24.2	24.0	25.9	24.1

TABLE V.

COMPOSITION OF PARTS OF PIGS. AIR-DRY.

	Bone ash.		Blood.		Leg muscle.		Liver.		Brain.	
	% P	% Ca	% P	% Ca	% P	% Ca	% P	% Ca	% P	% Ca
Basal, 411	18.48	37.16	0.24	0.035	0.93	0.030	1.43	0.020	1.49	0.08
Inorganic, 408	18.26	36.91	0.31	0.031	0.81	0.029	1.34	0.030	1.57	0.10
Whole bran, 412	18.00	37.12	0.33	0.038	0.77	0.025	1.35	0.066	1.54	0.09
Normal, 415	18.20	37.23	0.28	0.026	0.78	0.041	1.27	0.030	1.43	0.09

TABLE VI.

DRY WEIGHT OF SKELETONS AND BREAKING STRENGTH OF THIGH BONES.

	Basal, 411.	Phosphate, 408.	Whole bran, 412.	Standard, 415.
Weight of skeleton, grams .	1193	2371	1288	1609
Weight of animal, pounds .	84	123	102	138
Breaking strength of thigh bone, pounds per sq. mm.	0.63	1.80	1.84	1.69
Diam. mm. thigh bone at centre	18.00	23.90	18.50	22.00

TABLE VII.

SPECIFIC GRAVITY AND ASH CONTENT OF CORRESPONDING BONES OF THE SEVERAL
SLAUGHTERED ANIMALS.

	Basal, 411.	Phosphate, 408.	Whole bran, 412.	Standard, 415.
Sp. gr. bones	0.977	1.157	1.100	1.192
Ash (thigh bone)	31%	55%	53%	46%

EXPERIMENT II.

In the winter of 1907-1908 the work described in the foregoing pages was repeated and somewhat extended. Our previous plan included the use of but one form of inorganic phosphorus, namely, the precipitated calcium phosphate. It was our purpose to use in the present experiment two known tri-calcium phosphates, a bone ash and a crude ground phosphate rock, known as floats. The experiment with precipitated calcium phosphate was again repeated, as also were the standard mixture grain experiment, the whole bran experiment, and the basal or low phosphorus experiment. So striking had been the results of the previous year that it was considered necessary to repeat the work.

The rations. — The details of the ration used, type of experimental animals, and management of this experiment were in all respects similar to those of Experiment I, except that small outside enclosures, free at all times from vegetation, were provided each lot.

Collection of excreta. — Toward the end of the experiment an animal from each lot, except the standard lot, was placed in an especially constructed cage and the urine and feces separately collected. This was to afford data on the channels of phosphorus elimination and quantities absorbed and retained.

Sequence of feeding. — The ration carrying precipitated phosphate was made by adding 625 gm. of precipitated phosphate to 49½ pounds of the feed mixture. In the case of bone ash 625 gm. were added, and in the case of floats 875 gm. of the ground crude phosphate rock. This supplied approximately equal quantities of phosphorus in each ration. The experiment covered one hundred and twenty-three days.

The tables that follow are summarized in exactly the same way as was done in Experiment I.

Discussion of the data. — The history of this experiment is a repetition of Experiment I and will be but briefly discussed.

Basal lot. — This lot again repeated the phenomena observed in 1906-1907. With the low intake of phosphorus, 1.12 gm. daily per pig, they failed to develop into strong vigorous pigs. An increase of body weight occurred in all cases and was considerable in number, 517. The attacks of weakness of limbs, stupor, and a condition analogous to coma were somewhat delayed, owing possibly to the opportunity for exercise in the outdoor pen, but nevertheless were

strongly manifested after a lapse of three months. The twitching tendency of the muscles, dragging the hind quarters, and the peculiar attitude assumed when standing were shown by all the animals on this ration. After four months from the initial date loss of weight commenced, and the experiment was discontinued. One animal from this lot was killed, and the others again restored to an apparently normal condition by the use of milk and middlings.

The proportion of dry matter in the muscles examined was comparable with that of the animals receiving normal feeds.

The texture of the bones showed a decided variation from the bones of the animals of all other lots. They were spongy and loose in construction. Their breaking strength per square millimetre was but half that of the corresponding bones in the animals receiving either low phosphorus rations, supplemented with various forms of calcium phosphate, or normal feeds naturally supplying an abundance of this element. The specific gravity of the bones was less than 1, while in all other lots it was greater than unity. The thigh bones show but 33 per cent of ash, while the corresponding bones from all other lots contain from 46 to 57 per cent.

Lots 2, 3, and 4. — Certain individuals unfortunately did not do well on the rations. This was confined to no single lot. The somewhat unpalatable ration produced by such material as our prepared washed bran required animals of initial inherent strength and vigor. At no time, however, did any of the animals show symptoms of retrogression similar to Lot 1. No condition of body weakness, stupor, or coma manifested itself. An examination of the table will show that certain individuals made as large gains in weight as those on standard feeds, and in every respect were as vigorous and strong.

On March 20 one animal from each lot was slaughtered. The proportion of dry matter in the two muscles examined was practically identical with that of the animal receiving normal feeds. The thigh bones from the pigs of all these lots were practically identical in breaking strength, while the ash content was from 46 to 57 per cent, as compared with but 33 per cent in the basal lot.

Lots 5 and 6. — In lot 5 the ingested form of phosphorus was very largely in the form of phytin, while in lot 6 the ingested phosphorus was more largely in the form of nucleins, lecithins, and phytin, with a predominance of no single form. The animals gained in weight regularly and in every way were vigorous and strong. On the 20th of March one animal from lot 5 was slaughtered. The

dry matter in the muscles was comparable with that of all other animals examined. The bones were hard, silky in appearance, with a breaking strength equal to that of the animals on rations receiving an equivalent of phosphorus in inorganic form. Their specific gravity was 1.14, and the ash content of the thigh bone 54 per cent.

Discussion of data on intake and outgo of phosphorus. — Toward the end of the experimental period a single animal from all lots except the standard ration was placed in a cage and an accurate balance of income and outgo of phosphorus determined. The first few days of collection were discarded. Only after the animal had become well accustomed to its surroundings were records commenced. Variation of intake of phosphorus was due of course to the variable amounts of food consumed daily.

Pig receiving the basal ration. — While the intake of phosphorus averaged 1.08 gm. daily, the average amount retained was but one half, or 0.53 gm. daily. This, manifestly, was insufficient for complete nutrition. The presence of inorganic phosphorus in the feces indicates that the total absorption of ingested phosphorus has been greater than the figures for retention show. This animal was at a low ebb of vitality and certainly suffering from phosphorus starvation. It is a significant fact that even under such conditions there was an appreciable output of phosphorus, representing in all probability phosphorus waste. The low content of ash in the skeleton strongly supports the view that the bones were serving as a reserve of calcium phosphate, from which the animal was drawing in order to supply the deficiency in the ingested feeds. This would easily account for the long-delayed retrogression of the animal, and for the fact that it maintained the phosphorus and calcium content of its tissues equal to that of a pig receiving an abundance of these elements.

Pig receiving the basal ration, supplemented with inorganic phosphates. — The average intake of total phosphorus in the three lots varied from 4 to 5 gm. daily. At least 80 per cent of the phosphorus was in inorganic form. The average amounts retained daily ranged from 1.50 gm. in the bone-ash-fed pig to 2.35 gm. in the float-fed pig. It is perfectly clear, of course, that the rate of growth will in a large measure control the demand for phosphorus and calcium, causing thereby a variation in the amounts retained dependent upon this factor. The proportion of calcium to phosphorus in the food supply is an additional factor in regulating the

amount of phosphorus retained by the growing young, as was pointed out by Ingle. Just how far a plentiful supply of bases other than calcium can function in the retention of phosphorus for skeleton formation has not been determined. But an examination of the data reveals the fact that where the preponderating supply of phosphorus has been as a calcium phosphate, there the percentage ash content of the bones was not appreciably larger than in cases where a very low supply of calcium to phosphorus for formation of a calcium phosphate existed. However, it is evident that in most cases the animals receiving calcium phosphates show a tendency to the production of heavier skeletons. It is, of course, clear that for bone formation a proper proportion of calcium for ultimate formation of calcium phosphate must accompany the phosphorus intake. The fact that the proportion of phosphorus to calcium in tri-calcium phosphate is as 1:1.9, while in wheat bran it is as 1:.09, at least suggests the thought that an added lime supply in the form of calcium carbonate could induce an increased proportional retention of the phosphorus of wheat bran for bone formation.

There were days when the data showed as high a retention as 3.11 gm. in the float-fed pig. These variations in daily retention would depend largely upon the quantity of excreta voided and on the rate of growth. The data indicate that an intake of 3 gm. of phosphorus daily for a growing 50-pound pig is the safe minimum quantity allowable. An additional supply of 1 to 2 gm. daily for purposes of gut regulation through the action of the salts of phosphoric acid, for the replacement of waste, and for as yet undetermined functions, appears much safer. The more insoluble floats did not fail to supply the required phosphorus, but appeared in every way to have been as efficient as precipitated phosphates for these animals. Of the excreted phosphorus 85-88 per cent passed through the gut. The form of this excreted phosphorus was almost entirely inorganic.

The extended investigation on the composition of farm animals at the Rothamsted Experiment Station affords data of a general character worth our consideration at this point. It was found at that station that the amount of ash in the entire animal (store pig) was 2.67 per cent, about 17.2 per cent of which was phosphorus. In a 50-pound pig this would approximate 105 gm. When the animal had increased to 150 pounds weight, allowing that at that weight the proportion of ash in the soft tissues was not decreasing,

the phosphorus content would then be 315 gm. If this increase had been made in one hundred days, then the daily retention would have been about 2.1 gm. of phosphorus. A more rapid increase in the growth would have increased this amount. With allowance for waste, indicated by the behavior of our animals under phosphorus starvation, the total daily requirements would not be far from the figures given by us.

Pig receiving whole bran ration. — In this ration the form of phosphorus consisted of about 80 per cent phytin phosphorus, with the remaining 20 per cent in the form of nucleins and lecithins. No inorganic phosphates were contained in the intake. The average retention was 2.36 gm. daily, or about 42 per cent of the intake. The phosphorus excreted in the feces was almost wholly inorganic in form. It is very probable that the excreted phosphorus represented phosphorus that had already been absorbed. The fact that the blood contains a phytin-splitting enzyme,¹⁰ while the ordinary intestinal enzymes are incapable of producing this cleavage, strongly supports this view. From this point of view it is manifestly impossible to measure absorbed or "available" phosphorus compounds by simply estimating the phosphorus content of the feces and subtracting this amount from the total ingested phosphorus.

Ash in the bones of a pig of 40 pounds weight. — To make sure that the decreased ash content in the skeletal tissues in those animals receiving the low phosphorus ration was incident to lack of supply in the feeds, and not the normal amounts in the bones when the pigs were placed in the experiment, a normally fed animal of 40 pounds weight was slaughtered. The air-dried thigh bones showed 54.3 per cent of ash, confirming our conclusions that the skeletal tissues were furnishing the calcium phosphate supply on the ration of low phosphorus intake.

DISCUSSION OF THE TWO EXPERIMENTS.

The data secured from the two experiments support the following deductions, which are briefly summarized below.

1. *Influence of supply of phosphorus.* — A low phosphorus ration carrying 1.12 gm. of phosphorus daily could not sustain growing swine. They became weak, with loss of the use of their limbs, and

¹⁰ E. V. McCOLLUM and E. B. HART: *Journal of biological chemistry*, 1908, iv, p. 497.

completely collapsed after a period of three to four months. The supply of phosphorus for the nutrition of parts other than the skeleton was drawn from the skeleton during this low phosphorus intake. The skeleton lost its phosphorus, in company with calcium, probably as a calcium phosphate. The soft parts of the animal maintained the proportions of calcium and phosphorus very similar to the corresponding tissues of animals receiving an abundance of these elements.

An abundant supply of phosphorus in organic or inorganic forms had no influence on the proportion of phosphorus contained in the soft parts of the body. When the animal body is flooded with large quantities of calcium phosphate, the skeleton tissues not only become normally impregnated with the salts, developing strong bones, but up to a limiting capacity inherent in the animal, can still further add to the weight, size, and density of these tissues. This is evidenced by the fact that when the calcium and phosphorus intake of pigs receiving a normal standard ration was increased by 25 gm. daily of either precipitated phosphate, bone ash, or floats, for a period of three months, the breaking strength of the thigh bones became per square millimetre 2.16, 1.84, and 2.19 pounds, respectively, while those not receiving this addition remained at 1.77 pounds. These results were secured from animals of the same breed and age, and as nearly alike in every respect as it was possible to select them.

These results, so far as the soft tissues are concerned, are in entire harmony with the conclusions of LeClerc and Cook,¹¹ who have shown that with dogs and rabbits there is not an increased retention of phosphorus by the soft tissues when the salts of this element are added to a food containing normal amounts of phosphorus. However, over long periods of feeding with additional supplies of calcium phosphate the ash content of the skeletal tissues of pigs is increased.

2. *Influence of form of phosphorus when the organic supply, as nucleins and lecithins, is low.* — There appears to be no superiority, as source of supply, of any one form among the forms of phosphorus tested, when supplementing rations extremely low in this element. This holds true for swine. It may not be true of other classes of animals. The forms tested were precipitated calcium phosphate, bone ash, floats, and the organic form, phytin. All were

¹¹ LE CLERC and COOK: *Journal of biological chemistry*, 1906, ii, p. 203.

able to furnish the necessary supply for vigorous healthy development when the intake of nuclear and lecithin phosphorus was low.

3. *Retention of various forms of phosphorus and minimum supply necessary.* — The amount of phosphorus retained by growing pigs was not so much influenced by the form of phosphorus administered as by the vigor and rate of development of the animal. The average amounts retained daily when the supply was entirely organic was 2.36 gm., and when 80 per cent of the supply was in the form of floats, it was 2.35 gm. daily. It is apparent from this data that a safe minimum requirement of at least 3 gm. daily is necessary for a vigorous growing pig of 50 pounds weight. It is also manifest that even in phosphorus starvation there is an appreciable daily excretion of this element. This indicates a continuous catabolism of phosphorus-holding bodies in the animal tissues, and that such portions as have been catabolized escape further use and are eliminated. It is probably safe, for the reason stated above, to estimate the daily requirement for a growing 50-pound pig at from 4 to 5 gm. of phosphorus.

4. *Catabolism of phytin.* — When the food supply of phosphorus was entirely organic and 80 per cent of it consisted of phytin, the form of the excreted phosphorus was almost wholly inorganic. Pepsin or trypsin does not possess the power to cleave this body into an inorganic form, and the conclusion of Scofone¹² is in harmony with the view that none of the enzymes of the digestive tract are capable of inducing this change. The presence of a phytin-splitting enzyme, phytase, in the blood and liver of mammals is strong evidence that this body is absorbed from the intestinal tract and afterwards undergoes cleavage, while the excess and unnecessary quantities are again largely eliminated through the gut in inorganic forms.

5. *Evidences of synthesis of organic forms of phosphorus from inorganic forms.* — The data do not furnish conclusions of a definite character on the question of the synthesis of organic phosphorus bodies from inorganic phosphates by the animal cell. At no time was the supply of organic phosphorus in the basal ration low enough to give data on this point. While the addition of inorganic phosphates supplied the necessary factor to the basal ration for successful growth, yet the intake of organic forms would supply at least 1 gm. per day and one half of this was retained. Facts are available which allow us to calculate intelligently on an assumed case. A pig of

¹² SCOFONE: Abstract in *Biochemisches Centralblatt*, 1905, iii, p. 606.

50 pounds increased its weight to 100 pounds in two months on our basal ration, provided with inorganic phosphates. It has been found with steers¹³ that, exclusive of blood and the contents of the stomach and intestines, the soft tissues represent about 51 per cent of the total body weight. With swine the proportion is larger, probably not far from 60 to 65 per cent. Assuming that 60 per cent of the increased live weight is soft tissue and that 0.1 per cent is the average total organic phosphorus content in such tissues, then the total organic phosphorus necessary for the development of these tissues at the end of 60 days was but 13.6 gm.; yet the supply of organic phosphorus from our basal ration over a period of two months had been at least 60 gm. This calculation does not take into consideration the amount of organic phosphorus daily catabolized by the animal. There exist no data on the amount of phosphorus broken down into inorganic forms by the metabolic processes in the nuclei, when the animal is receiving a ration free from phosphorus but otherwise satisfactory. That this amount is not inconsiderable is strongly suggested by the data in Table VII, Experiment II. The pig receiving a supply of phosphorus too small to permit of a normal development of muscular and skeletal tissues was still excreting on an average 0.55 gm. of phosphorus every day. While this figure cannot be taken to represent the amount daily catabolized by the animal, it does point toward the inability of the animal to reduce this phosphorus loss below a certain level. The work of Grindley¹⁴ has shown that the average total phosphorus content of beef muscle is approximately 0.25 per cent, and that 75 per cent of it is water soluble. If we allow that all the insoluble phosphorus exists in organic forms, which is probably very near the truth, and that the soluble inorganic phosphorus is 0.08 per cent, then the total organic phosphorus is approximately 0.14 per cent. Our own determinations on the lean meat of swine placed the total phosphorus content as considerably lower. From this it is clear that our allowance of 0.1 per cent of organic phosphorus in the soft tissues is, if anything, high.

The final solution of the problem of whether the function of the synthesis of organic phosphorus-bearing bodies from inorganic phosphorus and organic radicals is resident in the animal cell, or whether these bodies must be supplied as such in the feeding materials, is

¹³ Jordan, Me., Agricultural Experiment Station, Report 1895, p. 36.

¹⁴ GRINDLEY: *Journal of the American Chemical Society*, 1906, xxviii, p. 25.

under way in this laboratory. It is clear that should further research prove that this synthetic function is a part of the animal's equipment, then the problem of form of supply for production, so far as the phosphorus-bearing bodies are concerned, becomes a secondary matter, and whatever enhancement of growth accrues from the use of special types of phosphorus-bearing bodies, this enhancement in production must result from the physiological effect of the non-phosphorus-bearing complexes to which the phosphorus is attached.

TABLE I
COMPOSITION OF MATERIALS USED.

	Water. Per cent.	Protein. Per cent.	Fat. Per cent.	Phosphorus. Per cent.
Rice	13.60	8.20	0.38	0.105
Wheat gluten	7.80	73.00	1.50	0.205
Washed bran	7.60	12.20	4.10	0.110
Whole bran	10.80	14.50	4.60	1.470
Corn ¹	10.60	10.30	5.00	0.313
Oats ¹	11.0	11.80	5.00	0.355
Middlings ¹	12.1	15.60	4.00	0.870
Oil meal ¹	10.1	33.20	3.00	0.789
Calcium phosphate	15.75
Bone ash	16.38
Floats	11.66

¹ HENRY: Feeds and feeding, Table of average composition of American feeding stuffs.

TABLE II.
THE INITIAL RATION OF EACH LOT.

LOT 1.			LOT 2.		
Feeds.	Amount per pig daily in pounds.	Total phosphorus in grams.	Feeds.	Amount per pig daily in pounds.	Total phosphorus in grams.
Ground rice . . .	1.24	0.56	Ground rice . . .	1.20	0.54
Washed bran . . .	0.65	0.32	Washed bran . . .	0.63	0.30
Wheat gluten . . .	0.26	0.24	Wheat gluten . . .	0.25	0.23
Sugar-salt mixture	0.048	Sugar-salt mixture	0.046
Total	2.198	1.12	Precipitated phosphate	0.059	4.22
			Total	2.185	5.29
LOT 3.			LOT 4.		
Ground rice . . .	1.20	0.54	Ground rice	1.20	0.54
Washed bran . . .	0.63	0.30	Washed bran . . .	0.63	0.30
Wheat gluten . . .	0.25	0.23	Wheat gluten . . .	0.25	0.23
Sugar-salt mixture	0.046	Sugar-salt mixture	0.046
Bone ash	0.059	4.38	Floats	0.078	4.13
Total	2.185	5.45	Total	2.204	5.20
LOT 5.			LOT 6.		
Ground rice . . .	1.26	0.57	Ground corn . . .	0.67	0.95
Washed bran	0.67	4.47	Ground oats . . .	0.67	1.07
Wheat gluten . . .	0.27	0.24	Wheat middlings .	0.67	2.64
			Oil meal	0.22	0.79
Total	2.20	5.28	Total	2.23	5.45

TABLE III.
GAINS IN WEIGHTS IN POUNDS.

BASAL.				
Number of animal	516	517	518	
Initial weight, Oct. 29	52	54	39	
Weight, Nov. 29	54	68	40	
Weight, Dec. 29	68	92	45	
Weight, Jan. 29	68	108	49	
Weight, Feb. 29	75	120	58	
Weight, Mar. 20	74	109	57	
Gain	22	55	18	
Average gain			32	
PRECIPITATED PHOSPHATE.				
Number of animal	510	511	512	Pig from old experiment.
Initial weights, Oct. 29	50	49	50	39
Weight, Nov. 29	58	58	61	48
Weight, Dec. 29	80	75	65	67
Weight, Jan. 29	87	93	64	72
Weight, Feb. 29	102	108	64	78
Gain	52	59	14	
Average gain			42	
BONE ASH.				
Number of animal	513	514	515	Pig from old experiment.
Initial weight, Oct. 29	67	45	38	28
Weight, Nov. 29	80	46	39	31
Weight, Dec. 29	110	54	45	38
Weight, Jan. 29	133	56	49	42
Weight, Feb. 29	145	57	53	
Gain	78	12	15	
Average gain			35	

TABLE III, *Continued.*
GAINS IN WEIGHTS IN POUNDS.

FLOATS.				
Number of animal	507	508	509	Pig from old experiment.
Initial weight, Oct. 29 . . .	60	42	46	37
Weight, Nov. 29	70	47	47	45
Weight, Dec. 29	90	62	57	56
Weight, Jan. 29	104	66	69	66
Weight, Feb. 29	125	71	80	
Gain	65	29	34	
Average gain			43	
WHOLE BRAN.				
Number of animal	504	505	506	
Initial weight, Oct. 29 . . .	53	51	43	
Weight, Nov. 29	72	68	45	
Weight, Dec. 29	91	82	59	
Weight, Jan. 29	103	100	66	
Weight, Feb. 29	125	115	80	
Gain	72	64	37	
Average gain			58	
STANDARD.				
Number of animal	501	502	503	
Initial weight, Oct. 29 . . .	58	43	48	
Weight, Nov. 29	75	52	59	
Weight, Dec. 29	94	65	78	
Weight, Jan. 29	106	87	95	
Weight, Feb. 29	125	107	114	
Gain	67	64	66	
Average gain			66	

TABLE IV.

1908. PER CENT OF SOLIDS DRIED AT 100° FOR FIVE HOURS.

Part.	Basal.	Precipitated phosphate.	Bone ash.	Floats.	Whole bran.
Number of animal	516	512	514	506	509
Leg muscle	24.5	24.4	25.3	23.2	27.1
Tenderloin muscle	22.7	25.0	21.8	25.3	22.5

TABLE V.

DRY WEIGHT OF SKELETON AND BREAKING STRENGTH OF THIGH BONES.

	Basal.	Precipitated phosphate.	Bone ash.	Floats.	Whole bran.
Number of animal	516	512	514	509	516
Weight of skeleton	870 gm.	950	950	1495	850
Weight of animal at slaughter	77 lbs.	87	58	82	87
Breaking strength of thigh bone, lbs. per sq. mm.	0.87	1.70	1.77	1.65	1.86
Diam. mm. thigh bone at centre	16.0	16.0	15.5	20.00	17.00

TABLE VI.

ASH CONTENT OF CORRESPONDING BONES FROM EACH ANIMAL.

	Basal.	Precipitated phosphate.	Bone ash.	Floats.	Whole bran.
Number of animal	516	512	514	509	516
Sp. gr. bone	0.984	1.15	1.12	1.19	1.14
Ash (thigh bone)	33%	46%	53%	57%	54%

TABLE VII.
PHOSPHORUS BALANCE SHEET.

BASAL.					
Date.	Total phosphorus ingested in grams.	Total phosphorus urine in grams.	Total phosphorus feces in grams.	Total phosphorus (inorganic) feces in grams.	Total phosphorus retained in grams.
February 4	0.779	0.0104	0.705	0.145	0.064
" 5	0.806	0.0157	0.465	0.069	0.326
" 6	0.540	0.021	0.271	0.117	0.248
" 7	0.837	0.021	0.363	0.216	0.453
" 8	1.258	0.0182	0.375	0.093	0.865
" 9	1.270	0.0179	0.593	0.238	0.660
" 10	1.270	0.0167	0.797	0.215	0.457
" 11	1.208	0.170	0.469	0.189	0.722
" 12	1.121	0.0257	0.611	0.192	0.485
" 13	1.281	0.0204	0.505	0.186	0.756
" 14	1.281	0.0367	0.595	0.201	0.650
" 15	1.283	0.0274	0.548	0.156	0.708
Total . .	12.934	0.2481	6.297	2.017	6.394
Average .	1.08	0.02	0.52	0.168	0.53

TABLE VIII.
PHOSPHORUS BALANCE SHEET.

PRECIPITATED PHOSPHATE.				
Date.	Total phosphorus ingested in grams.	Total phosphorus urine in grams.	Total phosphorus feces in grams.	Total phosphorus retained in grams.
March 21	4.61	0.257	2.52	1.84
" 22	5.17	0.461	2.26	2.45
" 23	5.76	0.214	2.48	3.07
" 24	4.67	0.585	2.39	1.80
" 25	4.90	0.377	2.59	1.94
Average . .	5.02	0.378	2.45	2.22

TABLE IX.
PHOSPHORUS BALANCE SHEET.

BONE ASH.				
Date.	Total phosphorus ingested in grams.	Total phosphorus urine in grams.	Total phosphorus feces in grams.	Total phosphorus retained in grams.
March 28	3.27	0.206	1.99	1.08
" 29	3.51	0.270	2.30	0.94
" 30	4.16	0.185	1.81	2.17
" 31	4.87	0.256	2.76	1.86
April 1	4.39	0.500	2.50	1.39
" 2	4.28	0.273	2.23	1.78
Average . .	4.08	0.281	2.26	1.54

TABLE X.
PHOSPHORUS BALANCE SHEET.

FLOAT LOT.				
Date.	Total phosphorus ingested in grams.	Total phosphorus urine in grams.	Total phosphorus feces in grams.	Total phosphorus retained in grams.
March 3	4.73	0.471	1.40	2.86
" 4	3.28	0.380	1.50	1.40
" 5	4.39	0.295	1.59	2.50
" 6	4.98	0.187	1.69	3.11
" 7	3.70	0.098	1.57	2.03
" 8	4.80	0.187	1.86	2.76
" 9	3.95	0.158	1.96	1.83
Average . .	4.26	0.253	1.65	2.35

TABLE XI.
PHOSPHORUS BALANCE SHEET.

WHOLE BRAN.					
Date.	Total phosphorus ingested in grams.	Total phosphorus urine in grams.	Total phosphorus feces in grams.	Total phosphorus inorganic feces in grams.	Total phosphorus retained in grams.
March 13	4.98	0.538	2.36	2.29-97%	2.09
" 14	5.75	0.500	2.50	2.75
" 15	5.75	0.483	1.39	1.34-97%	3.88
" 16	5.75	0.628	2.88	2.31-80%	2.24
" 17	5.75	0.705	2.78	2.45-88%	2.27
" 18	5.75	0.660	2.83	2.58-91%	2.26
" 19	5.75	0.730	3.07	3.07-100%	1.95
" 20	5.75	0.688	3.62	3.25-90%	1.45
Average	5.65	0.666	2.66	2.36

SUMMARY.

1. On the ration extremely low in phosphorus, pigs made as large gains up to 75 or 100 pounds when starting at weights of from 40 to 50 pounds as animals receiving an abundance of this element. After reaching this point loss of weight began, followed by collapse.
2. When such low phosphorus rations as induced the above symptoms were supplemented with calcium phosphates, no untoward results appeared. Animals fed a low phosphorus ration, supplemented with inorganic phosphates, made as vigorous a development as others receiving their phosphorus supply wholly in organic form.
3. Precipitated calcium phosphates, a mixture of di- and tri-calcium phosphates, gave no better results than did floats, a crude tri-calcium phosphate.
4. Phytin as the supply of phosphorus gave no better results than the inorganic phosphates.

5. A young animal of 40 pounds weight receiving inorganic phosphates, together with other salts as supplementary to a ration very low in mineral constituents, grew to be an animal of 280 pounds weight, bore a litter of fairly vigorous pigs, which on the same ration completed the cycle back to 80 pounds, while animals on the same ration less the inorganic phosphates collapsed in three months, with loss of weight accompanied by a loss of the use of their limbs.

6. Determinations of calcium and phosphorus in the principal organs and tissues of the animals on the low phosphorus ration showed that they maintained the proportion of these elements constant and comparable to that of normally fed pigs.

7. The percentage of ash in the skeleton of pigs on the depleted phosphorus ration was reduced to nearly one half that of pigs receiving a normal ration, or a phosphorus poor ration supplemented by an inorganic phosphate.

8. The marked reduction in the quantity of ash of the bones of the animal receiving an insufficient supply of calcium phosphates, together with the ability of the animal to build up a skeleton very rich in calcium phosphate when an abundance of the latter is supplied in inorganic forms, strongly points to the possession of a synthetic power by the animal which enables it to convert inorganic forms of phosphorus into the organic forms demanded by its body.

9. When the animals were starving for phosphorus, they drew this element from the skeleton, but removed calcium and phosphorus in the proportions found in tri-calcium phosphate.

10. The daily phosphorus supply for a 50-pound growing pig should be at least 3 gm. A supply of four to five gm. is probably a safer quantity.

11. The data furnish no positive evidence of the synthesis of nucleo-proteids or other organic phosphorus-bearing complexes from inorganic phosphates in the animal body.

STUDIES IN EXPERIMENTAL GLYCOSURIA.—IV. THE
CAUSE OF THE HYPERGLYCÆMIA PRODUCED
BY ASPHYXIA.

By J. J. R. MACLEOD.

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THAT glycosuria is one of the results of asphyxiation was, first of all, clearly shown by Claude Bernard. It was also found by this worker that prolonged asphyxia led to the disappearance of the glycogen stored in the liver. Dastre¹ confirmed the former of Bernard's discoveries and found, besides, that the percentage of reducing substance in the blood greatly rose when an animal (dog) was made to breathe for some time in a confined space. Lack of oxygen was ascribed as the cause of the increased production of sugar. Similar results were obtained by temporarily connecting a cannula in the trachea with a limited air space. The hyperglycæmia was quite transitory in the latter case.

The next important contribution to the subject came from Araki,² who, working under Hoppe-Seyler's direction, found that it is deprivation of oxygen rather than excess of carbon dioxide which is the cause of asphyxial glycosuria. In Araki's experiments animals (dogs, rabbits, hens) were placed in a practically air-tight box provided with a potash-absorbing apparatus, so that the carbon dioxide exhaled by the animal could not accumulate in the box, whereas the percentage of oxygen became steadily less, its space being taken by air. Unfortunately, analyses of the atmosphere in the box were not conducted, so that it is uncertain whether the carbon dioxide was adequately absorbed. It was found that glycosuria invariably became established provided that the animals were well fed, but did not appear when they had been starved for some time previous to the experiment. In all cases lactic acid also appeared in the urine. Exactly similar results were obtained by causing animals to breathe

¹ DASTRE: *Comptes rendus de l'Académie des Sciences*, 1879, lxxxix, p. 669.

² ARAKI, T.: *Zeitschrift für physiologische Chemie*, 1891, xv, p. 335.

air containing carbon monoxide, which, as had been previously pointed out by Zuntz, produced its effect by causing displacement of the oxygen in the blood. Araki concluded that the deficiency of oxygen causes increased production of sugar from the glycogen stored in the liver.

Lépine and Boulud³ ascribed the accumulation of reducing substance in the blood in asphyxia to quite another cause from that given by Araki, namely, to its diminished destruction rather than to its increased production. They claimed that the blood of asphyxiated animals possesses a less glycolytic power than does normal blood. In confirmation of this, Lépine and Boulud³ state that they were able, by means of alcohol, to extract from the blood of asphyxiated animals a leucomaine which, when added to normal blood, caused depression of its glycolytic power, and when hypodermically injected into guinea pigs was followed by glycosuria. These observers further explain the glycosuria which results from ligation of a large blood vessel as due to the production of such leucomaines in the occluded vascular area. That occlusion of a large blood vessel does produce glycosuria had previously been shown by Schiff,⁴ who, however, ascribed the cause to the action of some ferment, produced in the occluded portion, on the production of sugar in the liver. This hypothesis of Lépine and Boulud assumes that normal blood has a considerable glycolytic power, which, according to numerous researches of subsequent workers, has been rendered highly improbable. It would be out of place to go into this subject further here, a good review of the work having recently been given by Cohnheim⁵ and by Embden and Claus.⁶

Edie⁷ has more recently taken up the question as to the cause of asphyxial glycosuria. He kept animals in an air-tight metabolism chamber connected with an oxygen tank and having provision for the absorption of carbon dioxide. In the various experiments the percentage of carbon dioxide was regulated in the atmosphere of the chamber either by controlling the rate of absorption of carbon

³ Cf. LÉPINE et BOULUD: Comptes rendus de l'Académie des Sciences, 1902, cxxxiv, p. 582, and p. 1341.

⁴ SCHIFF: *vide* LÉPINE et BOULUD, *Loc. cit.*

⁵ COHNHEIM, O.: Zeitschrift für physiologische Chemie, 1903, xxxix, p. 330.

⁶ EMBDEN and CLAUS: Beiträge zur chemischen Physiologie und Pathologie, 1905, vi, p. 214.

⁷ EDIE, E. S.: The bio-chemical journal, 1906, i, p. 455.

dioxide by means of varying amounts of soda lime, or by delivering the pure gas into the chamber from a Kipp's apparatus. Analyses of the composition of the air in the chamber were made from time to time. He concluded, in the case of dogs, cats, and rabbits, that it is the high percentage of carbon dioxide rather than lack of oxygen which is the cause of asphyxial glycosuria. He found, for example, when 10 to 15 per cent by volume of carbon dioxide was present in the atmosphere of the chamber, that glycosuria occurred even when the percentage of oxygen was greater than that in atmospheric air; on the other hand, as low a percentage of oxygen as 6 did not, in the absence of an excess of carbon dioxide, cause any glycosuria. The above amount of carbon dioxide also caused anæsthesia of the animals, so that it is claimed by Edie that carbon dioxide owes its glycosuria-producing effect to the fact that it is an anæsthetic, all of which cause more or less glycosuria. It is suggested that this general action of anæsthetics is due to their combining with proteids at the expense of the carbohydrate groups which are thus set free. Such an hypothesis assumes that in asphyxial glycosuria it is not the production of sugar by the liver which is at fault, but rather an abnormal liberation of sugar elsewhere in the organism.

Underhill⁸ suggests that asphyxial hyperglycæmia may be the result of depressed activity of the tissue oxidases, which are very easily affected by deficiency in oxygen supply.

It is thus seen that there are three views concerning the cause of the accumulation of reducing substance in the blood in asphyxia: one of them ascribing it to excessive production of sugar by the liver; another, to diminished destruction in the blood or tissues, and the third, to a setting free of dextrose from glucoproteids.

It is evident that the most decisive way by which an answer can be given to this question is by eliminating the liver from the circulation. By so doing, the main source of sugar supply is removed, although there still remains the glycogen stored in the muscles as a possible substitute. That the latter source of sugar can be disregarded in the present connection is, however, made evident by the results which we will next consider, namely, those relating to the behavior of the reducing substances in the blood and urine after removal of the liver in various forms of experimental diabetes, especially in that due to removal of the pancreas.

⁸ UNDERHILL: *The journal of biological chemistry*, 1905, i, p. 113.

The first of these experiments was performed by Schiff,⁹ who showed that ligation of the blood vessels of the liver, in frogs that had been rendered glycosuric by piqûre, caused the sugar to disappear from the urine. Bock and Hoffmann¹⁰ then found, by cutting off the blood supply to the liver, that the reducing power of the blood greatly diminished in a normal animal, and that the hyperglycæmia which usually appeared when curare was injected or piqûre performed did not set in, or, if previously present, disappeared, as a result of the occlusion. To ensure removal of the liver from the circulation these workers, in most of their experiments, besides ligating the abdominal aorta and vena porta, obstructed the inferior vena cava just above the liver, by inserting into it, from below, a metal catheter covered at its free end with a gall bladder which, by inflation, acted as a plug. Seegen¹¹ repeated these experiments on curarized dogs and found a similar decrease in the reducing power of the blood. Hédon¹² found that removal of the liver from the circulation did not cause diminution of the reducing power of the blood in depancreated dogs. This result is, however, exactly the opposite to those obtained by Chauveau and Kaufmann,¹³ by Kaufmann,¹⁴ and by Montouri,¹⁵ all of whom found that removal of the liver from the circulation in depancreated mammals caused marked hypoglycæmia. It is also at variance with the results of Kausch,¹⁶ who removed the liver entirely from depancreated geese and ducks, with the result that the usual hyperglycæmia resulting from depancrea­tion gave way to hypoglycæmia which was as marked as that observed in geese from which the liver alone had been removed. Kausch found that the glycogen of the muscles disappeared after removal of the liver from the circulation, both when the pancreas was also absent and when it was present. This last-mentioned

⁹ SCHIFF: *vide* Recent advances in physiology and bio-chemistry, 1905, Longmans Green & Co.

¹⁰ BOCK and HOFFMANN: *Experimentalstudien über Diabetes*, Berlin, 1874, *vide* p. 18. *Cf.* also PAVY and SIAU: *The journal of physiology*, 1903, xxix, p. 375.

¹¹ SEEGEN: *Die Zuckerbildung im Thierkörper*, Berlin, 1890, p. 184.

¹² HÉDON: *Archives de physiologie*, 1892, Series V, iv, p. 245.

¹³ CHAUVEAU and KAUFMANN: *Comptes rendus de la Société de Biologie*, 1893, p. 23.

¹⁴ KAUFMANN: *Archives de physiologie*, 1896, Series V, viii, p. 151.

¹⁵ MONTOURI: *Archives italiennes de biologie*, 1896, xxv, p. 122.

¹⁶ KAUSCH: *Archives für experimentelle Pathologie und Pharmakologie*, 1897, xxxix, p. 219.

result shows that, when deprived of its hepatic source of sugar supply, the organism does call on that of the muscles, but that the supply of sugar from this latter source is not in itself sufficient to make good the loss of sugar.

It seems clear, from these results, that the cause of hyperglycæmia after depancreation is not that the normal destruction of sugar in the organism is interfered with, but that its production is increased. In all these experiments, however, the removal of the liver was made within a comparatively short time after the depancreation (the longest period being five days, Chauveau and Kaufmann), and it is quite uncertain whether at later periods a similar result would have been obtained. It is, for example, possible, during the later stages of depancreation, when all the glycogen of the liver has been used up, that the persistent hyperglycæmia is due to deficient destruction.

Lastly, Schenck¹⁷ has found that the usual hyperglycæmia which follows removal of large quantities of blood in rabbits does not occur when the liver is excluded from the circulation by tying a mass ligature around all of its vessels. The increase of reducing power of the blood in these cases must therefore be due to increased production of sugar by the liver.

A considerable amount of work is on record regarding the appearance of reducing substances in the urine following subcutaneous or intravenous injection of curare.

That such a result frequently follows injections of the drug is undoubted, but it is by no means constant, and there exists no satisfactory explanation of its cause. This effect of curare was discovered by Claude Bernard,¹⁸ who also found hyperglycæmia to be produced. Schiff thought interference with the respiratory movements to be the immediate cause of the hyperglycæmia; but Bernard, in reply, demonstrated that the drug still produces glycosuria when injected in such dosage as to interfere only with the action of the voluntary muscles, but not with those of respiration. As a result of further investigation of this action of curare, several important points have been indubitably established and several views have been advanced as to the exact cause, the more important of which are briefly as follows:

¹⁷ SCHENCK: *Archives für die gesammte Physiologie*, 1894, lvii, p. 553.

¹⁸ BERNARD: *Leçons de physiologie expérimentale*, Paris, 1855, i, p. 363; SCHIFF: *Loc. cit.*

1. That the result is very inconstant, the same dose in different animals of the same species giving sometimes a negative and sometimes a positive result (Langendorff,¹⁹ Morishima²⁰).

2. That the hyperglycæmia is not the result of the action of curare itself, but results indirectly through the interference with respiration, or with body heat regulation, or because of vascular disturbance, etc. (Zuntz,²¹ Sauer,²² Araki,²³ Schiff,²⁴ etc.).

3. That the hyperglycæmia is dependent upon there being a good supply of glycogen in the liver (Saikowsky,²⁵ Luchsinger²⁶).

4. That the hyperglycæmia is not dependent upon the supply of glycogen in the liver (Dock,²⁷ Morishima²⁸), but that it can occur when the liver is removed from the circulation (Langendorff²⁹).

5. That it is also independent of the supply of glycogen in the muscles (Morishima²⁸).

The above brief review of the literature makes it clear that for curare diabetes, as well as for that which follows asphyxia, very great uncertainty indeed prevails as to whether increased production or diminished destruction is the cause of the accumulation of dextrose which takes place in the blood in these conditions. The fact that carefully controlled artificial respiration in curarized animals has sometimes been noted to prevent the glycosuria (Penzoldt and Fleischer,³⁰ Sauer³¹) even when large doses of the drug were administered has been considered by some as conclusive evidence that curare and mechanically produced asphyxia bring about hyperglycæmia in the same way: that is, by rendering the blood asphyxial in character. However, no definite opinion exists as to how the asphyxial blood causes hyperglycæmia.

¹⁹ LANGENDORFF: Archiv für Physiologie, 1886, Supp. Bd., p. 269, and 1887, p. 138.

²⁰ MORISHIMA: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xlii, p. 217.

²¹ ZUNTZ: Archiv für Physiologie, 1884, p. 380.

²² SAUER: Archiv für die gesammte Physiologie, 1891, xlix, p. 423.

²³ ARAKI: Zeitschrift für physiologische Chemie, 1891, xv, p. 359.

²⁴ SCHIFF: *Loc. cit.*

²⁵ SAIKOWSKY: quoted from Morishima, *Loc. cit.*

²⁶ LUCHSINGER: quoted from Morishima, *Loc. cit.*

²⁷ DOCK: Archiv für die gesammte Physiologie, 1872, v, p. 581.

²⁸ MORISHIMA: *Loc. cit.*

²⁹ LANGENDORFF: *Loc. cit.*

³⁰ PENZOLDT and FLEISCHER: quoted from Morishima.

³¹ SAUER: *Loc. cit.*, p. 425.

Turning now to the present investigation, we shall consider the results obtained under several headings:

1. The behavior of the reducing power of the blood and urine and the rate of urine formation in asphyxiated (anæsthetized) dogs, and in dogs poisoned by curare.

2. The source of the increase in reducing substances in the blood in asphyxia and in curare poisoning.

As a result of the investigations under the second heading, it has been found that the source of the increased amount of reducing substance is the glycogen of the liver. This leads us to the next question, namely:

3. Does the asphyxial blood (or the blood of curarized animals) act directly on the liver cells, or does it act on these through the intermediation of the nervous system?

We shall see in answer to this last question that the blood acts directly on the liver cells. It remains, therefore, for us to investigate:

4. The property of asphyxial blood which gives it a stimulating effect on hepatic glycogenolysis.

BEHAVIOR OF THE REDUCING POWER OF THE BLOOD AND URINE, AND THE RATE OF URINE FORMATION IN ASPHYXIATED DOGS AND IN DOGS POISONED BY CURARE.

That asphyxia causes intense hyperglycæmia and glycosuria has been sufficiently well established by previous workers. It seemed advisable in this connection, however, to investigate two effects of asphyxia which have not received sufficient attention and which will be of importance in helping us to decide whether or not the hyperglycæmia, etc., following stimulation of the splanchnic nerve, is due to a local asphyxia in the liver. These are: (1) the relationship of the increase of the reducing power of the blood to that of the urine and to the rate of excretion of the latter, and (2) the length of time that it takes after removal of the cause of the asphyxia for the reducing power of the blood, etc., to return to normal.

Two experiments of such a nature were conducted, the results of which are given in Table I.

In the first recorded experiment (No. 43) marked hyperglycæmia became established within thirty minutes after starting the asphyxiation and steadily increased during the two and a half hours of the observation. No distinct amount of reducing substance, however, appeared in the urine until the second half-hour, and towards the end of the observation it fell off in amount. Marked diuresis became established in about thirty minutes after the beginning of asphyxia and persisted unabated throughout. In the other experiment (No. 44) the cause of the asphyxia was removed after ninety minutes and the animal then allowed to breathe oxygen-rich air. During the asphyxiation period the percentage of reducing substance in both blood and urine became proportionally increased, and marked diuresis occurred. During the following period the percentage of reducing substance in the urine only slowly decreased, being still distinctly over 1 per cent after two and one-half hours, when it was also found that the hyperglycæmia had not disappeared. The diuresis during this time, instead of abating, became much more evident and did not disappear until about two hours. It is evident that asphyxia produces results in these particulars which are almost identical with those obtained when the great splanchnic nerve is stimulated.³² Both conditions produce a hyperglycæmia which becomes marked, and which persists for some considerable time after removal of the exciting cause. In both cases, also, glycosuria and most marked diuresis result. As to whether or not the marked diuresis is partly due to the rhythmical changes of blood pressure induced in both types of experiments, or whether it is merely due to the diuretic effect of the excess of dextrose in the blood, is uncertain.

Three experiments are recorded in Table I relative to the behavior of the reducing power of the blood after the intravenous or subcutaneous administration of curare. In these cases precautions were taken to prevent asphyxia, and indeed in one of them (No. 34) in which the drug was administered subcutaneously, the effect on the respiratory muscles was not sufficient to completely paralyze them until thirty minutes after injection. The artificial respiration bellows was then employed, but no oxygen administered. In the other two cases artificial respiration by the bellows and oxygen administration were given freely. In No. 93 there was quite a free flow of urine containing reducing substances, but in the other

³² MACLEOD, J. J. R.: This journal, 1908, xxii, p. 373.

TABLE I.
THE PERCENTAGE OF REDUCING SUBSTANCES IN THE BLOOD AND URINE, AND THE RATE OF URINE EXCRETION IN ASPHYXIA AND CURARE POISONING.

No. of experiment.	Weight of dog. kgm.	Reducing substance in blood.		Time periods. minutes.	Urine.		Remarks.	
		Before. per cent.	After. per cent.		Reducing substance. per cent.	Amount excreted per minute. c.c.		
43	8.0	0.296	30	trace	0.06	Fed with meat and sugar for two days before experiment.	
			0.350		1.25			0.17
			0.424		0.6			0.17
44	8.7	0.163	0.294	60	1.7	0.34	Fed day before with meat and sugar. Asphyxiation stopped and oxygen administered.	
			0.374	90	2.0	0.35		
				30 ¹	2.2	0.6		
				90 ¹	1.66	0.6		
				150 ¹	0.230	1.1		0.15
34	6.2	0.158	0.613	60	None?	Few drops	Curare <i>subcutaneously</i> injected in such dosage as to gradually stop the respiratory movements. Artificial respiration performed for 30 min. prior to taking blood.	
92	6.4	0.163	0.214	60	Distinct amount	0.1	Curare <i>intravenously</i> injected until respiratory movements stopped. Artificial resp. and large amount of oxygen by Hirsch method. B. P. fell from 150 mm. to 80 mm. Hg.	
			0.232	90				
93	7.9	0.263	60	Distinct amount	0.25	Fed day before with bread and meat. Curare <i>intravenously</i> inj. until resp's just stopped. Artif. resp. and oxygen by Hirsch meth.	

¹ After removal of clamp.

two cases only a small amount of urine was produced, and in one of these (No. 34) the few drops collected in the course of the experiment did not distinctly reduce Fehling's solution. In No. 34 artificial respiration was not performed more than was necessary to keep the animal alive, and in this case the hyperglycæmia was most marked indeed. In the other two cases (Nos. 92 and 93) in which the artificial respiration was more actively employed and oxygen given, there was only slight hyperglycæmia. It may be that the natural respirations which are paralyzed by curare were not adequately replaced by these means, and that curare owes its hyperglycæmia-producing action solely to the respiratory paralysis which it induces. It would be rash to give a final conclusion on this question from the above data.

THE SOURCE OF THE INCREASE IN THE REDUCING SUBSTANCES IN THE BLOOD IN ASPHYXIA AND IN CURARE POISONING.

As has been pointed out in the review of previous literature on this subject, there are two possible causes for the accumulation of sugar in the blood in asphyxial conditions, namely, an increased production by the liver and a decreased destruction in the tissues. We have seen that considerable uncertainty prevails as to which of these is the responsible cause. The fact that the inhalations of oxygen-rich air should cause a previously established asphyxial hyperglycæmia to disappear, — although slowly, as we have shown, — and the fact that such inhalations prevent the appearance of the hyperglycæmia which usually follows the administration of certain drugs which otherwise appear to bring about an asphyxial state, are, of course, no aid in arriving at a decision of the question.

If we disregard for the present the presence of glycogen in the muscles, it is evident that an answer to this question will be furnished by finding in what way removal of the liver from the circulation influences the increase of the reducing power of the blood in asphyxia.

We will now proceed with a consideration of certain experiments of such a nature.

Dogs fed for one or two days with excess of carbohydrate food were anesthetized with pure ether, and an anastomosis of the portal vein with the inferior vena cava established by the method already

described.³³ The hepatic artery was then ligated. In this way the liver was removed from the circulation. Samples of blood for analysis were taken usually from a cannula inserted in the carotid artery, although in one or two instances the femoral artery was chosen instead. When taken from the latter vessel, a considerable amount of blood had first of all to be allowed to escape, since the venous return from the lower limb had been cut off by the ligation of the vena cava involved in the operation.

In some preliminary experiments (Table II, Nos. 80 and 81) nothing further was done, since it was necessary to see what effect on the reducing power of the blood the operative procedure itself might have. As already pointed out in the first part of this article, previous workers had found that removal of the liver in dogs and geese causes a gradual diminution of the percentage of reducing power in the blood. These estimations were not, however, made until some hours after the removal of the viscus, so that they are not directly serviceable for our present purpose. In the remaining experiments (Nos. 82-91) the animal was asphyxiated by clamping the respiratory tube or curare was injected usually in such dosage as to cause cessation of the respiratory movements. In all cases the arterial blood pressure was recorded from the carotid, so as to furnish an index of the extent of asphyxiation.

Observations on the reducing power of the blood after anastomizing the portal vein to the vena cava but leaving the hepatic artery untied, have already been published in the third communication of this series.³⁴ In the five experiments there recorded in which this determination was made (namely, Table I, Nos. 2, 3, 4, 6, and 9) no hyperglycemia was noted in three, a slight amount in one, and in the remaining experiment a distinct amount. In this last case, however, a clot formed in the cannula, so that the portal blood did not get into the general circulation, the intestines remaining intensely cyanotic, and the condition of the animal being very low indeed. The results of the two further experiments of this nature in Table II shows that in one of them there was slight hyperglycemia thirty minutes after removal of the liver. The operation in this case was a difficult and lengthy one.

On the whole, taking the above results in conjunction with those reported by previous workers, it is evident that the amount of sugar

³³ MACLEOD: *Loc. cit.*

³⁴ MACLEOD and RUH: This journal, 1908, xxii, p. 397.

in the blood becomes subnormal by removal of the liver from the circulation. In the two cases of both series of experiments in which the reducing power was found somewhat increased, there must have been, for some reason, a production of sugar from the glycogen stores in the muscles which exceeded in amount that used up in the organism.

Coming now to the results of those experiments in which asphyxiation was induced after removal of the liver (viz., Nos. 82, 83, 84, 85, 88, 89, and 91), we see that in six of them no hyperglycæmia occurred. In the remaining experiment, on the other hand, the percentage reducing power of the blood rose to 0.330, but it is uncertain whether or not all the vessels to the liver had been completely tied off, since, on account of an oversight, no *post mortem* was conducted, and the operation was performed in a hurry.

Concerning the curare experiments (Nos. 86, 87, and 90), it will be seen that results in keeping with the above were obtained, a subnormal percentage reducing power being found in the blood after the dog had been profoundly under the effect of the drug for thirty-five minutes in one case, and for one hour in the other two cases. This result is, perhaps, the most striking of all, since curare under other conditions causes so remarkable an increase in the reducing power of the blood.

These results definitely show that the main cause of increase in the reducing power of the blood in (mechanical) asphyxiation is increased output of sugar from the liver. The same is true for those forms of hyperglycæmia caused by the administration of curare. They further show us that in these conditions no excessive amount of sugar can be derived from the stores of glycogen in the muscles. Although the results show that deficient destruction of dextrose in the tissues is not the cause of asphyxial hyperglycæmia, yet they do not make it impossible that this process may be somewhat depressed.

IN THUS CAUSING INCREASED OUTPUT OF DEXTROSE FROM THE LIVER, DOES THE ASPHYXIAL BLOOD ACT DIRECTLY ON THE LIVER CELLS, OR DOES IT ACT ON THEM THROUGH THE INTER-MEDIATION OF THE NERVOUS SYSTEM?

The pronounced stimulating action of asphyxial blood on the various medullary and spinal nerve centres at once suggests that

TABLE II
 THE PERCENTAGE OF REDUCING SUBSTANCE IN THE BLOOD OF ASPHYXIATED OR CURARIZED DOGS AFTER REMOVAL OF THE LIVER
 FROM THE CIRCULATION.

No. of experiment.	Weight of dog.	Nature of experiment.	Minutes after establishment of fistula at which blood removed.	Reducing substance in blood.		Vessel from which blood taken.	Remarks.
				per cent			
80	kilos. 8.4	Fistula, but no asphyxia	min. 50	0.129	0.134	Femoral	B. P. 60-65 mm. Hg. Artif. resp. and O ₂ inhalations occasionally. <i>Post mortem</i> : Hepatic artery tied.
81	"	30	0.220	0.252	"	B. P. 55 mm. Hg. Operation unsatisfactory. O ₂ inhalations throughout. <i>Post mortem</i> : Hepatic artery tied.
82	10.7	Fistula and asphyxia by clamping	35	0.151	0.144	"	B. P. fell twice to zero during experiment, making artificial respiration necessary. <i>Post mortem</i> : Hepatic artery tied.
83	4.2	"	30 60	0.219	0.199	" Carotid and heart	<i>Post mortem</i> : Hepatic artery tied.
84	8.5	"	30	0.210		Femoral and heart	B. P. low throughout so that asphyxiation had to be performed with care. <i>Post mortem</i> : Hepatic artery tied.

85	7.8	"	45	0.334 0.326 0.304 0.301	Femoral Carotid	B. P. 95 mm. Hg. Operation quickly performed, but hepatic artery possibly not tied. <i>Post mortem</i> : not made.
88	6.6	"	30	0.162 0.152	Femoral Carotid	B. P. showed marked asphyxial effect eight times during half hour. Asphyxia kept up last time until B. P. began to fall. <i>Post mortem</i> : All structures to hilus cut.
89	10.3	"	30	0.196 0.155 0.151	" "	B. P. 55 mm. Hg. <i>Post mortem</i> : all structures to hilus cut.
91	9.4	"	30	0.192 0.179	" "	The pancreatico-duodenal branch of portal was intentionally left unligated. <i>Post mortem</i> : all structures to hilus cut unless above branch of portal v.
86	6.6	Fistula and cur- rare injections until muscles paralyzed	35	0.112	Carotid and heart	B. P. 60-70 mm. Hg. <i>Post mortem</i> .
87	11.1	"	60	0.141	Carotid	B. P. 90-95 mm. Hg. <i>Post mortem</i> : Hepatic artery tied.
90	10.4	"	30 60	0.160 0.132 0.140	" "	Curare effect profound. Artificial respiration discontinued for some time before removing blood samples. <i>Post mortem</i> : All structures to hilus cut and tied.

it may be by a similar action of asphyxial blood on the hypothetical nerve centres controlling the liberation of dextrose from the liver that asphyxial hyperglycæmia is produced. If such be the mechanism involved, then, after section of the nerves running to the liver, asphyxia would not be expected to cause hyperglycæmia.

On the other hand, it is possible that the asphyxial blood does not act through the nervous system, but directly on the hepatic cells, in which case asphyxiation following section of the nerves would cause the usual hyperglycæmia.

Table III gives the results of experiments conducted on dogs in solution of this question.

The hepatic nerves were cut by placing several pairs of mass ligatures around all the structures running to the hilus of the liver, except the portal vein, and cutting between the ligatures. The outer coat of the portal vein was also carefully crushed by forceps so as to destroy any nerve fibres running in it. The occlusion of the hepatic artery and of the bile ducts involved in this procedure have already been shown to have no effect on the amount of reducing substance in the blood.³⁵

After removing a sample of blood for analysis, the animal was asphyxiated or curare injected until the respiratory movements had disappeared, and, after a varying period of time, further samples of blood were removed.

Five experiments of this nature were performed. In one of these (No. 36) in which the dog was pregnant and had not received any food on the day before the experiment, no hyperglycæmia was produced by the asphyxiation. The asphyxia in this animal was not intense. In another experiment (No. 41) only a slight hyperglycæmia occurred in seventy-five minutes after starting asphyxiation, and there was only a trace of reducing substance in the few drops of urine which collected from ureter cannulas during the course of the observation. In the remaining three experiments (Nos. 42, 73, and 79) very marked hyperglycæmias were observed within one hour after starting the asphyxiation. One of the last-mentioned experiments, in which curare was given (No. 42), showed, besides the hyperglycæmia, a very remarkable polyuria and an intense glycosuria, this result being perhaps due to the fact that considerable amounts of cane sugar were given along with flesh

³⁵ MACLEOD, J. J. R.: *Loc. cit.*

TABLE III.
THE PERCENTAGE OF REDUCING SUBSTANCE IN THE BLOOD AND URINE AND THE RATE OF URINE EXCRETION IN ASPHYXIA AND CURARE POISONING AFTER CUTTING THE HEPATIC NERVES.

No. of experiment.	Weight of dog. kilos.	Reducing substance in blood.		Reducing substance in urine. per cent.	Urine excreted per minute. c.c.	Whether asphyxiation or curare poisoning.	Remarks.
		Before.	After.				
		per cent.	per cent.				
36	6.2	0.158	50 min. 0.202	0.202 0.192 0.187	c.c.	Asphyxia (slight)	Not fed day before experiment. Pregnant. Asphyxiation kept up till slight asphyxial rise in B. P. appeared. <i>Post mortem</i> : Hepatic nerves all cut.
			75 min. 0.187				
41	9.85	0.190	75 min. 0.230	Mere trace	Few drops	Asphyxia	Asphyxiation till vagus pulse became marked. <i>Post mortem</i> : no record?
42	17.9	0.289 (after operation)	60 min. 0.470	60 min. 8.0 90 min. 2.5	0.7 0.28	Curare	Fed day before with flesh and cane sugar. Curare administered hypodermically until respiratory movements very feeble. Respiratory tube clamped until curare effect developed. <i>Post mortem</i> : Hepatic nerves all cut.
73	9.75	0.183	60 min. 0.323 90 min. 0.323	30 min. none 60 min. none 90 min. trace	Asphyxia	No food on day before experiment, but previous to that abundantly fed. <i>Post mortem</i> : Hepatic nerves all cut.
79	7.0	0.163	45 min. 0.331 0.333	Asphyxia	Fed day before experiment with bread and flesh. Some MgSO ₄ solution got into artery about 20 min. after starting asphyxia, as a result of which respiratory movements stopped rendering artificial resp. necessary. <i>Post mortem</i> (?).

as food on the day preceding the experiment. In another case (No. 73) no food whatsoever had been given on the day preceding the experiment, and it was found that, despite the hyperglycæmia, practically no reducing properties were acquired by the urine, and the amount of this latter excreted was extremely small. In the remaining experiment the animal had been fed on bread and meat, but unfortunately no observations were made on the urine.

These results show conclusively that asphyxia or curare still produces hyperglycæmia when the liver is isolated from the central nervous system. They show us that the asphyxial blood must act directly on the hepatic cells. It might be argued, however, that the above result is no proof that asphyxial blood acts directly on the liver cells, since the nerve centres still remain in connection with the other great *dépôt* of glycogen, namely, the muscles. It might quite well be, for example, that, in the absence of any nerve path to the liver, stimulation of these centres would act through the efferent paths to the muscles instead of through the hepatic nerves. There is nothing in my experiments which absolutely disproves this hypothesis, although the probability of such is rendered slight by the fact that the hyperglycæmia also occurs in curare poisoning (with the hepatic nerves cut), in which case, therefore, the motor nerve endings are paralyzed and the glycogen of the muscles therefore removed from nervous control. It is in this regard that the curare experiment is of great importance in the present connection.

If a nerve centre controlling the production of sugar from the liver does exist, — which as yet cannot be stated with certainty, — then of course it may, like other nerve centres, be stimulated by asphyxial blood, but its effect in causing hyperglycæmia will be masked by the local action in the same direction going on in the liver.

WHAT PROPERTY OF ASPHYXIAL BLOOD IS IT THAT STIMULATES THE GLYCOGENOLYTIC FUNCTION OF THE HEPATIC CELL?

The possibilities to be considered in this connection may, for simplicity's sake, be grouped into three:

1. The deficiency of oxygen.
2. The excess of carbon dioxide.
3. The presence of some chemical substance produced in the tissues as a result of the asphyxia.

The method employed for determining whether deficiency of oxygen or excess of carbon dioxide in blood can stimulate the glycogenolytic activities of the liver cell was as follows:

The liver of a dog or rabbit which had been fed the evening before with a large amount of cane sugar was excised after the animal had been killed by bleeding. It was immediately placed in a freezing mixture and minced through an ice-cold mincing-machine, the mince being then kept almost frozen. Into three pairs of small Erlenmeyer flasks, which had previously been weighed, were placed equal amounts of the animal's defibrinated blood, and to each flask was then added an amount of minced liver equal to that of the blood (about 20 gm. in most cases).

The amount of glycogen in the contents of one pair of flasks was then determined by the Pflüger-Nerking process. The remaining flasks were closed by india-rubber stoppers, each provided with two glass tubes, one of which extended to near the bottom of the flask, the other being short. The long tubes were connected, in the case of one pair of flasks, with an oxygen cylinder, and in the case of the other pair with a Kipp's apparatus generating either hydrogen or carbon dioxide gas, or with a gas pipe. These four flasks were fitted with sinkers, and were then submersed in a water bath kept at 38° C. and the gas allowed to slowly bubble through their contents for a period of from one to four hours. After this time the flasks were simultaneously removed from the water bath, and the proper amounts of 60 per cent potassium hydroxide solution added for the estimation of glycogen.

The results of these experiments are given in Table IV.

The first column in the table gives the percentage amount of dextrose of glycogen found in the liver and blood before incubating; the second, the amount found after incubation in the presence of oxygen, and the third, the difference between the first two, that is, the amount of glycogen which disappeared during incubation. This last result is then calculated as percentage of the original amount of glycogen, and the value thus found given in column four. Columns five, six, and seven correspond with columns two, three, and four, except that the incubation took place in the presence of hydrogen or carbon dioxide. In one of the experiments (No. IX) one pair of flasks was merely closed by a rubber stopper and the other had oxygen passing through it.

An examination of the results will show that the duplicates agree

sufficiently well to justify a definite conclusion being drawn from what at first sight may seem a small number of observations. Some-

TABLE IV.

COMPARISON OF THE RATE OF DISAPPEARANCE OF GLYCOGEN FROM SAMPLES OF LIVER AND ARTERIAL BLOOD INCUBATED IN THE PRESENCE OF OXYGEN WITH SIMILAR SAMPLES INCUBATED IN THE PRESENCE OF HYDROGEN OR CARBON DIOXIDE OR COAL GAS.¹

No. of exp.	1 Amount in liver before incubation.	Amount in liver after incubation.						8 Duration of incubation.	9 Nature of atmosphere in flasks not containing oxygen.
		2 In presence of O ₂ .	3 Amount disappeared.	4 Amount disappeared.	5 In absence of O ₂ .	6 Amount disappeared.	7 Amount disappeared.		
	gm.	gm.	gm.	per cent.	gm.	gm.	per cent.		
5.	5.178	2.904			2.155			4 hrs.	CO ₂
	5.544	2.830			2.260				
6.	5.361	2.867	2.494	46.5	2.207	3.154	58.8	2 hrs.	CO ₂
		9.320			8.080				
7.	12.160	9.400	2.800	23.02	8.330			3 hrs.	H
		9.360			8.20	3.960	32.57		
8.	9.520	4.26			3.900			3 hrs.	H
	9.900				4.020				
9.	9.710	4.26	5.450	56.12	3.96	5.750	59.21	3 hrs.	H
					5.576				
4.	8.92	5.358	3.255	37.30	5.570	3.155	36.16	3 hrs.	Closed flask. Coal gas.
	8.53	5.582			5.564				
4.	8.725	5.470			5.570			3 hrs.	Closed flask. Coal gas.
					3.155				
4.	5.22	3.070	1.585	32.70	3.453	1.531	31.66	50m.	Coal gas.
	4.45	3.430			3.304				
4.	4.835	3.250			3.304				
4.	7.56	5.44	2.12	28	5.06	2.50	33		

¹ Results given as dextrose of glycogen in 100 gm. of moist liver.

times, indeed, there appears an error of about 6 per cent (Column I of V, VII, and VIII), but this occurs in cases where it is of no consequence in the main result. The duplicates in columns two and five, which are important, do not show an error of more than

about 3 per cent, whereas the conclusions drawn from the results are for much greater percentages than this.

The rate of glycogenolysis is found to be the same in the presence of hydrogen as in that of air (Nos. VII and VIII), but to be greater in the presence of carbon dioxide than in air (Nos. V and VI). In the case of one of the latter experiments 46.5 per cent of the glycogen disappeared during a four hours' incubation in air, whereas in the same time 58.8 per cent of glycogen disappeared in the presence of carbon dioxide, a difference of 12.3 per cent of the original amount of glycogen; in the other experiment, in which incubation lasted for only two hours, the above values were, for air, 23.02 per cent and for carbon dioxide 32.57, a difference of 9.55 per cent of the original glycogen. The slight differences noted in the hydrogen experiments are within the experimental error as above indicated, and, moreover, these differences do not occur constantly in the same direction.

From the above experiments it is evident that the glycogenolytic effect of venous blood is due, in part at least, to the excess of carbon dioxide which it contains, and not to its deficiency of oxygen.

Before, however, drawing such a conclusion, several other facts must be considered. In the first place, it must be pointed out that in the above experiments a very large excess of carbon dioxide was present, probably a much larger amount than could possibly exist in the blood even in the most intense asphyxia. Indeed there was probably a considerable amount of free acid (H^+ ions) present in the contents of the flask. That the presence of a very small amount of any acid accelerates the rate of transformation of starch into dextrose by the action of malt diastase has been shown by Detmer,³⁶ by Kjeldahl³⁷ and others, and that carbonic acid is especially active in this regard has been shown by Detmer and by Müller-Thurgan.³⁸ Schierbeck³⁹ has found that the action of ptyalin and of pancreatic diastase on most forms of starch is also greatly accelerated by the presence of carbonic acid, provided that the reaction of the solution be neutral or faintly alkaline to start with. This worker has also found that the accelerating action of carbon dioxide is, within cer-

³⁶ DETMER, W.: Zeitschrift für physiologische Chemie, 1883, vii, p. 1.

³⁷ KJELDAHL: *vide* SCHIERBECK, Skandinavisches Archiv für Physiologie, 1892, iii, p. 344.

³⁸ MÜLLER-THURGAN: *vide* OPPENHEIMER, Die Fermente, Leipzig, 1903, p. 20.

³⁹ SCHIERBECK: Skandinavisches Archiv für Physiologie, 1892, iii, p. 344.

tain limits, proportional to the concentration of this gas in the atmosphere, and therefore to the amount dissolved in the liquid. He has, for example, found that the action of ptyalin on starch paste in neutral reaction is distinctly accelerated by 0.75 per cent CO_2 in the atmosphere when compared with pure air, and that the maximum amount of hydrolysis occurs when the percentage of CO_2 is only about 3 per cent of an atmosphere. Beyond this percentage the acidity of the solution becomes raised and the amylolytic activity depressed. Therefore CO_2 depresses the amylolytic activity in acid reaction. When, to start with, the solution is distinctly alkaline in reaction, then a certain amount of the CO_2 is used up to neutralize the alkali. This accelerating effect of carbonic acid is therefore due to the fact that it is a weak acid. That ptyalin acts best in faintly acid reaction was, first of all, clearly shown by Chittenden and his pupils.⁴⁰ Schierbeck, by using lactic acid of various strengths, has confirmed this, finding indeed that the accelerating influence is even much greater than had previously been supposed. The presence of free alkali, even of Na_2CO_3 , causes marked depression in the activity of ptyalin.

In one group of observations Schierbeck tested the rate of hydrolysis of a 1 per cent solution of glycogen by the action of ptyalin in the presence or absence of carbon dioxide. He found that in faintly alkaline reaction the carbon dioxide accelerated the hydrolysis, whereas when the reaction was faintly acid the carbon dioxide had a depressing effect. The results were therefore exactly like those obtained on starch paste. They are confirmatory of previous results obtained by O. Nasse,⁴¹ who also found that the accelerating influence of CO_2 manifests itself when its partial pressure is only 8 per cent

Researches of a similar nature to the above had previously been recorded by Ebstein,⁴² who found that carbon dioxide depresses the activity of glycogenase and all other animal diastases. His results, however, are not of much value, since, as pointed out by Schierbeck, many of the solutions were faintly acid in reaction to start with, and, as we have seen above, this causes carbon dioxide to have a depressing rather than an accelerating effect on diastatic action.

⁴⁰ CHITTENDEN and GRISWOLD, *American chemical journal*, 1881, iii, p. 305; CHITTENDEN and ELY: *Ibid.*, 1882, iv, p. 107.

⁴¹ NASSE, O.: *Archiv für die gesammte Physiologie*, 1877, xv, p. 471.

⁴² EBSTEIN: *Die Zucker Harnruhr: ihre Theorie und Praxis*, Wiesbaden, 1897.

Ebstein, as a result of his findings, propounded an hypothesis of the cause of diabetes which is briefly as follows: under normal conditions the diastatic ferments in the body are depressed in activity by the carbon dioxide of the blood; in diabetes there is less carbon dioxide expired than in health, signifying that there is less of this substance in the blood, and that, therefore, the diastatic ferments are less inhibited than is normally the case, so that more sugar is produced.

There can be no doubt that the action of ptyalin on starch paste corresponds with that of glycogenase on glycogen, so that we are warranted in concluding that there are at least two properties of venous blood, as compared with arterial, which could account for its stimulating effect on glycogenolysis. These two properties are (1) an increased amount (and tension) of carbon dioxide; (2) an increased amount of acid substances, that is, a diminished alkalinity; indeed it is almost certain that both these properties owe their stimulating effect to the same cause, namely, the diminished alkalinity of the blood.

That the alkalinity of the blood diminishes in asphyxia has been shown by Araki.⁴³ As already pointed out, this worker placed animals in an air-tight chamber and caused asphyxia by diminishing the amount of oxygen in the atmosphere. He found, on examination of the urine and in some cases of the blood, that besides dextrose relatively large quantities of lactic acid were formed. He thought the source of this to be incomplete oxidation of carbohydrates. More recently Galeotti⁴⁴ has found that after several days' sojourn in high altitudes the alkalinity of the blood of man diminishes by 40 per cent. In the case of animals subjected for several hours to an atmosphere containing a deficiency of oxygen, a less marked depression in alkalinity was also noted.

In reviewing the literature on glycosuria we have seen that there are two opposing views regarding the exact mode of action of asphyxial blood in causing hyperglycæmia. The one refers it to a deficiency of oxygen; the other, to an excess of carbon dioxide. From the above considerations it is plain that both views may be correct; for the deficiency of oxygen leads to the production of acids which, if they do not themselves accumulate in sufficient amount to have a stimulating effect, will, by diminishing the alkalinity of

⁴³ ARAKI: *Loc. cit.*

⁴⁴ GALEOTTI: *Archives italiennes de biologie*, 1904, xli, p. 80.

the blood, greatly assist the carbon dioxide in stimulating hepatic glycogenolysis.

There is no account in the literature of glycosuria being a noteworthy effect of acid intoxication, but in most of these experiments it is possible that a hyperglycæmia may have been produced which was not of sufficient intensity to cause glycosuria. The matter will require more thorough investigation. It is a question as to whether curare and other drugs that produce hyperglycæmia do so by diminishing the alkalinity of the blood or by causing some other unknown substance to appear which also has a stimulating effect on hepatic glycogenolysis. That artificial respiration and oxygen inhalation do not entirely prevent the appearance of hyperglycæmia after curare poisoning would suggest the possibility that in this case some non-oxidizable chemical substance is present in the blood, which substance stimulates the glycogenolytic function of the liver.

The acid bodies developed in the blood during asphyxiation are undoubtedly most readily oxidized. In any attempt to demonstrate their stimulating effect on hepatic glycogenolysis outside the body, therefore, it is necessary to use every precaution to prevent contact of the blood with air. This has been done by receiving blood from an asphyxiated dog into a flask filled with hydrogen, defibrinating in this flask and then transferring some of the defibrinated blood through tubing filled with hydrogen to the incubation flasks containing liver, and having a constant and abundant stream of hydrogen passing through them. Other portions of the same liver, after being mixed with arterial blood from the same dog, were incubated in the presence of an air stream for the same length of time as the samples in asphyxial blood. The other details of technique were exactly as in the previous experiments. The results of two experiments of this nature did not show asphyxial blood to have any more marked a glycogenolytic effect than arterial (Table V).

Such a result can scarcely be considered as disproving the hypothesis that in asphyxia acid substances other than carbon dioxide accelerate hepatic glycogenolysis. All that the experiments demonstrate is that in a limited quantity of blood removed from an animal in asphyxia there is not a sufficient amount of such substances to have any effect on hepatic glycogenolysis *in vitro*, probably because the above precautions were insufficient to prevent their destruction.

If, as has been suggested, stimulation of the great splanchnic nerve causes hyperglycogenolysis because it brings about a local

asphyxia in the liver, then it must follow that there is produced in the blood, as it passes through this organ, either a considerable amount of acid or a considerable amount of carbon dioxide. The absolute test of this hypothesis rests upon an examination of the blood flowing from the hepatic vein, and until this is done a further discussion of the nature of the glycogenolytic fibres in the splanchnic nerve can be of no value.

TABLE V.

COMPARISON OF THE RATE OF DISAPPEARANCE OF GLYCOGEN FROM SAMPLES OF LIVER AND ARTERIAL BLOOD INCUBATED IN THE PRESENCE OF AIR, WITH SAMPLES OF LIVER AND ASPHYXIAL BLOOD INCUBATED IN THE PRESENCE OF HYDROGEN.¹

No. of exp.	1 Amount in liver before incubation.	Amount in liver after incubation.						8 Duration of incubation.
		2 In arterial blood in air.	3 Amount disappeared.	4 Amount disappeared.	5 In asphyxial blood in hydrogen.	6 Amount disappeared.	7 Amount disappeared.	
	gm.	gm.	gm.	per cent.	gm.	gm.	per cent.	
10.	8.93	4.56			4.30			3 hrs.
	9.30	4.88			5.10			
11.	9.11	4.72	4.39	48.	4.70	4.41	48	3 hrs.
	4.40	2.35						
	4.20	2.33						3 hrs.
	4.30	2.34	1.96	45.5	2.46	1.84	42.7	

¹ Results given as dextrose of glycogen in 100 gm. of moist liver.

CONCLUSIONS.

The percentage reducing power of the blood and urine, and the rate of excretion of urine in anesthetized dogs, which are asphyxiated by constricting the respiration tube connected with the trachea, behave in very much the same way as they do during stimulation of the great splanchnic nerve.

The hyperglycemia, glycosuria, and diuresis thus induced do not pass off for some time after the cause of the asphyxiation has been removed.

Curare produces most marked hyperglycæmia, which can be only partially prevented by careful artificial respiration and oxygen inhalations.

Removal of the liver from the circulation is followed by hypoglycæmia both in normal animals and in animals which are asphyxiated or injected with curare. The source of the increased amount of reducing substance in the blood in asphyxia and curare poisoning must therefore be the glycogen of the liver.

The asphyxial (and curare) blood acts on the hepatic cells directly, and not through the intermediation of the central nervous system, for the usual hyperglycæmia, etc., follow asphyxiation (or curare injections) after all the hepatic nerves are cut.

By estimating the rate of disappearance of glycogen from pieces of liver mixed with defibrinated blood and incubated in flasks kept at body temperature, it was found that in an atmosphere of carbon dioxide glycogenolysis is more rapid than in air or oxygen. On the other hand, an atmosphere of hydrogen has no influence on the rate of glycogenolysis. It is therefore concluded that the property of asphyxial blood which stimulates hepatic glycogenolysis in the intact animal is the excess of carbon dioxide and not the deficiency of oxygen which it contains. It is pointed out that acids other than carbon dioxide in asphyxial blood most probably have a similar effect to that of carbon dioxide. The stimulating effect of such acids could not, however, be demonstrated, probably because they are very readily oxidized.

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THE ABSORPTION, EXCRETION, AND DESTRUCTION OF STROPHANTHIN.

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INTRODUCTION.

THE literature of strophanthus embraces comparatively few investigations of the problems of absorption from the alimentary canal, the channels of elimination, and the degree to which the drug is destroyed in the organism.

Haynes¹ secured very rapid absorption from the stomach of the cat, but he used large doses of the undiluted tincture, in which case absorption bears very little relation to the therapeutic dose.

Maurel,² Schulz,³ Hatcher and Bailey,⁴ and others have shown that strophanthin is very much more toxic when injected subcutaneously or intravenously than when administered by the stomach to different animals. Maurel found the toxicity for the rabbit by vein to be one hundred times as great as that by the stomach, but I have not found the rabbit so tolerant as he did after oral administration. Emesis frequently saves the cat and dog from very large doses of strophanthin given by the stomach, but an amount equal to several times the fatal dose by vein may be given to these animals by the stomach without causing any perceptible effect, and even larger amounts may remain in the alimentary canal for some hours before the absorption occurs of an amount equal to the fatal dose by vein.

Hatcher and Bailey⁵ succeeded, however, in inducing toxic symptoms in cats with doses given by the stomach which under slightly different conditions caused no perceptible effects (see cats M, 3 and 4, Table I).

¹ HAYNES: Biochemical journal, 1906, i, p. 62.

² MAUREL: Société de Biologie, 1901, p. 837.

³ SCHULZ: Vierteljahrsschrift für gerichtliche Medizin, 1901, xxi, p. 293.

⁴ HATCHER and BAILEY: This article will appear shortly in the Journal of the American Medical Association.

⁵ HATCHER and BAILEY: *Loc. cit.*

While some therapeutists recognize the difference in the activity of strophanthus by the mouth and by subcutaneous injection, the commonly accepted dose of the official strophanthin for oral administration is actually only one third as large as that now advised by the vein, but in the latter case only one dose is given in a day.

It is not surprising under the existing circumstances that the results of the clinical use of strophanthus by the mouth have been variable and often unsatisfactory; and this must continue to be so until we know more of the conditions which influence absorption.

Fraenkel and Schwartz⁶ recently recommended the use of the amorphous strophanthin by intravenous injection and reported brilliant results from its employment in this way.

It is not necessary to discuss here the relative advantages of the oral and intravenous administration of strophanthin, for no one will deny that it is preferable to give drugs by the mouth when satisfactory results can be obtained thereby, and the tincture of strophanthus is not well suited for intravenous and subcutaneous injection.

I have tried to discover why strophanthus has not proved so satisfactory after oral administration as its pharmacology indicates that it should be, and have been led to believe that differences in absorption, excretion, and destruction in the body, rather than unreliable preparations, are responsible for the discordant clinical reports, for I have shown⁷ that the tincture of strophanthus is now remarkably uniform in potency, though it was formerly extremely variable.

The official strophanthin made by different manufacturers was also probably variable at one time, leading clinicians to distrust it, but I am inclined to think that the belief in its variability arose, at least in part, from a want of uniformity in the methods used in testing different samples, for previous to the experiments made in this laboratory I can find no record of an accurate and trustworthy comparison of the toxicity of several samples of different manufacturers. Some observers used cats and dogs with one specimen, while others used frogs or rabbits with another, and the results were taken for comparison of the toxicity. The fallacy of this becomes apparent when one learns that different species of animals

⁶ FRAENKEL and SCHWARTZ: *Archiv für experimentelle Pathologie und Pharmacologie*, 1907, lvii, p. 79.

⁷ HATCHER: *Journal of the American Medical Association*, 1907, xlvi, p. 1177.

show very great differences in susceptibility; thus the rat is nearly one thousand times as resistant as the cat to strophanthin by subcutaneous injection, and I have been unable to kill the rat when strophanthin is added to the food. The rabbit occupies an intermediate position between the cat and the rat in this respect.

Thoms added to the confusion concerning strophanthin when, having isolated the ouabain of Arnaud from *Strophanthus gratus*, he called it *gratus strophanthin*, or *g-strophanthin*, proposing that every strophanthin shall have prefixed the initial letter of the species of *strophanthus* from which it was obtained. This has not been generally adopted, because, for one reason, it is impossible to obtain the different *strophanthus* seeds free from contamination with closely allied species.

There is little excuse for the confusion that exists in regard to the strophanthin of Thoms, or *g-strophanthin*, since it is a crystalline substance having the formula $C_{30}H_{46}O_{12} + 9H_2O$, while the official product is amorphous, and differs from the crystalline by having a methyl group replacing an H of the crystalline. It is methyl ouabain and has the formula $C_{31}H_{48}O_{12}$.

Schulz⁸ investigated the toxic action of the crystallized strophanthin and reported that it did not differ essentially from that of the amorphous. Hatcher and Bailey found the crystalline to be about two and a half times as toxic as the amorphous to cats, dogs, rabbits, and guinea pigs by subcutaneous injection.

At the present time the official strophanthin of the principal manufacturers is of very nearly uniform activity, and the crystalline is a perfectly definite and uniform substance.

I think it may be said definitely that so far from *strophanthus* and strophanthin being uncertain in action when introduced into the circulation, they are among the most uniform of remedial agents. This physiological action is modified less by extraneous conditions, such as anesthesia, poor general condition of the animal, etc., than the action of any other official substance, so far as I know. The fatal dose per kilogram of animal is diminished by an excess of fat, and this is the only modifying influence that I have encountered.

The uniformity of this action on cats is such as to suggest their use for the physiological standardization of *strophanthus* preparations and for the quantitative estimation of strophanthin in the body tissues and excreta.

⁸ SCHULZ: *Loc. cit.*

The method of employing the test is very simple and depends upon the fact that one tenth of a milligram of the crystallized strophanthin, or three and a half milligrams of the strophanthus seed, per kilogram of cat will give rise to a fairly uniform train of symptoms when injected intravenously, including vomiting, purging, convulsions (except during narcosis), and death of the animal within two hours. Subcutaneous injections may be substituted for the intravenous, but in the latter case the fatal dose is about 20 per cent greater.⁹ The toxicity of the amorphous strophanthin for the cat has not been determined with precision, so far as I am aware.

A difference of 10 per cent in the toxicity of pure solutions can be detected usually; hence a difference of one hundredth of a milligram of the crystalline strophanthin can be determined when a cat of one kilogram weight is used. The reaction is not quite so delicate in the presence of colloids with subcutaneous injection, but they do not exert sufficient influence to prevent the employment of this method in most cases.

Frogs afford a test for much smaller amounts of strophanthin than the cat does, and differences of a thousandth of a milligram of crystallized strophanthin may be detected by their use, but the train of symptoms is not nearly so characteristic as in the case of the cat, and their use is more tedious. I hope to employ them in some work of this character in the future.¹⁰

I think it must be admitted that the uncertainty of the therapeutic action of strophanthus is not due to any want of uniformity

⁹ Cats of not more than 3 kg. in weight may be injected by one man, in the absence of an assistant. The skin on top of the head between the ears is grasped between the tips of the ring and little fingers and the palm of the left hand, the cat's head is then drawn backward, until the skin over the spine near the root of the tail may be grasped between the tips of the thumb and middle and index fingers. This leaves the right hand free to manipulate the hypodermic syringe. The cat tries to hold to the table with its claws and cannot bite when held thus.

¹⁰ When a number of frogs are injected and the length of time before the heart stops in systole is to be determined, it will be found convenient to identify the frogs by means of the markings on the back. A rough outline of these spots (about five in number on the grass frogs commonly supplied here) can be made in a moment, and these are quite different for each frog. This is more convenient than tying strings to their legs, as recommended by Cushny, and it is applicable to any number. The web of the foot is examined under a low power of the microscope from time to time. A very slow circulation is maintained for a time after the heart ceases to beat, but with care this source of possible error will be avoided.

in the physiological action, and this is also indicated by the clinical results obtained by the intravenous use of strophanthin.

On the other hand, the questions of absorption, excretion, and destruction of strophanthin in the body assume especial importance in connection with the oral administration.

ABSORPTION.

It is obvious that strophanthin is not absorbed readily into the circulation from the alimentary canal under the usual conditions, since animals commonly show no symptoms after the oral administration of amounts equal to several times the fatal dose by vein, and since the absorption of such a dose into the circulation is always fatal in a short time.

There are two factors operating in strophanthin poisoning by the stomach which tend to prevent the absorption of a fatal dose in the case of the cat and dog. — emesis, already mentioned, and diarrhea, — but these do not occur in the case of rodents, which are, nevertheless, even more tolerant than the cat and dog.

Emesis and diarrhea are among the earliest of the toxic symptoms after the administration of strophanthin to the cat or dog, as well as to man, and since they usually occur promptly when much less than the fatal dose has been absorbed, they serve to remove any poison remaining in the gastro-intestinal tract, and thus lessen the chance of absorption.

While the emetic and purgative actions of strophanthin are protective, they are far from being uniform in degree or time of onset, and they interfere with any attempt to estimate absorption from the alimentary canal of the intact animal. Even when they are prevented in the dog by the previous administration of ether or morphin, the results of the oral administration of strophanthin are variable, but, on the whole, they point to a very slow absorption after large, but not fatal, doses.

I have sought to investigate the subject by placing definite amounts of strophanthus and crystalline strophanthin in different portions of the alimentary tract which had been tied off, and allowing them to remain for a suitable length of time or until the death of the animal. The characteristic symptoms and the death of the animal indicated the absorption of approximately that amount

which would be fatal by vein. If the animal survived the period of observation without showing toxic symptoms, it proved that less than that amount had been absorbed into the circulation. The amount remaining in the lumen of the intestine at the close of the experiment was determined in some cases, and confirmed these results.

The results of a number of the experiments on intact animals and with different portions of the alimentary canal are given in Table I.

The most striking fact shown in these experiments is the extreme variability in the rate of absorption. This is to be expected in those intact mammals in which emesis and diarrhea play such an important rôle; thus, 6.6 mg. of strophanthus per kilogram of weight given in concentrated tincture with a little alcohol by stomach (the equivalent of twice the fatal dose by vein) caused toxic symptoms in cats M, 3 and 4, while 50 mg. per kilogram (fourteen times the fatal dose by vein) only caused emesis in cats Q and R, and 20 mg. strophanthus per kilogram by stomach caused practically no less effect in dog S than did 100 mg. per kilogram (twenty-five times the fatal dose by vein) administered in the same way to dog A.

An examination of the table shows that the absorption of a fatal dose from the lumen of the dog's small intestine within a period of four hours did not depend upon the presence of a mere excess of the poison. An amount equal to four times the fatal dose by vein was distributed throughout the small intestine of dog G-2 and allowed to remain there for more than four hours without the absorption occurring of a sufficient amount to induce toxic symptoms.

That absorption was largely independent of the extent of surface participating is shown by the results with dogs I and J-2 in which fatal doses were absorbed from short loops of the intestine in thirty-six and forty-four minutes respectively, and a toxic dose was absorbed from a loop in dog G in one hour.

Absorption from the small intestine appears to be much more sharply controlled by the total amount of strophanthin injected. When the amount injected into the small intestine, either the whole length or into a loop, did not exceed five times the fatal dose by vein, then less than a fatal dose was absorbed in four hours (G-2, M-2, I-2); but when more than six times the fatal dose by vein

TABLE I.
ABSORPTION OF CRYSTALLIZED STROPHANTHIN.

Animal.	Exp.	Mode of administration.	Dilution.	Dose. mg. × kg.	Times fatal dose by vein.	Absorption.
Cat	18	Stomach	1-2000	1.0	10.0	24 hrs., no symptoms.
Cat	26	Stomach	1-500	1.0	10.0	17 minutes, emesis.
Cat	21	Stomach	1-2000	2.0	20.0	After 1° 30' ¹ (Alc.). 14 minutes, emesis — 48. ¹
Dog	B-2	Small intestine	1-10,000	1.53	12.0	1° 11', toxic.
Dog	C-2	Small intestine	1-10,100	1.76	14.0	1° 48'. ¹
Dog	9	Rectum	1.0	8.0	1°, none.
Dog	15	Rectum	1.0	8.0	After 1° 11'. ¹
Dog	9	Rectum	2.0	16.0	Purgation.
Rabbit	15	Stomach	10.0	30.0	3° 35' ¹ (Alc.).
Rat	1	In food	1600.0	No symptoms.

STROPHANTHUS SEED.

Cat	M	Stomach	1-10	6.6	1.9	Cat previously sick. After 2° 30' ¹ (Alc.)
Cat	3	Stomach	1-10	6.6	1.9	15', emesis (Alc.).
Cat	4	Stomach	1-10	6.6	1.9	36', emesis (Alc.).
Cat	C	Stomach	25.0	7.0	55', emesis (Alc.).
Cat	F	Rectum	25.0	7.0	After 63' before 2° 18', ¹ 10', emesis.
Cat	H	Stomach	1-1000	35.0	10.0	No symptoms.
Cat	Q	Stomach	1-100	50.0	14.0	1° 22', emesis.
Cat	R	Stomach	1-200	50.0	14.0	1° 15', emesis.
Dog	B	Stomach	1-1000	10.0	2.5	No symptoms.
Dog	A	Stomach	1-200	100.0	25.0	20', emesis.
Dog	L	Stomach	1-100	50.0	12.5	} 6° 45', not toxic (morph. given to depress vom. centre).
Dog	L-2	Stomach	25.0	6.25	

¹ Died.

TABLE I (Continued)

Animal.	Exp.	Mode of administration.	Dilution.	Dose. mg. × kg.	Times fatal dose by vein.	Absorption.
Dog	M	Stomach	50.0	12.5	5°, emesis; after 7°. ¹
Dog	F-2	Small intestine	1-500	27.0	6.75	1° 45'. ¹
Dog	E-2	Small intestine	1-500	31.0	7.75	38'. ¹
Dog	H-2	Small intestine	1-500	30.6	7.5	1° 27', not toxic.
Dog	G-2	Small intestine	1-1000	19.0	4.75	4° 24', not toxic.
Dog	M-2	Small intestine	1-1000	20.0	5.0	4° 25'. ¹
Dog	I-2	Small intestine	1-1000	20.0	5.0	4°, not toxic.
Dog	I	Loop 50 cm.	1-100	50.0	12.5	36'. ¹
Dog	G	Loop 20 cm.	1-100	44.6	11.0	1°, toxic.
Dog	J-2	Loop 50 cm.	1-100	53.0	13.0	44'. ¹
Dog	J	Large intestine	30.0	7.5	1° 20', not highly toxic.

¹ Died.

was injected, either into a loop or into the whole length, then a toxic dose was absorbed within two hours when the observations were continued for that period.

Closely analogous results have been observed in clinical cases where the amounts given by mouth considerably exceeded the fatal dose by vein. A patient in a New York hospital received daily for two days 120 minims of tincture of strophanthus, representing 12 grains of the seed, which is about equal to three times the fatal dose by vein (if the toxicity for man may be calculated by that for the cat), without showing any effect. Hochheim¹¹ gave a man 75 mg. of crystallized strophanthin daily by the mouth for two days; this is equal to ten times the fatal dose by vein, if calculated as just mentioned. It resulted in severe diarrhea with blood and mucus in the stools.¹²

¹¹ HOCHHEIM: *Centralblatt für innere Medizin*, 1906, xxvii, p. 65.

¹² The vomiting and diarrhea from strophanthin are both of central origin apparently, for very much smaller doses cause these symptoms, and the action is very much

EXCRETION.

One reason which has been advanced to explain why strophanthin is less toxic when given by the mouth than when injected subcutaneously or intravenously is that excretion keeps pace with absorption. Since the presence of a certain amount in the circulation — and that a very small amount — is fatal, it follows that excretion and destruction in the body must very nearly equal absorption, but I know of no quantitative estimation of the amount of strophanthin excreted.

That absorption from the various portions of the alimentary canal may be very much more rapid than excretion and destruction is proved by the experiments in cats M, 3 and 4, and those on dogs in which moderately large amounts were placed in ligatured portions of the small intestine and death resulted promptly. Haynes' results with large doses of the undiluted tincture have been mentioned, but in this connection I refer to doses comparable to those which have been used in therapeutics, and in fact the dose which induced toxic symptoms in three cats, M, 3 and 4, was only two thirds as large per kilogram of weight as that administered to the patient already referred to, in which no perceptible effect was produced. There are cases recorded of death from the oral administration of strophanthus, but none of these was under the direction of a physician, and the fatal dose for man is not known.

The amount of strophanthin which proves quickly fatal to the cat or dog is so small that the rat was chosen for the experiments in excretion, because that animal survives extraordinarily large doses either by the mouth or subcutaneous injection.

Preliminary tests showed that the urine, as well as extracts of the tissues and of the gastro-intestinal contents of the rat, could be injected subcutaneously into the cat without causing perceptible effects in twenty-four hours.

Experiment I. — White rat, chloroformed; contents of gastro-intestinal tract added to a little normal saline, this was diluted with an equal volume of

more rapid after the subcutaneous injection than when given by the stomach. Both of these manifestations are frequently absent after large doses have been injected by the vein. They appear to paralyze the centres before emesis and diarrhea can be induced, for they are often absent in such cases, even when the animal survives for a longer time than the interval before their occurrence with much smaller doses.

alcohol to facilitate filtration, the mixture filtered and evaporated at a gentle heat in a current of air to half the original bulk.

Cat A, male. Wt., 2.47 kg.

3.45 P. M. Injected subcutaneously the extract just described. Cat remained perfectly normal in appearance for twenty-four hours.

Cat B. Wt., 2.38 kg. Injected subcutaneously about 5 c.c. of rat's urine, after simple dilution and filtration. Cat remained perfectly normal.

Cat C, male. Wt., 3.15 kg. Injected subcutaneously extract representing about 6 gm. of heart and liver of rat. Cat remained perfectly normal in appearance.

In order to determine whether strophanthin is excreted by the kidneys, a rat (B) was given 50 mg. of crystallized strophanthin with the food; this was eaten within an hour, the urine was collected for twenty-two hours and injected subcutaneously into a cat, but it produced no perceptible effect. Had the rat excreted by the kidneys as much as 1 per cent of that eaten, the urine would have been fatal to the cat.

Since I had no proof that strophanthin is absorbed at all from the alimentary tract of the rat, another one was given a subcutaneous injection of crystallized strophanthin, after which the urine was examined and found to contain a notable amount of strophanthin but much less than that injected. The contents of the gastrointestinal tract was then examined and found to contain a much larger portion of the strophanthin, showing that the excretion of crystallized strophanthin into the intestine of the rat is much more active than by the kidneys. The volume of urine in this case was not sufficient to permit of a more exact determination of the amount of strophanthin contained, but the intensity of the symptoms induced in the cat used for the test indicated that excretion by the kidneys was less active than that by the intestine.

Sixty-six per cent at least of the total amount of strophanthin injected into rat I was recovered from the intestine after twenty-four hours. While the amount of strophanthin excreted by the kidneys of this animal was probably greater than in the case just mentioned, the dose for this animal was very nearly fatal, the rat remaining very much depressed throughout the experiment and passing practically no feces, whereas in the previously mentioned case the injection produced much less depression. It is possible that a part of the strophanthin excreted into the intestine was reabsorbed and excreted by the kidneys in this case, but the feeding experi-

ment already mentioned does not support such an idea. The intestines and the kidneys are both capable of excreting the poison, and it is not at all probable that the same ratio of activity between them should be maintained when the animal is severely poisoned, as in this case, or even in all normal animals. The rate of the urinary secretion in the rat was found to be very variable from time to time, and the details of Experiment II show that the strophanthin was excreted by the kidneys much more rapidly in the first five hours of the experiment than in the succeeding nineteen hours.

Experiment II. — Common gray rat. Wt., 180 gm.

3 P. M. 15 mg. crystallized strophanthin injected subcutaneously.

8.30. Collected about 5 c.c. urine, diluted to 35 c.c.

11.00 A. M. Collected about 9 c.c.

11.30 A. M. Collected about 3 c.c. Mixed urine diluted to 35 c.c.

3.45 P. M. Rat (which had been much depressed) was chloroformed, and gastro-intestinal contents removed and the tract washed with normal saline, then with alcohol; the contents and washings mixed and made up to 100 c.c. The mixture was filtered, the alcohol evaporated as in Experiment I, and the loss made up with normal saline. Labelled "Intestinal Extract."

Cat A, female. Wt., 2.0 kg.

11.10 A. M. Injected subcutaneously 10 c.c. of diluted first portion of urine (two sevenths of that secreted in five hours and thirty minutes).

12.40. Cat died after usual symptoms.

Cat B, female. Wt., 3.15 kg.

11.18 A. M. Injected subcutaneously 14 c.c. of dilute second portion of urine (two fifths of that secreted between 8.30 P. M. and 11.30 A. M.). Cat remained perfectly normal in appearance.

Cat C. Wt., 3.48 kg.

3.48 P. M. Injected subcutaneously 10 c.c. of mixed dilute urine (one seventh of total for twenty-four hours, none secreted after 11.30 A. M.).

5.20 P. M. Nearly fatal. Cat died before 8 A. M.

Cat D, female. Wt., 2.41 kg.

10.36 A. M. Injected subcutaneously 1 c.c. per kg. of intestinal extract of rat.

5.00 P. M. Very nearly fatal after usual symptoms.

Cat E, female. Wt., 1.98 kg.

1.53 P. M. Injected subcutaneously 1.25 c.c. per kg. intestinal extract.

4.08. Cat died after the usual symptoms.

Since 1.25 c.c. of the intestinal extract represented 0.125 mg., 100 c.c. represented 10 mg. Since one seventh of the urine was fatal to a cat of 3.48 kg., the whole of the urine would have been fatal to 24.36 kg., representing 3 mg. crystallized strophanthin; the total amount recovered being 13 mg.

In Experiment II the effort was made to estimate the total amounts of strophanthin excreted by the kidneys and by the intestine with a greater degree of accuracy than in most of the others. Five cats were used and 87 per cent of the strophanthin injected was accounted for. As a matter of fact, it is almost certain that a larger percentage could have been found in the intestine had a further series of tests been made, but the determination of a slight difference did not seem to justify the sacrifice of animals.

The extract of the intestinal contents of the normal dog is somewhat toxic for the cat when injected subcutaneously, and it was not considered feasible to apply the physiological test on cats to determine the amount of strophanthin excreted into the intestine of the dog after subcutaneous injection, the amount which could be so recovered being of necessity very small, and the results would be obscured by the toxic action of the intestinal contents. I hope to perfect the technic of this test and to employ it for the purpose.

DESTRUCTION.

Strophanthin is a glucosid, and therefore is destroyed readily in acid media, and many glucosids, notably the purgative principles of the anthracene group, are decomposed in the intestine; hence it would seem reasonable to suppose that some of the strophanthin given by the mouth would be destroyed in the gastro-intestinal tract, and this would help to explain why so small a percentage of that administered orally is absorbed into the circulation. It would also help to explain why very large amounts, a part of which could escape destruction, are so rapidly fatal.

The preliminary experiments, in which the digestive ferments acted on strophanthin in suitable media, make improbable any considerable destruction of strophanthin in the stomach and small intestine of the dog, nor is it destroyed in the dog's liver.

Experiment III. — Digestion.

One milligram of crystallized strophanthin was placed in each of three test tubes and treated as follows: 10 c.c. of 0.2 per cent hydrochloric acid and a little active pepsin were added to the first, 10 c.c. of 0.1 per cent acid and pepsin to the second, and 10 c.c. of 0.1 per cent sodium carbonate and active pancreatin to the third. These were digested at 38° C. for two hours and tested as follows:

A cat received 1.5 c.c. of the first per kilogram subcutaneously, and died in one hour and six minutes after the usual symptoms. Two other cats received 1.25 c.c. respectively of the second and third. The second cat had a convulsion; the dose was evidently very nearly fatal, but the cat recovered; the third cat also recovered after severe symptoms.

These cats had 0.15, 0.125, and 0.125 mg. of strophanthin per kilogram respectively, minus any that was destroyed. The latter amounts of pure strophanthin are just fatal to the cat, and the recovery of these two indicates that some destruction occurred; that the amount destroyed was very small indeed is shown by the result of the first, and by the fact that the other two doses were so very nearly fatal. This was the first case where I had seen a cat survive a convulsive dose of strophanthin.

Tincture of strophanthus gave precisely similar results with pepsin and pancreatin.

In order to determine the amount of strophanthin remaining in the dog's small intestine after a definite dose had been injected, the physiological test on cats was again resorted to, the preliminary tests having demonstrated that it can be estimated quantitatively after extraction from the contents of the small intestine.

Experiment IV. — Bitch. Wt., 3.8 kg. Control.

9:37 A. M. Dog chloroformed; small intestine tied at cecum and pylorus, excised, slit open, contents removed to dish and mixed with enough of the washings of the intestine to make 60 c.c. Labelled "Intestinal Extract." Twenty c.c. of the intestinal extract was mixed with a solution of 2 mg. of crystallized strophanthin and diluted to 40 c.c. Labelled "Extract A."

Twenty c.c. of the intestinal extract was diluted to 40 c.c. and labelled "Extract B."

Ten c.c. of Extract A was diluted with an equal volume of alcohol to facilitate filtration, filtered, and 12 c.c. of the filtrate was evaporated at a gentle heat in a current of air to 6 c.c. Labelled "Extract A-1." Each

cubic centimetre of this extract represented $\frac{1}{20}$ of a milligram of the strophanthin.

Cat A, female. Wt., 1.91 kg.

11.40 A. M. Injected subcutaneously 5 c.c. Extract A-1 (0.13 mg., per kilogram).

1.20 P. M. Cat died after usual symptoms.

The remainder of Extract A was filtered.

Cat B, female. Wt., 2.13 kg.

11.45 A. M. Injected subcutaneously 5 c.c. of Extract A (0.117 mg. per kilogram).

1.42 P. M. Cat died after usual symptoms.

Cat C, female. Wt., 2.81 kg.

11.57 A. M. Injected subcutaneously 15 c.c. of Extract B.

3.00 P. M. Cat nearly normal.

The next day and for some days the cat was greatly depressed, but it recovered.

This experiment demonstrates that practically all of the strophanthin may be recovered from the contents of the dog's small intestine.

Various amounts of strophanthin, either the crystalline or in the form of the diluted tincture, were placed in the stomach or in different portions of the dog's small intestine, which was ligatured above and below, and allowed to remain there for a certain length of time or until the animal died, after which the amount remaining in the lumen was determined. The death of the animal, with the typical symptoms which have been described frequently, showed fairly accurately how much strophanthin had passed into the circulation. Any difference, above the fatal dose by vein, between that injected and that found should represent the amount destroyed.

Owing to the nature of the tests, they were limited in number, in some cases but one was made, but the rapidity and the intensity of the action gave a very good indication of the approximate amount injected into the test animals.¹³ As a rule, the death of the cat was taken as indicating the minimum of the amount recovered.

In the experiment on dog B-2 the loss is really less than 26 per cent, but more than 9 per cent, but the dog received more than 1200 per cent of the fatal dose by vein; hence such small losses

¹³ This is very well illustrated in the tests on cats D and E of Experiment II.

are of minor importance in the question of absorption at least. The loss in the experiment on dog G-2 was less than 20 per cent, but the dog had not absorbed a toxic dose in more than four hours, though 480 per cent of that amount had been injected into the intestine.¹⁴

TABLE II.
DESTRUCTION.

Dog.	Duration and effect.	In-jected.	Found in intes-tine.	Disap-peared from in-estine.	Toxic dose.	Lost.	Lost.	Times fatal dose by vein.
		mg.	mg.	mg.	mg.	mg.	per cent.	
A-2 ¹	2	1.95	0.05	0.05	2.5
A	2	2.13	0.13 ²	0.13 ²	6.5 ²
B-2	1° tox.	11	7.3 8.8	3.7 2.2	0.9 1.125	2.8 1.075	26.2 9.8	12.0
F-2	1° 40' ³	200	185.0	15.0	26.0 33.0	11.0 ² 18.0 ²	5.5 ² 9.0 ²	6.6
G-2	4° 24', none	130	80.	50.0	23.0 30.0	27.0 20.0	20.2 15.4	4.8
H-2	1° 35', tox.	230	160.0	70.0	26.0 34.0	44.0 36.0	19.1 15.6	7.7
I-2	4°, none	138	96.0	42.0	22.0 31.0	02. 11.0	15.0 8.7	5.0
J-2	44' ³	250	188.0	62.0	16.0 21.0	46.0 41.0	18.4 16.4	14.0
K-2	2° 5' tox.	200	154.0 174.0	46.0 26.0	27.0 33.0	19.0 7.0 ²	9.5 3.5 ²	6.5
L-2	3° 15', tox.	331	228.0	103.0	46.0 60.0	57.0 43.0	17.2 13.0	6.0

¹ A-2, A and B-2 received crystallized strophanthin; the others had strophanthus seed.
² More indicated than injected. ³ Died.

The result of the experiments given in Table II do not settle the question definitely concerning the destruction of small amounts of strophanthin in the stomach and small intestine of the dog, and indeed we must suppose that a small amount at least is destroyed there, but the amount so destroyed is wholly insufficient to account for the failure of those organs to absorb a fatal dose in more than four hours when five times the fatal dose by vein is presented for absorption.

¹⁴ The fatal dose for the dog by the circulation is calculated at one sixth to one eighth of a mg. per kilogram (HATCHER and BAILEY, *Loc. cit.*).

Cloetta and Fischer¹⁵ found that digitoxin is fixed and destroyed by the normal heart, and they conclude that a further amount is fixed, but not destroyed, by the heart after it has been poisoned by digitoxin. I have been unable to obtain evidence by the physiological test that the rat's heart stores strophanthin in greater proportion than the rest of the tissues. The preliminary test (Experiment V) shows that strophanthin may be recovered almost quantitatively from the rat's tissues.

Experiment V. — White rat. Wt., 72 gm.

11.13 A. M. Injected subcutaneously 2 mg. crystallized strophanthin into the muscles of the thigh.

11.18 A. M. Rat chloroformed; contents of the alimentary canal, and the skin and tail removed, the remainder of the tissues (46 gm.) hashed, extracted several times with water and twice with alcohol, the extracts mixed, made up to 120 c.c., and filtered. The filtrate was evaporated at a gentle heat in a current of air to 60 c.c.

Cat A, male. Wt., 4.05 kg.

4.20 P. M. Injected subcutaneously 5 c.c. of extract of tissues per kilogram.

5.20 P. M. Cat died after usual symptoms.

5 c. c. of extract equals 0.125 mg. crystallized strophanthin.

60 c.c. of extract equals 1.5 mg. crystallized strophanthin.

The cat died so soon after the injection that it is very probable that it received more than the minimal lethal dose, in which event the extract contained somewhat more than that indicated.

Experiment VI. — White rat. Wt., 126 gm.

9.40 A. M. 50 mg. crystallized strophanthin added to a small amount of food.

6.00 P. M. All the strophanthin eaten.

8.30 P. M. Collected about 3 c.c. urine.

11.30 A. M. Collected about 7 c.c. urine.

11.45 A. M. Rat chloroformed; skin and tail removed, chopped fine, and extracted with alcohol, alcohol evaporated and residue taken up in water. Labelled "Extract A."

The gastro-intestinal tract was excised, the contents and feces extracted, made up to 120 c.c. Labelled "Extract B."

¹⁵ CLOETTA and FISCHER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1906, lxiv, p. 294.

The remaining tissues were hashed, extracted, and made up to 400 c.c. Labeled "Extract C."

Cat A, female. Wt., 1.8 kg.

11.43 A. M. Injected subcutaneously 3.5 c.c. of second portion of rat's urine. Cat remained normal.

Cat B, male. Wt., 2.47 kg.

3.42 P. M. Injected subcutaneously one fourth of total Extract A. Cat remained normal.

Cat C, male. Wt., 2.76 kg.

2.36 P. M. Injected subcutaneously one third of 1 per cent of Extract B for each kilogram of weight. Cat vomited many times and recovered.

Cat D. Wt., 2.98 kg.

3.30 P. M. Injected subcutaneously one third of 1 per cent of Extracts B and C and of mixed urine. Cat was not quite so much affected as cat C.

The extract of the tissues added nothing to the toxicity of the dose, but the increased amount of colloid present appeared to retard absorption and actually to lessen the toxicity slightly. The results of several of the experiments on rats are given in Table III.

TABLE III.

EXCRETION AND DESTRUCTION OF STROPHANTHIN IN RATS.

ADMINISTERED BY THE MOUTH.					
Rat.	Dose.	Duration of experiment.	Found in urine.	Found in intestine.	Loss.
	mg.	hours.		mg.	per cent.
B	50	22	-30.0 ¹	+40 ²
H	50	26	-37.5	+25
ADMINISTERED SUBCUTANEOUSLY.					
C	10	5½	Some	+2.3	
G	10	18	+0.85	4.0	
I	15	24	3.0	10.0	13

¹ - Indicates that the figure is too high. ² + Indicates that the figure is too low.

DISCUSSION OF RESULTS.

Experiment VI affords pretty strong evidence of the destruction of strophanthin in the rat's body or alimentary canal, the tests on cats C and D indicating the loss of at least one fourth of the total amount administered, or 12.5 mg., since 50 mg. of strophanthin would be fatal to 400 kg. of cat, and less than enough to kill 300 mg. was recovered. Since none appeared in the urine after such a large dose, one must suppose that very little was absorbed unchanged into the circulation, and this is supported by the fact that the examination of the tissues proved negative.

The amount recovered from the urine and gastro-intestinal contents after subcutaneous injection approximated much more closely to that administered (see Experiment II).

If the unpoisoned heart is capable of destroying digitoxin, it is quite possible that the heart (and other tissues) of the rat may destroy strophanthin under similar conditions, and that the slow absorption from the alimentary canal permits of the destruction of all that is absorbed, thus explaining why practically none is excreted by the kidneys after the oral administration.

Rat I showed great depression after the subcutaneous injection of strophanthin, and it is quite possible that under such conditions the tissues behave toward strophanthin as Cloetta found the heart to behave toward digitoxin after having been poisoned by it.

For the present I cannot attempt to answer the question, but if the strophanthin is destroyed in such large quantities in the alimentary canal after feeding, it seems strange that that which is excreted into the alimentary canal after subcutaneous injection is not also destroyed, particularly since the amount in the latter case is so very much less.

We have abundant evidence that strophanthin may be absorbed from the alimentary tract under certain conditions, and the experiments on the dog's intestine tend to show that the rate of absorption is disproportionately rapid with the increase of the dose above a certain point.

If 120 minims of the tincture of strophanthus may be given to a man daily without producing perceptible effects, it proves that only a small proportion of that dose is absorbed into the circulation; if disproportionately less of 20 to 30 minims is absorbed, it is diffi-

cult to understand how any therapeutic action will follow such a dose, and as for the commonly advised doses of the official strophanthin, one third to one fifth of a milligram, there is no reason to suppose that they are capable of producing any effect whatsoever when given by the mouth.

Since an amount of strophanthin equal to several times the fatal dose by vein may lie unabsorbed in contact with a surface certainly capable of absorbing it under very slightly different conditions, and since we know that the intestine of the rat is capable of excreting strophanthin, it seems very probable that strophanthin is actually absorbed fairly rapidly from the intestine of the cat and dog and immediately excreted into the intestine again before it can enter the general circulation in sufficient amount to cause toxic symptoms, unless the amount administered is excessive (more than five times the fatal dose by the vein for the cat, dog, and man); in the latter case the rate of absorption may far exceed excretion and a toxic dose may accumulate in the circulation.

The fatal dose of strophanthin for the rabbit by subcutaneous injection is much greater in proportion to its weight than is that for the dog, while the rat is nearly a thousand times as tolerant as the dog, and we find that while ten times the fatal dose by vein is toxic for the dog by the mouth, the rabbit may take as much as thirty times the fatal dose by vein before enough accumulates in its circulation to induce toxic symptoms, and I have not succeeded in inducing any decided symptoms in the rat when twenty times the toxic dose by subcutaneous injection was added to the food, and eaten in a few hours.

This may be put somewhat more clearly, perhaps, in this way: The dog dies when approximately one ten-millionth of his weight of strophanthin enters the circulation, whereas the rat will tolerate in its subcutaneous tissues an amount approximately equal to one ten-thousandth of its body weight. It seems probable that a ten-millionth of the body weight of a foreign substance may escape excretion and destruction much more easily than one ten-thousandth can.¹⁶

One is tempted to suppose that we have here a fundamental bio-

¹⁶ I have not determined the fatal dose of strophanthin for the rat by the vein, but that which is injected subcutaneously is rapidly excreted, and one must suppose that it is carried by the circulation.

logic principle involved. The herbivorous animal frequently must take in with his food small amounts of toxic vegetable substances which are absorbed with the products of digestion and against which it protects itself in part by excretion into the intestine, in part by destruction in the body, whereas the carnivora have less occasion to be protected against this particular class of poisons.

CONCLUSIONS.

1. The absorption of strophanthin is very rapid when more than six times the fatal dose by vein is placed in the ligatured small intestine of the dog.

2. The rapidity of absorption is dependent on the total amount injected into the alimentary canal to a far greater degree than upon the total surface of intestine concerned in absorption.

3. It is probable that the smaller the dose of strophanthin that is given by the mouth, the more nearly does excretion keep pace with absorption. This usually prevents toxic symptoms unless an amount more than equal to five times the fatal dose by vein is given. If an amount equal to ten times the fatal dose by vein is administered, toxic symptoms result.

If vomiting and diarrhea are prevented in such a case in the dog (morphin, ligature), death results as a rule.

4. The absorption of strophanthin in man appears to follow a rule similar to that in the dog.

5. Strophanthin is excreted rapidly into the intestine of the rat after being injected subcutaneously. It is excreted more slowly by the rat's kidney.

6. While strophanthin may be destroyed to a limited extent in the alimentary canal, the rate of destruction in the stomach and small intestine of the dog is insufficient to account for the failure of these organs to absorb a toxic dose when moderately large amounts have been injected.

7. Strophanthin can be extracted from the contents of the stomach and small intestine of the dog, and from the whole of the alimentary canal and the tissues of the rat, and the amount be determined by the physiological test on cats.

8. This test permits of the estimation of amounts of strophanthin

which cannot be determined by chemical means (one tenth of a milligram) probably accurate to within about 10 per cent.

9. The oral administration of strophanthin (and of strophanthus) in therapeutics is irrational in the present state of our knowledge concerning absorption and excretion in the alimentary canal, and destruction within the animal organism.

THE ELIMINATION OF TOTAL NITROGEN, UREA, AND AMMONIA FOLLOWING THE ADMINISTRATION OF GLYCOCOL, ASPARAGIN, AND GLYCYL-GLYCIN-ANHYDRIDE.

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RECENT work on the metabolism of aminoacids in the organism of omnivorous animals has necessitated a revision of the older views on the process of protein catabolism and protein regeneration.

The two practical general points on which the views of the physiologists of to-day differ from those of their predecessors are, first, the extent to which protein is disintegrated in the gastrointestinal tract, and, second, the nature of the protein fragments*from which the regeneration of tissue protein takes place.

Drechsel,¹ and later Kutscher,² were the first to bring to light the power of proteolytic enzymes to cause a deterioration of the protein molecule into its basic elements. Kutscher and Seeman³ then demonstrated that after a meal containing protein the elementary components of the protein molecule are found in the intestinal tract. These observations were corroborated by most later investigators. With these observations as a basis, it was assumed that through the action of digestive glands ingested protein undergoes complete cleavage into mono- and diaminoacids.

Meanwhile it became known, through the work of Fischer and his students and also through the observations on precipitin formation, that individual proteins differ not only in the nature and in the

¹ DRECHSEL: See HEDIN in DU BOIS REYMOND'S *Archiv für Physiologie*, 1891, p. 273.

² KUTSCHER: *Die Endprodukte des Tyrosinverdaung*. Habilitationsschrift, Strassburg, 1899.

³ KUTSCHER and SEEMAN: *Zeitschrift für physiologische Chemie*, 1901-1902, xxxiv, p. 528.

number of aminoacids which enter into their molecules, but also in the order in which individual aminoacids are linked together in each molecule. In the light of this knowledge protein assimilation could no longer be regarded as a process of mere retention of foreign protein, but had to be looked upon as the transformation of a substance with a given order of aminoacids into a substance containing the acids in a different order.

It was therefore natural to accept, on the basis of these speculative considerations, two distinct phases in the cycle of protein assimilation: the first, complete dissolution of protein into its elementary components in the intestinal tract, and the second, reconstruction of tissue protein from these fragments in the organs of the body. The experimental evidence which served as the basis for the first part of the assumption has already been referred to. In support of the second part, evidence was furnished by the work of Loewi,⁴ Henriques and Hansen,⁵ and Abderhalden⁶ and his co-workers.

The experiments of these investigators demonstrated that it was possible, for a short time at least, to maintain nitrogenous equilibrium by feeding animals with biuret-free cleavage products of protein. However, the details of their experiments warn emphatically against drawing definite conclusions of the degree of dissolution that a protein may undergo without losing its capacity for protein regeneration. Thus, on the one hand the observers found great difficulty in maintaining equilibrium by feeding animals on products obtained from the dissolution of proteins by means of mineral acids, while on the other hand this result was obtained without difficulty after feeding with the products of enzyme cleavage. The products obtained by the two methods differ in that the products of enzyme action contain intermediate products of hydrolysis of the nature of polypeptides, and aminoacids which on treatment with strong mineral acids undergo decomposition. Polypeptides and unstable aminoacids are not present among the products obtained by acid hydrolysis. The higher food value of the mixtures obtained by enzyme action can be attributed with equal probability to the presence in the mixture

⁴ LOEWI: *Archiv für experimentelle Pathologie und Pharmacologie*, 1902, *xlvi*, p. 393.

⁵ HENRIQUES: *Zeitschrift für physiologische Chemie*, 1904-1905, *xl*, p. 417, and 1906, *xlix*, p. 114.

⁶ Series of articles in *Zeitschrift für physiologische Chemie*, beginning vol. *xlii*.

of peptides, or to a greater number of aminoacids, or to the two factors together.

That in course of normal metabolism protein does not undergo complete dissolution in the gastrointestinal tract, but is in part absorbed in the form of peptides, is the view maintained by Falta ⁷ on the basis of his observations on the curve of nitrogen elimination after ingestion of various proteins. This author has observed that the time required by the organism of man to remove the nitrogen introduced in the form of a pure protein is not less than three days. On the other hand, Falta assumes, on the basis of experiments of Feder, that absorption from the intestinal tract is completed in a much shorter space of time. The delay of elimination is interpreted by him on the basis of the hypothesis that dissolution of protein in the organism takes place by degrees, that is, stepwise. Voigt ⁸ made similar observations on dogs, but is reluctant to accept the hypothesis of Falta. Regardless of their theoretical views, both authors agree that the curve of nitrogen elimination differs in its character in accordance with the nature of the ingested protein, and in this respect they corroborate an earlier observation of Praffenberger. The authors do not attempt to offer any explanation connecting the difference in the behavior of various proteins in the organism with differences in their chemical structure or composition. In fact, an interpretation of the observations on the curves of nitrogen elimination after protein ingestion may be based on either of the two following hypotheses: (1) That the peptides composing various proteins offer different resistances to proteolytic enzymes; and (2) that the individual aminoacids composing different proteins exhibit different behaviors in the organism.

There are scattered in literature abundant evidences favoring the view that individual aminoacids perform in the organism a specific function. The clearest expression to this assumption was given by Starling.⁹ Referring to the work of Willcox and Hopkins,¹⁰ who succeeded in maintaining nitrogenous equilibrium in a dog fed on zein to which tryptophane was added, Starling attributes to tryptophane the rôle of an agent stimulating the synthetic function of the

⁷ FALTA: *Deutsches Archiv für klinische Medicin*, 1904, lxxxi, p. 231; 1906, lxxxvi, p. 517.

⁸ VOIGT: *HOFMEISTER'S Beiträge*, 1906, viii, p. 409.

⁹ STARLING: *ASCHER and SPIRO, Beiträge*, 1906, v, p. 696.

¹⁰ WILLCOX and HOPKINS: *Journal of physiology*, 1906-1907, xxxv, p. 88.

tissues in a definite direction. Murlin has shown that the rôle of glyocol in the organism is determined not by its carbon content, but by the chemical nature of the molecule. The work of Hirsch,¹¹ Brügsch and Hirsch,¹² Stolte,¹³ and many others have demonstrated that the power of the organism to convert glyocol, alanin, leucin, etc. into urea is not the same. Hence the hypothesis connecting the behavior of a protein in the organism with the character of the composing aminoacids does not entirely lack an experimental basis. However, as yet we are not ready to reach a definite decision as to the nature of the factors determining the behavior of any protein in the organism, and we can hardly hope to be before we possess systematic information regarding the rôle of individual aminoacids in the organism. The work in that direction offers great difficulties at present, since we possess very few facts concerning the possible function of individual aminoacids. As already stated, Willcox and Hopkins and Starling regard tryptophane as the precursor of a specific hormone which stimulates the cells in their function of synthesizing protein. Others are inclined to view tyrosin and phenylalanin as the precursor of another hormone, namely, adrenin. Regarding the rôle in the organism of aminoacids of the aliphatic series, we possess neither actual knowledge nor hypothesis. To ascertain the parts of these acids we must employ indirect and even arbitrary methods. In the present investigation attention was directed towards the rate of speed of nitrogen elimination after administration of individual aminoacids and to the character of the nitrogenous compounds in which the nitrogen reappeared in the urine. It was reasoned that an aminoacid which serves the formation of a hormone-like substance will exhibit necessarily a slower rate of elimination than those that offer to the organism nothing more than calorific values. The conversion into urea of the two substances may be accomplished at different rates. Moreover, one would expect that this power to protect tissue protein would be determined by the relative importance of the hormone which they serve to form in the organism. Naturally, the manner in which the body disposes of a substance may also be determined by the state of nutrition of the

¹¹ HIRSCH: *Zeitschrift für experimentelle Pathologie und Therapie*, 1905, 1, p. 141.

¹² BRUGSCH and HIRSCH: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 638.

¹³ STOLTE: *HOFMEISTER'S Beiträge*, 1904, v, p. 15.

organism. A well-nourished animal receiving abundant food may usefully employ only the calorific energy of an additional aminoacid, while under other circumstances it may use the acid for constructive-synthetic purposes. On the other hand, it may occur that an organism in a poor state of nutrition may lose other elements required for the transformation of an aminoacid into the corresponding hormone, and therefore can benefit only by its calorific value. The observations of Brügsch and Hirsch¹⁴ on the combustion of glyocol, alanin, and leucin in the normal and in the fasting dog actually show these hypotheses to possess a basis of fact. These general considerations led us to perform our experiments on animals in a condition of nitrogenous equilibrium and of protein starvation.

METHODS.

Administration of food and of aminoacids.—The food of the animal weighing 9 kgm. consisted of 25 gm. of plasmon, 75 gm. of cracker meal, about 10 gm. of lard, and some salt. The food was made up in small balls and was slightly warmed in a pan. The dog after a while was trained so that it took the entire ration in less than five minutes. On the days of experiment the urine was collected in three-hour periods from nine A. M. until twelve P. M., and the last nine hours of the twenty-four constituted the last period. The curve of nitrogen elimination of the dog in nitrogenous equilibrium on the diet described was accepted as the standard curve. Additional food or aminoacids were added to the meal, and the curve of elimination was composed of the same periods as the standard.

Collection of urine.—The urine was received in the intervals between the individual periods in vessels containing hydrochloric acid and toluene. At the end of each period the bladder was carefully evacuated by catheterization and thoroughly irrigated. The urine and wash water were combined and subjected to analyses.

METHODS OF ANALYSES.

The total nitrogen was determined by the usual Kjeldahl-Gunning method; urea, by the method of Folin, and ammonia according to Folin-Shafer. Folin's method was subjected to a very careful trial

¹⁴ BRÜGSCH and HIRSCH: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 638.

during the preliminary stage of this work. The results obtained by the method were very satisfactory.

EXPERIMENTAL PART.

Period of standard diet. — During the period the intake of the dog contained 4.28 gm. of nitrogen. The period lasted twenty-six days. The highest output for twenty-four hours during this period reached 4.76 gm. of nitrogen (once) and the lowest 3.56 gm. (once). Generally the output of nitrogen approached very closely the intake, fluctuating between 4.40 and 4.60 gm. nitrogen per twenty-four hours. In Table I there is presented the course of elimination of total nitrogen, of urea, of ammonia, and of undetermined nitrogen in three-hour periods, the night period lasting nine hours.

Increased carbohydrate period. — It is generally accepted that the earliest phases of protein and of aminoacid catabolism consist in their desamidation. In the further course of catabolism of aminoacids they function as non-nitrogenous bodies, and as such may exercise a certain influence on the rate of combustion of the protein of the standard diet. For this reason it was considered advisable to precede the experiments with aminoacids by those with carbohydrates. Similar experiments had been performed in the past by Feder, and comparatively recently by Vogt. According to these writers the addition of carbohydrate to a pure protein diet causes the curve of nitrogen elimination to assume a less abrupt character and to approach the horizontal line nearer than the curve on a pure protein diet. Our experiment lasted four days; 50 gm. of starch were added to the standard diet. Tables II, III, and IV present the course of elimination of the various nitrogenous substances during three days of this period. This course does not differ materially from that of the standard diet. The explanation may lie in the fact that the standard diet contained in itself a quantity of carbohydrate sufficient to make possible a complete combustion of the protein, and that the excessive carbohydrates, especially in the first days of the period, are converted merely into glycogen without taking any further part in the immediate economy of the organism.

Increased fat period. — The influence of fat on the curve of nitrogen elimination has also been studied by older investigators, — by Panum, by Feder, and in recent years by Vogt. It was found that under the influence of fat the output of nitrogen by the urine was

more evenly distributed during the different periods of the day than it was on a fat-free diet. In our experiments the increased fat period lasted two days. Table V presents the distribution of nitrogenous substances in the urine collected in the same three-hour periods. Also in our experiment one can note the more even distribution of nitrogen during the various periods of the day. The nitrogen output in the early periods is lower than on the normal diet and higher in the later periods of the day. This difference in the nitrogen elimination can be interpreted by the retarding effect of fat on the protein absorption. There is some evidence in favor of this assumption.

The dog received daily 25 gm. plasmon, 75 gm. cracker dust, 40 gm. lard, 2 gm. salt.

Increased plasmon period. — Previous observers — Feder, Falta, and Vogt — noted that the curve of nitrogen elimination on a diet containing more protein than the standard diet was influenced by the nature of the protein. In our experiment the period of increased plasmon diet lasted two days. The dog received in addition to the standard diet 18 gm. of plasmon containing 2 gm. of nitrogen. Tables VI and VII contain the results of the analysis of the urine obtained at three-hour intervals. One can note that until the fourth period no increase in the nitrogen output occurs on the first day. The increase of the nitrogen output continues the following day, gradually declining during the first three periods, and the fourth period of the second day again shows a marked rise influenced by the diet of that day. A comparison of Tables VI and VII with Table I reveals also the significant fact that the catabolized part of the ingested protein is removed from the organism of the dog in the form of urea exclusively. Thus,

	T. N, gm.	Urea N, gm.	Ammonia N, gm.	Undetermined N, gm.
The urine of the first plasmon dog contained	4.48	4.13	0.137	0.198
The urine of the first normal period contained	3.87	3.47	0.149	0.264
Difference	0.61	0.66	0.012	0.066
The urine of the second plasmon day contained	5.22	4.94	0.140	0.130
The urine of the normal day contained	3.87	3.47	0.149	0.264
Difference	1.35	1.48	0.009	0.134

Glycocol and asparagin periods. — The older workers, Schultzen and Nentjki¹⁵ and Salkowski, have already noted that a great part

¹⁵ SCHULTZEN and NENTJKI: Zeitschrift für physiologische Chemie, 1906, xlvii, pp. 159-172.

of ingested aminoacids is converted into urea. Abderhalden and his co-workers have devoted to this subject a number of valuable investigations. They all arrived at the conclusion that aminoacids as well as simple peptides are converted in the organism of a dog principally into urea. However, an analysis of the figures contained in the tables illustrating the results of the experiments of Abderhalden and his pupils clearly shows that the facts which were in the possession of these authors did not justify any definite conclusion on the subject. Only in two experiments of Abderhalden and Teruuchi, in which small quantities of glycol and of alanin respectively were added to the standard diet, were the catabolized acids converted into urea to the extent of 100 per cent, in fact in the alanin experiment to 110 per cent. In many experiments there exists a complete lack of evidence that the acids or peptides were absorbed from the intestinal tract and catabolized in the organism. The most striking experiment of that nature is the one recorded by Abderhalden and Samuely, in which after the addition of 10 gm. of leucin containing 1 gm. of nitrogen the urine showed no increase in its nitrogen output. It therefore fails to bring forth evidence that the substance was at all absorbed. In other experiments recorded by Abderhalden and his co-workers the urine after administration of peptides or aminoacids shows only a slight increase in the nitrogen output, and the proportion of measured urea varied from 0 per cent to 11 per cent of the increase in the total nitrogen output. In our experiments the dog received on the glycin days 12.5 gm. of the acid, and on the asparagin days 10 gm. of asparagin, in addition to the standard food. In all our experiments the absorbed aminoacids were converted into urea to the extent of 100 per cent. Thus, on.

	T. N, gm.	Urea N, gm.	NH ₃ N, gm.	Undeter- mined N, gm.
The first glycin day the urine contained	6.07	5.65	0.225	0.196
The normal day the urine contained	3.87	3.47	0.149	0.264
Difference	2.20	2.18	0.076	-0.068
The second glycin day the urine contained	5.62	5.21	0.207	0.203
The normal day the urine contained	3.87	3.47	0.149	0.264
Difference	1.75	1.74	0.058	0.061

The same proportions are noted on the other two glycin days and also on the asparagin days. Thus, on

	T. N, gm.	Urea N, gm.	Ammonia N, gm.	Undetermined N, gm.
The first asparagin day the urine contained . . .	5.50	5.17	0.209	0.150
The normal day the urine contained	3.87	3.47	0.149	0.264
Difference	1.63	1.70	0.060	0.114
The second asparagin day the urine contained . .	5.50	5.12	0.165	0.205
The second normal day the urine contained . . .	3.87	3.47	0.149	0.264
Difference	1.63	1.65	0.016	0.059

It is clear from this experiment that proteins and the analyzed aminoacids are converted in the organism of the dog into urea completely. However, there exists a marked difference in the curve of nitrogen elimination on a diet containing increased protein and on that containing additional aminoacids. The rise of nitrogen elimination begins on the protein days only during the fourth period, and then the rise is comparatively low. On the aminoacid days the rise is already very considerable during the second period. There exists also a difference in the behavior of the two aminoacids. All the glycine nitrogen is removed within the first twenty-four hours. After five days of glycine feeding there was noted practically no retention of nitrogen. On the increased protein diet the rise in the nitrogen output persists to be very marked during the first three periods of the following day; according to Falta it lasts seventy-two hours. However, asparagin approaches protein in its behavior. There is a noted marked retention of nitrogen in the organism, although not to the same extent as on increased protein diet,¹⁶ and the rise in nitrogen elimination persists in the early periods of the day following the asparagin diet. Not only is the elimination through the urine of the absorbed asparagin retarded as compared with that of glycine, but also its absorption from the gastrointestinal tract is not quite so complete. This is evidenced by the higher nitrogen content of the feces.

Protein fasting period. — Ever since the classical experiments of Carl Voit physiologists have been conscious of the presence in the organism of two forms of protein. The difference of their behavior in the organism is evidenced most conspicuously at the time when the organism receives no protein material with the food. Under these conditions a portion of the body's protein is catabolized

¹⁶ Compare v. KNIERIEM: *Zeitschrift für Biologie*, 1874, x, p. 284, and MAUTHNER: *Zeitschrift für Biologie*, 1891, xxviii, p. 506.

at about the same rate of speed as the food protein, while the other portion possesses a great tenacity in preserving its own volume, its own rate of combustion, regardless of any physiological changes in the condition of the animal's nutrition. Nearly every investigator on this subject considered it his duty to modify the name given by his predecessors to these two forms of protein. In recent years it was pointed out, particularly by Folin,¹⁷ that the two forms of protein possess not only a different tenacity, but also a distinct character of catabolism. The more stable form (Organeiweiss of Voit, Stabile Eiweiss of Hofmeister, Endogenous protein of Folin) is converted into urea less completely than the other form. Since the observation was made that protein added to a standard diet is converted completely into urea, it seemed rational to expect that protein of standard diet was also transformed into urea completely. On the basis of this assumption one is led to the conclusion that the proportion of urea in normal dog's urine, fluctuating between 85 and 95 per cent, is conditioned by the fact that the so-called "Organeiweiss" is converted into urea in a still smaller proportion. Indeed Table XIV shows that after several days of protein starvation, when the total nitrogen output fell to 1.23 gm., the percentage of urea nitrogen suffered a very marked depression — to 68.8 per cent of the total nitrogen. On the other hand, deducting these values from those of the "normal" period, one notes that all the nitrogen removed in excess over the protein-starvation output is eliminated in the form of urea:

	Total N, gm.	Urea N, gm.	NH ₃ N, gm.	Undeter- mined N, gm.
Protein starvation	1.23	0.845	0.218	0.162
Difference	2.64	2.625	-0.069	-0.102

The diet during the protein-free period consisted of 60 gm. starch, 60 gm. sugar, 1 gm. diastase, 40 gm. lard, and 2 gm. of salt. The way of administration was in every detail the same as during the period of the standard diet.

Protein fasting and asparagin diet. — This period lasted five days. The intake contained 10 gm. of asparagin with 2.35 gm. nitrogen, and fat and carbohydrate in the same quantities as during the pervious experiment. The total output for the period was the following:

¹⁷ FOLIN: This journal, 1905, xiii, p. 66.

	Total nitrogen.		Total nitrogen.
First day	2.44 gm.	Fourth day	3.50 gm.
Second day	3.06 "	Fifth day	3.25 "
Third	3.50 "	Total	15.75 gm.
Total intake			11.75 "
Total loss in five days			4.00 gm.

On the day following fasting there was removed 0.75 gm. N in excess over the protein starvation value. Thus there remained a retention of about 2 gm. of nitrogen, or an average of 0.4 gm. per day. Approximately the same retention was observed when asparagin was fed in addition to a standard diet.

Also the conversion of catabolized asparagin into urea was as complete as during the days when asparagin was added to the standard diet. Thus during the period of protein starvation and asparagin diet the distribution of nitrogen in the urine was as follows:

	Total N, gm.	Urea N, gm.	NH ₂ N, gm.	Undeter- mined N, gm.
First day	2.24	1.91	0.150	0.179
Protein starvation	1.23	0.845	0.218	0.162
Difference	1.01	1.055	0.068	0.017
Fifth asparagin day	2.50	2.14	0.188	0.157
Protein fasting	1.23	0.845	0.218	0.162
Difference	1.27	1.285	0.030	0.005

Protein starvation and glycine diet. — Great difficulty was experienced in preventing the animal on this diet from vomiting. After a few days of vomiting the animal refused the food altogether. The animal was again placed on the standard diet of plasmon, cracker meal, and lard, and following several days of this diet again on a protein-free diet. It was then found that by adding to the protein-free diet about 20 gm. of cracker meal the dog retained 5 gm. of glycine per day. Larger quantities caused vomiting even under these circumstances. This period lasted three days. The total output of nitrogen during the period was as follows:

	Total N, gm.		Total N, gm.
First day	3.12 gm.	Third day	3.03 gm.
Second day	3.24 "	Total	9.39 gm.
Total intake for three days			4.92 "
Total loss			4.47 gm.

Hence the daily loss of nitrogen remained as it was on a pure protein-free diet. As is the case on the standard diet during protein starvation, the nitrogen of glycine is not retained in the organism.

The conversion of the substance into urea was also in this experiment complete.

	Total N, gm.	Urea N, gm.	NH ₃ N, gm.	Undetermined N, gm.
Protein free and glycine day	1.98	1.68	0.169	0.129
	1.23	0.845	0.218	0.162
	0.75	0.83	-0.049	-0.033

Protein fasting and glycyglycine anhydride diet. — This experiment lasted one and one-half days. The dog received with the protein-free diet 8 gm. of the anhydride.

The experiment was planned with a view to determine whether the greater retention of the nitrogen ingested in form of asparagine was conditioned by the presence in the molecule of the acid of the CONH₂ group. If this were true the nitrogen of glycyglycine anhydride which contains two CONH groups should be eliminated at a still lower rate of speed. However, it was noted that the anhydride was removed from the organism apparently without having undergone any change at all. Unfortunately, the dog died after the second feeding with the substance. It is impossible to be certain whether or not the death was caused by the anhydride or from another cause. The dog was perfectly normal the morning following the first experiment. However, the animal showed little desire to take food at the usual time, and took it about two hours after the usual time. The dog was found dead about one hour after feeding. Macroscopical and microscopical examination of the organs, made by Dr. Lamar of the Department of Pathology, failed to discover any definite lesion which could be regarded as the cause of death.¹⁸

Table XVIII clearly shows that there was absolutely no conversion of the substance into urea. Hence the substance had not been hydrolyzed into glycine. The table also shows that the increase in the nitrogen output began within the first three-hour period. In this respect the curve of nitrogen elimination resembles those of the days when the diet contained aminoacids. Thus, whenever amino-

¹⁸ The dog lost during the entire protein fasting period, lasting from May 2 to June 12, nearly 3 kilos. During that period it had received the standard ration from May 19 to May 23 and from June 3 to June 10.

acids or simple peptides are present in the gastrointestinal tract, they are absorbed and are removed through the urine in a comparatively short space of time, whether catabolized or unaltered. This leads to the conclusion that the absence of an increase in the nitrogen output after a protein-containing meal is caused by the fact that the hydrolysis of the protein into aminoacids or peptides had not yet taken place.

It is significant from this standpoint to consider the curve of nitrogen output on the first day of the period, when the diet contained plasmon in excess over the customary ration. The first three three-hour periods not only do not show any increase of the nitrogen output, but rather strike one by the slight depression of it. This might be interpreted in the light of the assumption that the ingestion of excessive quantities of protein may cause a retardation of the rate of its digestion.

TABLES I-XVIII.

TABLE I.

Standard diet.	Total nitrogen.	Urea.		NH ₃ .		Undetermined nitrogen.	
		Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.
I	0.475 +0.300 ¹	0.444	93.5	0.006	1.26	0.025	5.25
II	0.799 +0.643	0.756	94.6	0.013	1.63	0.030	3.76
III	0.802 +0.598	0.727	90.7	0.023	2.87	0.052	6.48
IV	0.610 +0.433	0.539	88.4	0.029	4.75	0.042	6.83
V	0.416 +0.275	0.347	83.4	0.017	6.50	0.042	6.50
VI	0.766 +0.392	0.649	84.6	0.051	6.66	0.066	8.60

TABLE II (STANDARD AND STARCH DIET).

I	0.596	0.553	92.6	0.007	1.17	0.036	6.53
II	0.720	0.645	94.5	0.005	0.69	0.070	4.81
III	0.842	0.811	96.5	0.017	2.08	0.024	1.42
IV	0.713	0.652	91.5	0.034	4.73	0.027	3.77
V	0.413	0.348	84.2	0.026	6.29	0.039	2.57
VI	0.844	0.705	83.5	0.075	8.90	0.064	7.60

TABLE III (STANDARD AND STARCH DIET).

I	0.429	0.390	91.0	0.006	1.30	0.033	7.70
II	0.687	0.648	94.3	0.003	0.44	0.036	5.26
III	0.761	0.713	93.6	0.010	1.32	0.038	5.08
IV	0.641	0.568	88.5	0.027	4.21	0.046	7.29
V	0.407	0.353	88.0	0.029	7.11	0.025	4.89
VI	0.845	0.713	84.3	0.082	9.71	0.050	6.00

¹ These figures show balance with the figures of the protein fasting days.

TABLES I-XVIII (Continued).

TABLE IV.							
Standard & starch diet.	Total nitrogen.	Urea N.		Ammonia N.		Undetermined nitrogen.	
		Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.
I	0.411	0.379	92.0	0.011	2.68	0.021	5.32
II	0.758	0.721	95.0	0.008	1.06	0.029	3.94
III	0.755	0.710	93.6	0.010	1.33	0.035	5.01
IV	0.602	0.545	90.5	0.030	4.98	0.025	3.52
V	0.418	0.369	88.0	0.027	6.45	0.022	5.55
VI	0.716	0.585	81.7	0.066	9.22	0.065	9.08

TABLE V (STANDARD AND LARD DIET).							
I	0.360 -0.115 ¹	0.343	95.0	0.010	2.78	0.007	2.22
II	0.459 -0.340	0.429	93.5	0.006	1.30	0.024	5.20
III	0.518 -0.284	0.483	93.3	0.004	0.77	0.031	5.93
IV	0.763 +0.153	0.712	93.30	0.013	1.70	0.038	5.00
V	0.550 +0.134	0.511	93.0	0.024	4.37	0.015	2.63
VI	0.930 +0.264	0.805	86.5	0.055	5.89	0.070	7.60

TABLE VI (STANDARD AND PLASMON DIET).							
I	0.465 -0.010 ²	0.425	91.3	0.010	2.16	0.020	6.54
II	0.717 -0.082	0.682	95.0	0.006	0.83	0.030	4.17
III	0.780 -0.022	0.744	95.5	0.011	1.41	0.025	3.09
IV	0.780 +0.170	0.730	93.5	0.010	2.05	0.034	4.45
V	0.547 +0.131	0.504	92.2	0.019	3.47	0.024	4.33
VI	1.190 +0.353	1.050	88.0	0.075	6.30	0.065	5.70

¹ These figures show balance with standard diet. ² Balance with standard diet.

TABLES I-XVIII (Continued).

TABLE VII.

Standard and plasmon diet.	Total nitrogen.	Urea N.		Ammonia N.		Undetermined nitrogen.	
		Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.
I	0.661 +0.186	0.624	94.3	0.010	1.50	0.027	4.20
II	1.016 +0.217	0.981	96.5	0.009	0.88	0.026	2.62
III	0.902 +0.100	0.884	96.0	0.010	1.11	0.008	2.89
IV	1.090 +0.480	1.030	94.5	0.020	1.83	0.040	3.67
V	0.590 +0.174	0.549	93.0	0.022	3.72	0.019	3.28
VI	0.964 +0.198	0.875	90.8	0.069	7.15	0.020	2.05

TABLE VIII (STANDARD AND GLYCIN DIET).

I	0.534	0.504	94.3	0.007	1.33	0.023	4.37
II	1.371	1.318	96.0	0.017	1.24	0.036	2.76
III	1.108	1.076	97.0	0.006	0.54	0.026	2.46
IV	1.126	1.065	94.5	0.027	2.40	0.034	3.10
V	0.706	0.641	91.0	0.044	6.28	0.021	2.72
VI	1.225	1.045	85.2	0.124	10.10	0.056	4.70

TABLE IX (STANDARD AND GLYCIN DIET).

I	0.450	0.417	92.5	0.007	1.55	0.016	5.95
II	1.250	1.206	96.5	0.009	0.72	0.035	2.78
III	1.340	1.276	95.0	0.024	1.79	0.040	3.21
IV	0.970	0.929	95.5	0.025	2.53	0.200	1.97
V	0.579	0.528	91.5	0.029	5.00	0.022	4.50
VI	1.030	0.858	83.4	0.112	10.85	0.060	5.75

TABLES I-XVIII (Continued).

TABLE X.							
Standard & glycin diet.	Total nitrogen.	Urea N.		Ammonia N.		Undetermined nitrogen.	
		Grams.	Per cent of total nitrogen.	Grams.	Per cent total of nitrogen.	Grams.	Per cent of total nitrogen.
I	0.591	0.566	95.6	0.009	1.52	0.016	2.88
II	1.240	1.197	96.4	0.013	1.05	0.030	2.55
III	1.560	1.454	93.1	0.026	1.67	0.080	5.23
IV	1.360	1.260	92.5	0.060	4.40	0.040	3.10
V	0.374	0.342	91.5	0.019	5.08	0.013	3.42
VI	1.060	0.920	86.8	0.081	7.65	0.060	5.55

TABLE XI (STANDARD AND GLYCIN DIET).							
I	0.559	0.491	87.9	0.035	6.25	0.033	6.85
II	1.231	1.170	95.5	0.017	1.38	0.037	3.15
III	1.375	1.269	92.0	0.021	1.53	0.085	6.46
IV	1.127	1.066	94.8	0.037	3.28	0.024	1.92
V	0.709	0.630	89.0	0.048	6.77	0.031	4.23
VI	1.010	0.830	82.1	0.120	11.90	0.060	6.00

TABLE XII (STANDARD AND ASPARAGIN DIET).							
I	0.485	0.461	95.0	0.011	2.27	0.013	2.73
II	1.381	1.344	97.3	0.013	0.94	0.024	1.76
III	1.317	1.265	96.0	0.022	1.70	0.030	2.30
IV	0.980	0.905	92.5	0.055	5.61	0.020	1.89
V	0.479	0.416	86.8	0.036	7.35	0.027	5.85
VI	0.878	0.780	89.0	0.072	8.20	0.036	2.80

TABLES I-XVIII (Continued).

Standard & asparagin diet.	Total nitrogen.	Urea N.		Ammonia N.		Undetermined nitrogen.	
		Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.
I	0.595 +0.120	0.559	94.0	0.005	0.83	0.031	5.17
II	1.23 +0.331	1.180	95.8	0.010	0.81	0.040	3.39
III	1.08 +0.278	1.035	95.7	0.015	1.39	0.030	2.91
IV	0.995 +0.385	0.923	92.4	0.037	3.72	0.035	3.88
V	0.549 +0.133	0.501	91.3	0.027	4.93	0.021	3.79
VI	1.048 +0.282	0.929	88.6	0.071	6.78	0.048	4.62

TABLE XIV (PROTEIN FREE DIET).

I	0.175	0.134	76.5	0.019	11.0	0.022	12.5
II	0.156	0.125	80.0	0.008	5.1	0.023	14.8
III	0.204	0.103	80.0	0.019	9.4	0.022	10.6
IV	0.177	0.131	74.0	0.023	13.3	0.023	12.7
V	0.141	0.095	67.0	0.026	18.7	0.018	14.3
VI	0.374	0.197	52.7	0.123	33.0	0.054	14.3

TABLE XV (PROTEIN FREE AND ASPARAGIN DIET).

I	0.166 -0.009	0.138	83.1	0.007	4.2	0.011	12.7
II	0.479 +0.323	0.420	87.8	0.022	4.6	0.037	7.6
III	0.454 +0.250	0.406	89.3	0.020	4.4	0.028	6.3
IV	0.403 +0.226	0.355	88.0	0.031	7.8	0.017	3.2
V	0.197 +0.056	0.163	82.8	0.019	9.6	0.009	7.5
VI	0.546 +0.172	0.433	79.2	0.057	10.4	0.056	10.4

TABLES I-XVIII (Continued).

TABLE XVI.							
Protein free and asparagin diet.	Total nitrogen.	Urea N.		Ammonia N.		Undetermined nitrogen.	
		Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.	Grams.	Per cent of total nitrogen.
I	0.147 -0.028	0.116	79.0	0.010	6.3	0.021	14.7
II	0.161 +0.005	0.130	80.8	0.013	8.1	0.018	11.1
III	0.350 +0.146	0.316	90.2	0.018	5.5	0.016	4.3
IV	0.453 +0.276	0.413	90.3	0.029	6.3	0.016	3.4
V	0.368 +0.227	0.320	87.0	0.026	7.1	0.020	5.9
VI	1.020 +0.646	0.844	82.8	0.092	9.0	0.066	8.2

TABLE XVII (PROTEIN FREE AND GLYCIN DIET).							
I	0.092 -0.083	0.067	72.5	0.015	16.8	0.010	10.7
II	0.294 +0.138	0.259	88.0	0.020	6.8	0.015	5.2
III	0.362 +0.158	0.323	90.5	0.017	4.6	0.017	4.9
IV	0.399 +0.222	0.355	89.2	0.027	6.8	0.017	4.0
V	0.273 +0.132	0.228	83.5	0.024	8.8	0.021	7.7
VI	0.560 +0.186	0.446	79.8	0.066	11.8	0.048	8.4

TABLE XVIII (PROTEIN FREE AND GLYCYLGLYCIN ANHYDRIDE DIET).							
I	0.218 -0.043	0.110	50.5	0.022	10.1	0.086	39.4
II	0.266 +0.110	0.113	42.5	0.021	7.9	0.132	49.6
III	0.463 +0.260	0.170	36.8	0.019	4.1	0.274	59.1
IV	0.521 +0.344	0.157	30.1	0.021	4.0	0.343	65.9
V	0.293 +0.152	0.095	32.4	0.014	4.8	0.184	62.8
VI	0.853 +0.479	0.357	42.0	0.085	10.0	0.409	48.0

TABLES I-XVIII.

TOTALS FOR TWENTY-FOUR HOURS.

No. of table.	Diet.	Total nitrogen.	Urea nitrogen.		Ammonia nitrogen.		Undetermined nitrogen.		Feces.	Output.	Intake.	Balance.
			Grams.	Per cent of total N.	Grams.	Per cent of total N.	Grams.	Per cent of total N.				
I	Standard	3.87	3.47	89.4	0.149	3.83	0.264	6.8	0.51	4.38	4.23	-0.10
II	Standard and starch	4.13	3.74	90.5	0.164	3.97	0.224	5.4	0.44	4.53	4.23	-0.30
III	Standard and starch	3.77	3.48	89.5	0.157	4.16	0.237	6.3	0.27	4.04	4.42	+0.38
IV	Standard and starch	3.66	3.31	90.3	0.152	4.16	0.201	5.5	0.15	3.81	4.30	+0.49
V	Standard and starch	3.58	3.28	91.5	0.111	3.10	0.185	5.4	0.50	4.08	4.30	+0.22
VI	Standard and plasmon	4.48	4.13	92.3	0.137	3.06	0.198	4.6	0.31	4.79	6.39	+1.60
VII	Standard and plasmon	5.22	4.94	94.9	0.140	2.63	0.130	2.4	0.29	5.51	6.39	+0.88
VIII	Standard and glycine	6.07	5.65	93.1	0.225	3.70	0.196	3.2	0.18	6.25	6.54	+0.29
IX	Standard and glycine	5.62	5.21	92.8	0.207	3.66	0.203	3.5	0.25	5.87	6.54	+0.67
X	Standard and glycine	6.18	5.72	92.7	0.208	3.36	0.239	3.9	0.21	6.39	6.54	+0.15
XI	Standard and glycine	6.01	5.46	91.0	0.278	4.60	0.264	4.4	0.44	6.45	6.54	+0.09
XII	Standard and asparagin	5.50	5.17	94.0	0.209	3.80	0.150	2.2	0.60	6.10	6.42	+0.32
XIII	Standard and asparagin	5.50	5.12	93.0	0.165	3.00	0.205	4.0	0.41	5.91	6.67	+0.86
XIV	Protein free	1.23	0.84	68.8	0.218	17.70	0.162	13.5	0.19	1.42
XV	Pr'n free and asparagin	2.24	1.91	85.3	0.150	6.70	0.179	8.0	0.20	2.44	2.35	-0.09 ²
XVI	Pr'n free and asparagin	2.50	2.14	85.8	0.188	7.52	0.157	6.2	0.75	3.25	2.35	-0.90 ³
XVII	Protein free and glycol	1.98	1.68	85.0	0.169	8.53	0.129	6.5	1.05	3.03	1.29 ⁵	-1.39 ⁴
XVIII	Protein free and glycol-glycin anhydride.	2.61	1.00	38.4	0.182	6.97	0.143	54.6	1.73 ⁷	0.35 ⁶
												...

¹ These figures show balance against the figures of the protein fasting days.
² Protein starvation = -1.45.
³ Protein starvation = -1.45.
⁴ Protein starvation = -1.45.
⁵ Glycoed.
⁶ Plasmon.
⁷ Glycylglycin.
⁸ Plasmon.

ACAPNIA AND SHOCK.—II. A PRINCIPLE UNDERLYING THE NORMAL VARIATIONS IN THE VOLUME OF THE BLOOD STREAM, AND THE DEVIATION FROM THIS PRINCIPLE IN SHOCK.

By YANDELL HENDERSON

(WITH THE COLLABORATION OF FELIX PERCY CHILLINGWORTH AND JAMES RYLE COFFEY).

[From the *Physiological Laboratory of the Yale Medical School.*]

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I. THE FACTORS DETERMINING THE OUTPUT OF THE LEFT HEART.

THE minute volume of the arterial blood stream is the sum of the systolic discharges of the left heart in the minute. The number of these discharges is the pulse rate, and is at all times easily determinable. Beset with difficulties, on the other hand, are the measurement of the absolute volume of the systolic discharge, and the estimation of the relative amplitude of the strokes of the heart at different rates of beat and under various conditions. Owing to these difficulties, present knowledge regarding the principle or principles underlying even the normal variations in the volume of the arterial blood stream is indefinite.

The extensive literature bearing upon this subject has recently been reviewed by Tigerstedt in the *Ergebnisse der Physiologie*¹ and need not here be discussed in detail. The investigations of Hering, Volkmann and Vierordt, Howell and Donaldson, Grehant and Quinquaud, Stolnikow, Stewart, R. Tigerstedt, Zuntz and Hage-

¹ TIGERSTEDT, R.: *Ergebnisse der Physiologie*, 1905, iv, p. 481, and 1907, vi, p. 265.

mann, Loewy and v. Schrötter, C. Tigerstedt, Elving and v. Wendt, and others have shown that the ratio between the heart rate and the minute volume of the arterial blood stream is subject to extensive fluctuations. This fact has led some writers to conclude that variations in the amplitude of the heart beat are as important an element in determining the total arterial blood supply of the body as are the alterations in rate, and that rate and amplitude are independent variables. We, on the contrary, have accumulated a mass of data in the form of volume curves, or plethysmograms, of the heart (of dogs) which indicate that when the venous supply to the right heart is ample the rate of beat is the fundamental variable. The principle according to which the amplitude of beat is dependent upon the rate has been formulated in a previous paper.² Its application to the minute volume of the blood stream is the topic of this article.

The volume of the systolic discharge of the left heart is dependent upon four principal sets of varying mechanical factors, — arterial pressure, the pulmonary circulation, the behavior of the heart itself especially in respect to the speed and extent of its diastolic relaxation, and the pressure and volume of the venous stream to the right heart.

1. The first of these factors exerts under physiological conditions a relatively slight influence. Within the limits of the normal variations in arterial pressure the systolic discharge against a high tension is as full as against a low. The output of the heart may vary widely, while arterial pressure is nevertheless maintained nearly uniform by the compensating alterations of peripheral resistance under the influence of the vasomotor nervous system. Mean arterial pressure is not an index of the volume of the blood stream.

2. Rhythmic alterations in the pulmonary circulation, induced mechanically by the changes in the size of the thorax, have been regarded by some writers as important factors in determining the variations in the output of the heart and in arterial pressure which occur during the successive phases of respiration.³ In a previous paper⁴ we have shown, however, that such is not the case, but that these variations are due to rhythmic alterations in the pulse rate

² HENDERSON, Y.: This journal, 1906, xvi, pp. 352 and 365.

³ For literature, see TIGERSTEDT, R.: *Ergebnisse der Physiologie*, 1903, ii, 2, pp. 560-565.

⁴ HENDERSON, Y.: This journal, 1906, xvi, p. 344. See also tracing 1 in Fig. 2 and tracings 1, 2, and 5 in Fig. 7 (pp. 352 and 369) of this paper.

induced through nervous channels. In a large number of experiments upon dogs the aortic pulse curve was recorded by means of a Hürthle manometer; then the thorax was opened, and the positive pressure respiration method of Brauer instituted. The volume curve of the ventricles of the heart was recorded, and simultaneously another record of the aortic pulse was taken. When the two pulse curves were similar in all respects, the pumping action of the heart before the opening of the thorax must have been identical with that shown in the volume curve. Careful study of this curve has convinced us that fluctuations in the pressure of the blood in the pulmonary vessels during the phases of respiration play mechanically only a secondary part in the variations in the volume of the systolic discharge of the heart.⁵

3. The behavior of the heart itself is under normal conditions the principal mechanical factor in determining the volume of the blood stream. According to the view to be here elaborated the speed and extent of the diastolic relaxation under the distending force of venous pressure are the principal elements in this behavior. They determine the maximum systolic discharge of which the heart is capable and the fractions of this volume which it ejects under various submaximal conditions. Engelmann,⁶ on the contrary, has postulated four distinct properties of the heart (among which diastolic distensibility does not appear to be included), each of which he holds may vary independently of the others. If under the ordinary conditions of life fluctuations of tonus and contractility (or *Treppe*) occur independently of each other and of the rate of beat, it would appear almost impossible that any mathematical expression of the variations in the volume of the blood stream could be formulated. In an earlier paper we have shown, however, that under certain conditions the amplitudes of diastolic relaxation and systolic contraction at all rates of beat are susceptible of simple geometrical expression.⁷ The essential element in these conditions is the maintenance of an ample venous supply to the right heart. In our experiments tonus and *Treppe* fluctuated widely at various rates of beat, but were always dependent upon the rate. These two functions varied not only in the same sense, but also nearly equally. Thus they prac-

⁵ A similar conclusion is reached by T. LEWIS: *Journal of physiology*, 1908, xxxvii, p. 233.

⁶ ENGELMANN, TH. W.: *Archiv für Physiologie*, 1900, p. 315

⁷ HENDERSON, Y.: *This journal*, 1906, xvi, p. 352.

tically neutralized each other in their influence upon the amplitude of the heart beat. The variations in the amplitude of systole were found to be determined by the duration of the preceding diastole. The relation is not, however, a simple proportion. The relaxation of the heart, like that of a skeletal muscle, describes a parabola. The durations of diastole and the volume of blood entering the ventricles during the relaxations (to be discharged by the succeeding systoles) bear the same relation to each other as do the abscissæ and ordinates of various points in this parabola. Under uniform external conditions the fundamental element in the behavior of the heart, upon which all other variables (*c. g.*, tonus, Treppe, amplitude of stroke, etc.) depend, is the rate of beat. Since the publication of these conclusions we have devised a diagram which summarizes these observations as to the amplitude of beat and the duration of systole⁸ and diastole at all rhythms of beat, although it has the defect of omitting tonus and Treppe variations. A photograph of the diagram adjusted to show a sudden change from a slow to a rapid heart rate is reproduced in Fig. 1. Its significance and its experimental justification will be more fully set forth in the discussion of the graphic records reproduced in Figs. 2 and 7. It illustrates the principle of the uniformity of the heart beat when the venous supply is ample.

4. For the maintenance of the full efficiency of the heart it is essential that the volume of the venous stream to the right auricle should be ample, and the resulting pressure competent for the distention of the right ventricle as fully and as rapidly as it relaxes in diastole. In the course of observations upon the volume curve of the heart of more than 60 dogs, we found that in a large majority of the experiments two distinct periods were clearly distinguishable. In the earlier period the graphic records conformed quite closely to the requirements of the diagram (Fig. 1), but in the later they always deviated more or less widely from this uniformity. If the distinction between these two periods were neglected our data, like those of many previous investigators and contrary to the view expressed in the preceding paragraph, might be interpreted as indicating an extreme inherent variability in the behavior of the heart. We have become convinced, however, that fluctuations in the func-

⁸ For literature bearing upon the duration of systole at various rhythms of beat, see W. P. BOWEN: This journal, 1904, xi, pp. 61 and 67.

tional capacity of the normal heart occur only as secondary results of vascular changes, and are never primary in the manner in which Engelmann believes. Examination of the abnormal volume curves of the later period in our experiments demonstrates that the diminu-

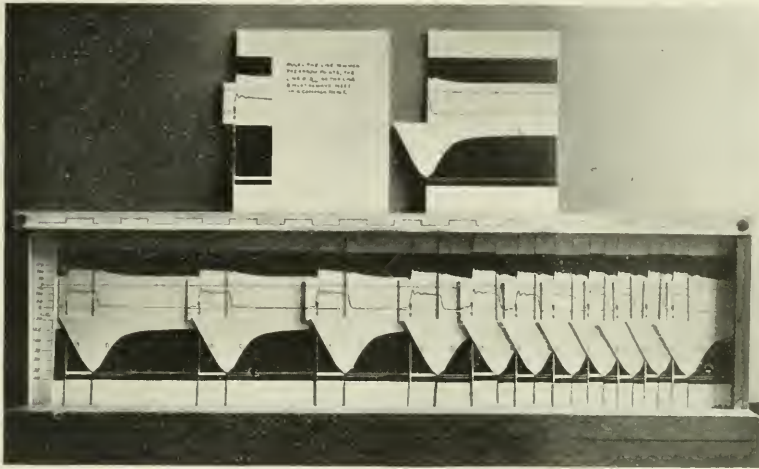


FIGURE 1. — Two sets of cards are placed alternately in the grooves of a frame, so that they can be slid apart or drawn together to represent the intervals between beats at slow or at rapid rates. Specimens of these cards are shown above the diagram. Upon one the systolic portion of the pulse and intraventricular pressure curves are drawn, and the opening of the semilunar valves is indicated by an arrow. Upon the other are shown the diastolic portions of these curves and the complete volume curve of the ventricles, as recorded at very slow rhythms of beat, consisting of the down-stroke (*S*) of the systolic discharge, the up-stroke (D_1) of the diastolic relaxation and refilling of the ventricles, and the horizontal portion of the curve (D_2), during which the ventricles are quiescent in diastasis. In order to bring the pulse and intraventricular pressure curves into correct relations with the volume curve, it is essential that the arrow should be placed directly above the point of intersection of the edge (*S*) of the card in front and the line (D_1D_2) of the card behind, *i. e.*, to the left. The intervals of time indicated at the top of the diagram may be taken as 0.15 to 0.20 second for a dog, or (probably) 0.25 to 0.30 for a man. The object of the diagram is to show how and why, because of the uniformity of the volume curve, the amplitude of every systole is determined by the duration of the diastole preceding and by the form of the relaxation curve (about one fifth the original size).

tion in the amplitude of the systolic contractions is the result of the incomplete filling of the ventricles during diastole. The diastolic upstrokes of these curves are less abrupt than normally.

The retarded in-flow of blood cannot be due to a lessened suction by the ventricles during diastole, for R. von den Velden has shown that the supposed post-systolic negative pressure in the ventricles

is merely an artefact.⁹ It cannot be the result of a diminution in the force of auricular systole, for the retardation is most marked during the early part of diastole, and is thus prior to auricular systole. Moreover, auricular systole plays only an inconsiderable part in the filling of the ventricles.¹⁰ Insufficiency of the venous supply is the cause of the phenomenon. This is demonstrated by the fact that, without exception, whenever saline was infused into a vein the diastolic up-stroke regained its normal abruptness and the amplitude of the volume curve (indicating the efficiency of the heart's action) was thus restored.¹¹

When the thorax is intact, the effective force of the venous stream to the right auricle is the difference between its absolute pressure and the negative pressure of the intra-pleural space. The minimum effective venous pressure which is necessary for the distention of the right ventricle as rapidly as its relaxation allows, is certainly not less than 3.0 mm. of mercury. We have found that, if the ventricles are enclosed in a plethysmograph into which air is gradually forced, arterial pressure falls whenever the difference between venous pressure and the extra-cardial pressure becomes less than 3.0 mm. of mercury.¹² In observations upon dogs, under operative conditions but with thorax intact, Burton-Opitz¹³ found pressures at the central end of the vena cava superior from -1.2 mm. to -4.8 mm. of mercury. The pleural pressure varied from -4.0 during the respiratory pause to -8.25 mm. at inspiration. These figures indicate that at times the effective venous pressure in anæsthetized animals is less than 3.0 mm. In four experiments in which the thorax had been opened Burton-Opitz recorded venous pressures of 2.4, 1.6, 1.4, and 0.8 mm., respectively. In two similar experiments upon large dogs whose hearts were beating efficiently we have found pressures of 5.0 and 6.5 mm., respectively. In healthy men the effective venous pressure is probably higher than these figures. We find (by Gaertner's method)¹⁴ that the veins in the back of the hand do not collapse until lifted above the level of the clavicles, with the subject sitting or standing. They remain distended up to 25 or 30 centimetres above the sternum when the subject is lying upon his back.¹⁵

⁹ VON DEN VELDEN, R.: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 432.

¹⁰ See tracings 6, 7, and 8 in Fig. 7 (p. 369) of this paper.

¹¹ HENDERSON, Y.: *This journal*, 1908, xxi, pp. 143-146.

¹² *Ibid.*, 1906, xvi, p. 307.

¹³ BURTON-OPITZ: *This journal*, 1903, ix, p. 201, and 1902, vii, p. 446.

¹⁴ GAERTNER, G.: *Münchener medizinische Wochenschrift*, 1904, lxxiv, p. 2038.

¹⁵ Cf. reference to HOOKER and EYSTER on p. 370.

These observations justify the presumption that under normal conditions the effective force of venous pressure (the pressure in the *venæ cavæ* aided by the pleural tension) is sufficient to insure the rapid distention of the right ventricle. But under anæsthesia and under operative procedures the measurements recorded by Burton-Opitz and by us show that venous pressure frequently falls below the force requisite for the maintenance of the full efficiency of the heart. The apparent failure of the heart in shock is really due to a failure of the venous supply. The fluctuations in the volume of the arterial blood stream independently of variations in the heart rate, which have been observed by previous investigators and by us also, are not due to alterations in the functional activity of the heart, induced through the nervous system, as the views of Engelmann suggest, but are the results of changes in venous pressure.

The behavior of the heart revealed by the volume curve of the ventricles at all rates of beat when the venous supply is ample — in other words, the principle underlying the normal variations in the volume of the arterial blood stream — will next be considered.

II. THE UNIFORMITY OF THE VOLUME CURVE AND ITS CONSEQUENCES.

Graphic records of the ventricular volume changes and of the simultaneous aortic or intraventricular pressure express the mechanics of the heart with the precision of the indicator diagram of a steam-engine. Rothberger¹⁶ in a recent paper has shown that when a plethysmograph is so placed as to include only the ventricles the volume curve affords an accurate measure of the volume of the systolic discharge and of the minute volume of the arterial blood stream. In a previous paper we have described devices by which under the positive pressure respiration method of Brauer or under artificial respiration the heart can be regulated to any desired rate of beat, and varied at the will of the operator.¹⁷ By these plethysmographic and cardio-regulative methods the data exemplified in Figs. 2, 5, and 7 were obtained. Observations of this character have been made by us upon 60 dogs. The behavior of the heart in the early period of these experiments is illustrated by the graphic records reproduced in Fig. 2.

¹⁶ ROTHBERGER: *Archiv für die gesammte Physiologie*, 1907, cxviii, p. 353.

¹⁷ HENDERSON, Y.: *This journal*, 1908, xxi, pp. 147 and 153.

In Fig. 2 are reproduced the carotid pressure and ventricular volume curves obtained at rhythms varying from a "vagus pulse" of one beat in one and a half seconds (record 5) up to a rate of four and a half beats in one second (record 4). Reading from left to right, the down-strokes of the volume curve express the systolic

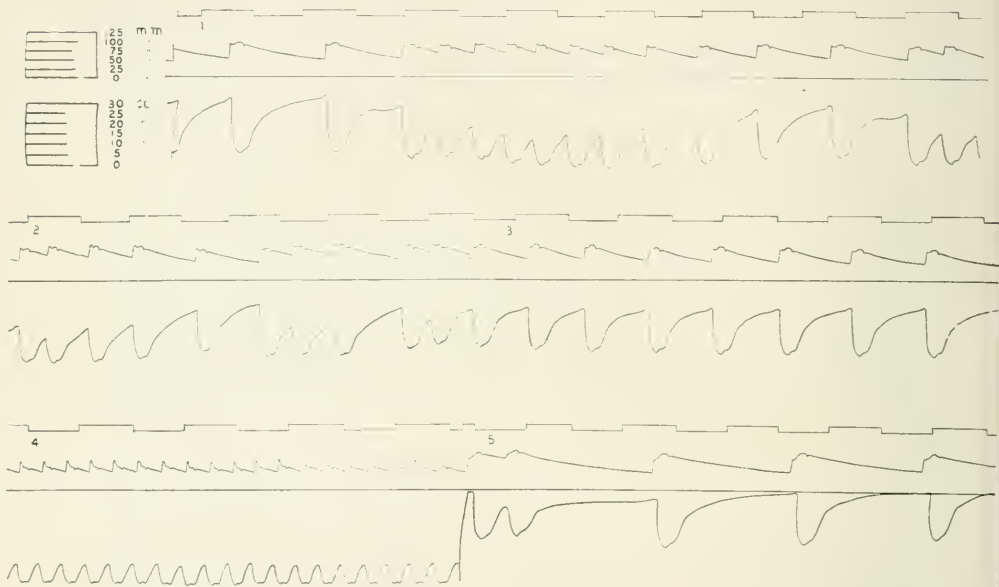


FIGURE 2. — Dog of 6.5 kilos. Morphin and ether. Respiration of compressed air (uniform pressure of 13 cm. water). Variations in heart rate from 40 up to 270 beats per minute induced by regulation of the supply of fresh air. Time record in 0.5 second. Carotid pressure recorded by a Hürthle manometer. Volume curve of the heart recorded by a tambour connected with a plethysmograph over the ventricles. The tonus and Treppe variations of the heart are shown by the distance of the volume curve beneath the base line. Thus the diastolic volume in record 3 is 13 c.c. less than that in record 5, and the systolic volume in record 4 is 20 c.c. less than in record 5. Nevertheless all the systolic down-strokes and diastolic up-strokes are merely longer or shorter arcs of the typical curve at the beginning of record 1. Note also that, owing to differences in the form of the pulse wave, the pulse pressure in record 4 is greater than in the inspiratory beats of record 1, although the systolic discharge in record 4 is the smaller. (About two fifths the original size.)

discharges, and the up-strokes the diastolic filling of the ventricles. At the slower rhythms a marked diminution in tonus involving an increase in both the systolic and diastolic volumes is indicated by the higher level of the entire curve. At the more rapid rhythms the Treppe variation and consequent diminution in the systolic vol-

ume of the heart is indicated by the lower level reached. But examination shows that the Treppe and tonus variations do not to any marked degree affect the amplitude of the beats. On the contrary, the volume curve at 270 beats to the minute consists merely of shorter arcs of essentially the same contraction and relaxation curves as those described in the full beats of the "vagus rhythm" of 40 per minute. The duration of the cardiac cycle at the various rhythms cuts off, so to speak, longer or shorter arcs of a nearly invariable volume curve. In other words, when the curves of a systole and the succeeding diastole at any rhythm are superimposed upon those of systole and diastole at any other rhythm, in the same manner as a geometrician superimposes angles, the lines are found to correspond. From this "superimposability" it follows that the volume of the systolic discharge of this heart at all rhythms can be summarized in the diagram shown in Fig. 3. In this diagram is reproduced a complete volume curve such as that occurring at the first heart-beat in Fig. 2. From various points in the relaxation curve such systolic down-strokes are drawn as would be described if the heart were to beat again after intervals of diastole corresponding to the time values of these points. Thus it becomes apparent that *the amplitude of any systole is the ordinate of that point in the complete relaxation curve for which the abscissa is the duration of the preceding diastole.* In order to show the relation of these amplitudes to the duration of the entire cardiac cycle (*i. e.*, to the heart rate), the vertical lines corresponding to the ordinates of the various points are drawn at the completion instead of at the onset of the systolic down-strokes.

The output of the left heart per minute at any uniform rhythm is equal to half the amplitude of the volume curve multiplied by the number of beats occurring in the minute. From the diagram (Fig. 3) it is evident that in a cardiac cycle of 1.0 second the amplitude of beat of the heart here discussed is nearly at its maximum. Therefore at all rates of beat below 60 per minute the volume of the arterial blood stream varies almost exactly in proportion to the rate. Indeed the output of the heart per minute holds nearly proportional to the rate up to a rhythm of 80 per minute, for the amplitude is only slightly lessened in a cardiac cycle of 0.75 second. At a rhythm of 120 per minute, however, the shorter cycle (0.5 second) causes a considerable diminution in the amplitude of beat, so that the output of the heart per minute, although still increasing

with each rise of rate, is no longer proportional to the rate. When the rate increases progressively from 120 to 240 per minute, the output is only slightly increased, for the amplitude of beat of a cycle of 0.25 second is little more than half that of a cycle of 0.5

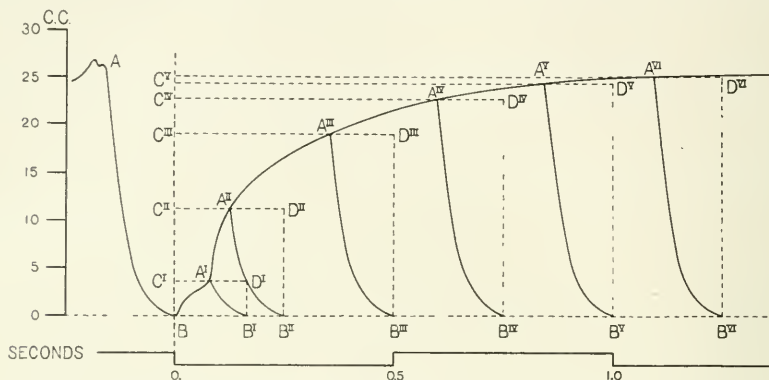


FIGURE 3. — The curve $ABA' A'' A''' A'''' A''''''$ is reproduced from the first volume curve in Fig. 2. Straight vertical coördinates have, however, been substituted for the arcs described by the stylus of the recording tambour, and corresponding corrections have been made in the volume curve. The systolic down-strokes $A_1 B_1, A_{II} B_{II}$, etc. are arcs of the full stroke of which AB is the type. The corresponding durations of diastole are $C_1 A_1, C_{II} A_{II}$, etc.; the durations of systole are $A_1 D_1, A_{II} D_{II}$, etc.; and the durations of the entire cardiac cycle are $C_1 D_1, C_{II} D_{II}$, etc., or their equivalents BB_1, BB_{II} , etc. The amplitudes of systole are $D_1 B_1, D_{II} B_{II}$, etc. The volume of the systolic discharges of the left ventricle is one half of these amplitudes. For example, at a uniform rate of 120 beats per minute each cycle would have the duration of 0.5 second or BB_{III} , and each systole would discharge from the left ventricle into the aorta 9.5 c.c. of blood, or one half $D_{III} B_{III}$. Similar relations hold true for all other rates of beat. The minute volume of the arterial blood stream thus determined for this dog at all heart rhythms is expressed in the curve VBS in Fig. 4.

second. At rates above 240 the output of the heart per minute is diminished in a progressively greater proportion by each increase of rapidity of beat.

The relations between the minute volume of the arterial blood stream and the rate of heart beat thus derived from the complete volume curve of the ventricles are plotted in the curve VBS in Fig. 4. These relations necessarily hold true only during uniform rhythms. During arrhythmia or during marked respiratory variations in the heart rate the minute volume of the output of the left heart may exceed or (more often) fall short of the curve VBS . Thus in record 1 of Fig. 2 the output during inspiration is 24 c.c. per second and that during expiration 14 c.c. The minute output

calculated from this tracing is expressed by the position of the small circle marked (1) in Fig. 4. The arrhythmia of record 2 (Fig. 2) affords an output corresponding to the circle (2) in Fig. 4. In record 5 (Fig. 2) occurs an extra-systole.¹⁸ It falls short of normal amplitude. Even if interpolated beats maintained full amplitude they must necessarily involve a considerable diminution in cardiac efficiency, because of the brevity of the preceding diastole and the duration of the succeeding compensatory pause. In respect to heart block it is a mistake to suppose that a ventricular systole following two or three auricular systoles has a greater amplitude than one of the same ventricular rate with normal auricular relations.¹⁹ Auricular systole is mechanically unimportant. The ventricles fill according to their own relaxation curve under venous pressure.²⁰

In this method of finding the normal relations of output to rate at all rhythms, there are two possible theoretical errors. Roy and Adami²¹ hold that during systole the auriculo-ventricular valves are drawn down into the cavity of the ventricles by the contraction of the papillary muscles and thus take an active part in the discharge of the blood into the arteries. If this is true, the volume curve of the exterior of the ventricles must show considerably less than the true volume changes of the heart. The measurements of Rothberger have demonstrated a very close agreement between the output of the heart as measured by means of a stromuhr inserted in the aorta, and as calculated from the volume curve of the ventricles.²² This concordance proves conclusively, it seems to us, that the contraction of the papillary muscles serves only to hold the valves in place and flat across their orifices, or, so to speak, "to take in the slack" as the ventricles shorten and the orifices narrow. It was shown by Chauveau that the base of the ventricles descends during systole. Indeed the entire mass of the ventricles must move caudad (*cf.* Rehfisch below). Porter demonstrated that the increase in the capacity of the auricles thus caused is an important element in the mechanics of the auricular reservoirs. But concurrence in this view does not involve admission that the mitral and tricuspid valves are pulled down into the ventricular chambers like the plunger of a syringe.²³

¹⁸ For another volume curve of arrhythmia, see *This journal*, 1906, xvi, p. 344.

¹⁹ *Cf.* CUSHNY and GROSH: *Journal of the American Medical Association*, 1907, xlix, 1259.

²⁰ See record 8 in Fig. 7 on p. 369.

²¹ ROY and ADAMI: *Practitioner*, 1890, xlv, p. 414.

²² ROTHBERGER: *Loc. cit.*

²³ PORTER: *Journal of physiology*, 1892, xiii, p. 537.

The other possible error referred to is due to the peculiar movements of the blood in the coronary circulation. The amplitude of the volume curve expresses the distention and compression of the coronary vessels in addition to the expansion and contraction of the ventricular chambers. At the beginning of diastole (between *B* and *A*₁ in Fig. 3) a notch occurs in the volume curve which is probably produced by the sudden inrush of blood from the aorta into the coronary arteries before the musculature of the heart has relaxed sufficiently for the inflow from the auricles into the ventricles to commence. It would be more accurate therefore, in determining the volume of the systolic discharge of the ventricles, to measure upward from the level of this notch than from the extreme lower point of the curve. The error involved in estimating the minute volume of the output of the heart at slow and normal rhythms without this correction is however small. Furthermore, with increasing rapidity of beat the size of this coronary notch in the volume curve diminishes (compare records 4 and 5 in Fig. 2), so that the error involved in neglecting this correction is probably no greater in rapid than in slow rhythms.

In passing from systole to diastole the heart is for a period of about 0.15 second in a relatively quiescent state. The duration of this period is practically the same at all rhythms, but its importance as an element in the efficiency of the heart at rapid and at slow rhythms is very different. In any cycle of a duration longer than 0.25 second the subtraction of this period leaves sufficient time for blood in the quantities determined by the abscissæ of the relaxation curve to enter and be discharged from the ventricles. But at a rate of 340 to 360 the cycle would be reduced to 0.17 or 0.18 second; and only 0.03 to 0.04 second would be allowed for the entrance and exit of blood. The heart must then come into a state which is practically that of tetanus, and its pumping action must cease.

In the course of a four-hour period of observation upon the dog, which, as a typical example of our experiments, is here under consideration, more than 20 metres of such tracings as are reproduced in Fig. 2 were obtained. From this mass of records of the behavior of the heart at rates of beat varying between 40 and 270 per minute the curves *DI'* and *SI'* in Fig. 4 have been constructed. They show the volume of blood in both ventricles at the beginning and end of systole at all rates of beat. The line of reference for the measurements of the top (or diastolic volume) and bottom (or systolic volume) of the volume curve in the records is the base line of the pressure curve, and the line of zero volume in Fig. 4 is therefore only an approximation of the complete emptiness of the ventricles which it should express. The tonus and Treppe variations amount

to 100 per cent of the maximum amplitude of beat. Thus at very slow rates of beat the ventricles discharge in a systole only half the blood contained in their chambers at the end of the diastole. At very rapid rates the diastolic volume is only one third of that in very slow rhythms, and is even considerably less than the systolic

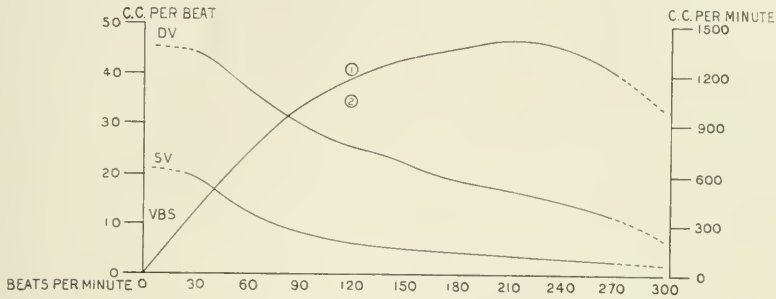


FIGURE 4. — A synopsis of the experiment illustrated in Fig. 2. The curve *DI* expresses the diastolic volume, and *SI* the systolic volume of the blood in both ventricles (measured in terms of the scale at the left), at rates of beat from 20 to 270. The vertical distance between these curves expresses the amplitude of beat at all rates. The curve *VBS* expresses the volume of the arterial blood stream per minute (measured in terms of the scale at the right) for this heart, and is obtained by multiplying the number of beats in the minute by half the amplitude of beat. The position of small circle (1) indicates the volume of the blood stream at the varying rhythm of record 1, and circle (2), that of the irregular rhythm of record 2 in Fig. 2.

volume in bradycardia. The amplitude of beat is expressed by the vertical distance between *DI* and *SI*, and the systolic discharge of the left ventricle is one half the amplitude. This half-amplitude multiplied by the rate of beat gives the volume of the arterial blood stream per minute summarized by the curve *VBS*.

At first sight it would appear from these curves that tonus and Treppe determine the amplitude of beat and as a consequence the volume of the blood stream at all rates. If such were the case, it would still be true that the volume of the systolic discharge and the output of the left heart per minute are functions of the rate of beat. Thus Fig. 4 shows that the systolic and diastolic volumes are determined by the rate. But on closer consideration it is evident that beyond 80 or 90 beats the line *DI* expresses the diminishing volume of abbreviated diastoles rather than the tonus of the heart. Thus the relations of the blood stream to the heart rate can be formulated, as has been done above in connection with Figs. 1 and 3, without taking the tonus and Treppe variations into account.

This simplicity is due to the fact, which we plan to discuss in a later paper, that the volume changes of the heart are directly proportional to the changes in the linear extension of the cardiac muscle fibres, instead of to the square or cube of their extension, as has generally been supposed. This supposition rests wholly on *a priori* considerations of the linear and cubic relations of geometrically similar bodies of different sizes. Such considerations are, however, robbed of significance for the heart by the well-established fact that the cross section of the base of the ventricles is not only smaller in systole than in diastole, but is also of quite a different shape in these two phases. Rehfish²⁴ finds that in systole *only one diameter* of the ventricles (*viz.*, the transverse) is shortened. The volume curve is essentially similar to an isotonic muscle contraction. Therefore the variations in the volume of the ventricular chambers are proportional to the linear changes in their walls. The volume of the systolic discharge (or amplitude of beat) at various rates of beat is comparable to the distance through which a muscle lifts a weight. The output of the heart per minute is dependent upon the rate of beat in the same manner that the total external work of a muscle in one minute in lifting a weight is determined by the number of stimuli it receives.

III. THE SYSTOLIC DISCHARGE OF A VAGUS BEAT AS A STANDARD.

The early investigators, Volkmann and Vierordt, attempted to estimate the volume of the arterial blood stream from measurements of the capacity of the dead heart. They assumed that the ventricles pass from extreme distention to entire emptiness in each beat and that the systolic discharge (*Schlagvolum*) is a constant quantity. Both assumptions are erroneous. The ventricles in extreme distention contain about twice as great a volume of blood as is discharged by even a maximal beat.²⁵ The living heart is never, except in vagus standstill, relaxed more than 80 per cent of this extreme, while at all ordinary rates of beat very considerable volumes of blood remain in the chambers at the end of systole. The work of many investigators has shown²⁶ that the systolic discharge is not a constant. According to the view of the behavior of the heart set forth in the preceding section, the normal amplitude of beat is not

²⁴ REHFISCH: *Archiv für Physiologie*, 1908, p. 1 (also bibliography).

²⁵ Cf. Fig. 4.

²⁶ See TIGERSTEDT'S reviews, *Loc. cit.*

the same (in the dog)²⁷ at any two pulse rates above 60 per minute. Nevertheless a study of ventricular volume curves indicates that for each individual heart there is a certain systolic discharge which may be adopted as the standard amplitude of beat. This unit of measurement is afforded by the so-called vagus beats occurring in partial asphyxia. These slow full strokes are induced by the action of CO₂ upon the cardio-inhibitory centre before the force of the heart begins to fail from lack of oxygen. At all rates slower than 60 per minute the beats are (within 10 per cent variation) uniform in amplitude. The beats occurring under direct stimulation of the vagus likewise have this amplitude, except when recorded immediately after tachycardia.

It is convenient not only to adopt the vagus beat as a standard, but to assign to it in all cases the arbitrary value 100. The relative amplitudes of the strokes at all rates more rapid than 60 per minute can then be derived by the graphic methods explained in connection with Figs. 2, 3, and 4, and can be expressed as percentages of this standard. By multiplying the percentage amplitudes by the corresponding rates of beat the relative volumes of the blood stream at these rates are obtained. Half the amplitude of the vagus beat when expressed in cubic centimetres and divided by the body weight affords a coefficient of the systolic discharge of the left heart. This quantity multiplied by the percentages yields data for the volumes of the strokes of the heart and of the blood stream in cubic centimetres per kilo.

The data of this character obtained from 12 dogs is summarized in the accompanying table. In the fifth column of section I of the table the coefficients of the systolic discharge exhibit considerable individual variation. In general they are much greater in small than in large animals, — the highest figure showing 2.6 c.c. per kilo in an animal of 8.0 kilos, and the lowest 1.3 c.c. in one of 16.5 kilos.²⁸ In the sixth to the eleventh columns are shown the relative amplitudes of beat at rates from 60 to 240 per minute. The fig-

²⁷ The average normal pulse of a dog at rest is 90 to 100 per minute with a minimum of 70 and a maximum of 120, according to RICHER'S *Dictionnaire de physiologie*, iii, p. 502.

²⁸ For observations indicating considerable individual differences in the volume of the systolic discharge in men, see V. HENDERSON, *This journal*, 1905, xiv, p. 287; corresponding variations in the relation of the weight of the heart to body weight have been shown by D. R. JOSEPH, *Journal of experimental medicine*, 1908, x, p. 521.

ures show that in passing from 90 per minute, which is approximately the normal rate for a dog, up to 180 per minute there occurs a diminution in amplitude of stroke of 30 to 40 per cent. In consequence of this lessening of the systolic discharge the relative volumes of the blood stream per second at these rates differ only in the proportion of 144 to 189, or 132 to 150 (section II of the table). Stated in terms of cubic centimetres per kilo body weight per second (in section III of the table), the output of the left ventricle, when the heart is pumping to its utmost power, is only 4.5, against 3.5 when it is beating at the normal pulse rate of 90 per minute. The range of adaptability thus shown is surprisingly small for the circulation of so active an animal as a dog.

The principal objection which can be raised to the theory of the behavior of the heart here tabulated is the fact that the data of previous investigators do not conform to it. Nearly all observers have noted that at times an increasing heart rate is accompanied by a diminishing systolic discharge. Often, however, a later observation at a slow heart rate has shown an amplitude of beat considerably smaller than that recorded at the beginning of an experiment. We shall not attempt to explain away these and other discrepancies by a review of the literature. On the contrary, the data from our own experiments afford numerous examples of nearly every type of deviation from the requirements of the theory of the uniformity of the volume curve. The explanation which serves to harmonize the discrepancies in our data with the theory as developed up to this point will be set forth in the next section of this paper. Doubtless it applies with equal force to the variations in the observations of previous investigators. It is necessary at this point, however, to emphasize the fact that the figures in the table do not represent measurements of the amplitudes of the volume curves at the heart rates above 60 per minute. In each experiment a single vagus beat alone was measured; and the figures for the percentage amplitudes were obtained from the volume curve of this beat. These data represent correctly,²⁹ we believe, the maximal efficiency of which the hearts were capable with an ample venous supply at the various rates. In none of our experiments have we ever observed such quantities to be exceeded. During the early period in most cases the amplitudes recorded corresponded closely to the theoretical re-

²⁹ The error in the data of the table may be 10 per cent plus or minus without invalidating the essential correctness of this view.

TABLE I.
DATA FROM THE VOLUME CURVES OF VAGUS BEATS IN TWELVE TYPICAL EXPERIMENTS.

Num-ber of ex-periment.	Date.	Body weight, Kilos.	Amplitude of vagus beat.		Percentage amplitude of beat at rates of						For graphic records see
			Both ven-tries, c.c.	Left ventri-cle, c.c. per kilo.	60 p. min.	90 p. min.	120 p. min.	150 p. min.	180 p. min.	240 p. min.	
1	May 4, 1905	6.5	27.0	2.1	100	95	80	71	63	44	Fig. 2. This journal, xvi, p. 349. Fig. 7. This journal, xvi, p. 351. This journal, xvi, p. 342. This journal, xvi, p. 146. This journal, xvi, p. 155. This journal, xvi, p. 340. Fig. 5. Calculated from the percentage amplitudes marked (*). Calculated from the percentage amplitudes marked (†). Calculated from Experiment 2. Calculated from Experiment 11.
2	May 17, 1905	8.0	42.0	2.6	100	90	76	70	57	40	
3	May 25, 1905	9.0	38.0	2.1	100	95	90	73	57	36	
4	Feb. 10, 1906	10.0	36.0	1.8	100	90	73†	62†	51	39	
5	Feb. 25, 1906	10.0	34.0	1.7	100	96	84	75*	63*	48	
6	Mar. 10, 1905	13.8	42.0	1.5	100	88†	80	63	50†	38	
7	July 10, 1905	14.0	50.0	1.8	100	91	83	73	63*	49*	
8	July 16, 1906	14.0	51.0	1.8	100	90	79	63	53	39	
9	May 15, 1906	15.0	42.0	1.4	100	95	85	75*	63	45	
10	Aug. 9, 1906	15.0	51.0	1.7	100	88†	74	62†	50†	32†	
11	July 11, 1905	16.5	42.0	1.3	100	94	77	69	62	44	
12	July 3, 1906	22.0	65.0	1.5	100	97*	92*	75*	60	42	
II.	Percentage amplitudes × rates of beat — the relative vol-umes of the blood stream at these rates.		Maximum	100	144	184	187	189	196		
	Minimum		100	132	146	155	150	128			
III.	Percentage amplitudes × rates of beat × systolic discharge of left ventricle in a vagus beat = volumes of blood stream in c.c. per kilo per second at these rates.		Maximum	2.6	3.5	3.9	4.5	4.4	4.1		
	Minimum		1.3	1.8	2.0	2.2	2.4	2.3			

quirements derived from the vagus beat. On the contrary, in all prolonged experiments a condition developed under which the amplitude of the volume curve was less than these theoretical maxima.

IV. THE DEVIATION CHARACTERISTIC OF SHOCK.

In the majority of our experiments the dogs passed sooner or later into a condition of shock. The arterial pressure records were of essentially the same character as those obtained in the investigations upon shock by Crile, by Howell, and by Porter. There is no reason to suppose that the failure of the circulation which developed was in any respect novel or peculiar. A study of the volume curves of the heart indicates, however, that the fall of arterial pressure was due to a cause different from that to which Crile in particular believes it to be assignable. During a more or less prolonged period, which always occurred as a stage preliminary to shock, arterial pressure was maintained at a normal level, but the up-stroke of the volume curve of the heart became less abrupt than the normal. This retardation of the diastolic filling of the heart was due to a diminution in the venous stream to the right auricle, for intravenous infusion of saline immediately restored the normal amplitude of the volume curve. Without infusion the diastolic filling of the ventricles became progressively more incomplete, and the systolic discharges were in consequence greatly reduced in volume. Finally, when the arterial blood stream had been thus diminished to about 30 per cent of the normal, arterial pressure fell rapidly. Evidently this fall was not due to an abolition of the peripheral resistance in the arterial system: since the arterioles were not relaxed, we must conclude that the vaso-motor nerve centres had not failed. On the contrary, the fact that arterial pressure was maintained until the blood stream was reduced below 40 per cent of the normal demonstrates that the vaso-motor mechanism was in a state of extreme activity in an effort to compensate the lessening output of the heart.³⁰ The failure of the circulation in shock is therefore fundamentally a venous stasis. The fall of the arterial pressure is secondary to this stagnation. A subnormal amplitude in the volume curve due

³⁰ These facts are in accord with the observations of PORTER, *This journal*, 1907-1908, xx, pp. 399, 444, and 500; SEELIG and LYON, *Journal of the American Medical Association*, 1900, lii, p. 45; MALCOLM, *Lancet*, 1905, i, ii, pp. 573, 618, 737, 922, and 1907, i, p. 497; and BOISE: *American journal of obstetrics*, 1907, lv, p. 1.

to the slow diastolic filling of the ventricles is the deviation characteristic of shock.

The underlying cause of the stagnation of the venous stream in such experiments as those here under discussion is, we believe, a diminution in the CO_2 content of the tissues of the body.³¹ The animals, after the opening of the thorax, were maintained under artificial respiration or under the positive pressure respiration of Brauer. A pulmonary ventilation which is even slightly in excess of the needs of the animal induces a progressive diminution in the CO_2 content of the blood. This Acapnia has a twofold influence upon the circulation: (1) It diminishes the activity of the cardio-inhibitory centre. As we have shown previously, the heart rate varies inversely as the CO_2 content of the arterial blood. (2) If a condition of arterial Acapnia is prolonged, the tonus of the venous system is abolished. In a later paper evidence³² will be presented to show that this effect is not due to alteration in the vaso-motor nervous system, but that it is peripheral and depends upon the CO_2 content of the tissues. At present we need consider only those observations which indicate that the heart under normal conditions must be supposed to conform to the principle of "uniformity of behavior," and that deviations from this behavior are not of cardiac but of venous origin.

In the early periods of nearly all of our experiments the volume curves at various rates of beat were "superimposable." Increase in the pulmonary ventilation caused an immediate and approximately proportional rise in the rate of the heart beat without distortion of the volume curve. It was only after the artificial hyperpnoea had been continued for considerable time that the deviation involved in a less abrupt up-stroke of the volume curve began to appear. If the pulmonary ventilation was then diminished, a slow heart rate was regained, in the course of half an hour, simultaneously with restoration of the CO_2 content of the arterial blood. The normal amplitude of the heart beat, on the contrary, was not restored until after a much longer period, — an hour or more according to the degree and duration of the preceding Acapnia. Other series of experiments in which the pressures in the femoral or jugular veins were determined have confirmed the inference that

³¹ Cf. Y. HENDERSON: This journal, 1907, xxi, p. 126.

³² For an abstract of this evidence, see the Proceedings of the American Physiological Society in this volume.

CO₂ is an element of primary importance in the maintenance of the venous stream toward the heart. We are led therefore to the following conclusions: (1) In the early periods of our experiments the hearts maintained uniformity of behavior because the venous pressures were normal and the volume of blood supplied to the heart sufficient to distend the ventricle as rapidly as it relaxed in diastole. (2) In the later periods the volume curves deviated from "superimposability" because of diminution in the venous supply. (3) The variations in the volume of the systolic discharge independently of alterations of heart rate which have been observed by previous investigators were due to similar changes in venous pressure, and do not indicate that the heart is endowed with a capacity of variability of behavior, as Engelmann believes. (4) The normal regulation of respiration by the CO₂ of the arterial blood, as shown by Haldane and Priestley, indirectly insures the maintenance of the venous stream in health. But under operative and other abnormal conditions the development of Acapnia induces venous stagnation, cardiac inefficiency, fall of arterial pressure, and shock.

In Fig. 5 are reproduced graphic records which illustrate these points. The analyses of the arterial blood gases performed in connection with this experiment have been reported in a previous paper, and the dependence of the heart rate upon the CO₂ content of the blood has been shown.³³ Examination of the graphic records shows that during the first hour after the opening of the thorax the volume curve maintains "superimposability," but that later the deviation of retarded diastolic filling develops (compare the records at 11.17 and 5.00 $\frac{1}{4}$). Consequently in the later periods of the experiment the amplitude of the volume curve at all rapid heart rates was greatly diminished (compare the records at 11.30 and 5.02). In this experiment the periods of excessive ventilation were not prolonged. Thus shock was avoided, although the animal was under observation for seven hours after the opening of the thorax. The temporary periods of Acapnia (indicated in the tracings by the resulting tachycardia) were sufficient to induce a marked diminution in the diastolic fillings of the heart, but not beyond the degree which the vaso-motor nervous system was able to compensate by increase of peripheral resistance in the arterial system.

The diminution in the volume of the arterial blood stream in the later as compared with the earlier parts of this experiment is

³³ This journal, 1908, xxi, p. 150.

shown in the diagram (Fig. 6). Here, as in Fig. 4, the curve expresses the maximal minute volume of the blood stream at all

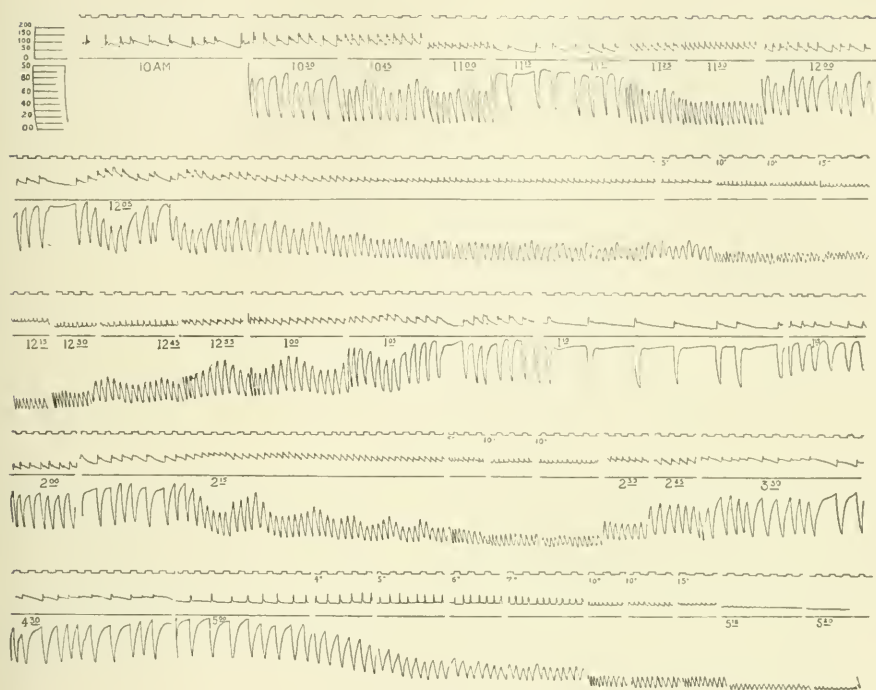


FIGURE 5. — Experiment of July 3, 1906. Dog of 22.0 kilos. Morphine and ether. Time in 0.5 second. Arterial pressure recorded by Hürthle manometer connected with carotid. Tracheotomized and thorax opened at 10.10. Cardiometer placed on ventricles, and volume curve (lower tracing) recorded with large tambour. Artificial respiration maintained with the apparatus described in this journal, 1908, xxi, p. 147. Heart rate repeatedly increased above 200 per minute and again slowed (once down to 20 beats per minute), by varying the pulmonary ventilation. Invert the tracing and note that the tonus, Treppe, and amplitude variations in the volume curve depend upon the rate of beat in the same manner as in a series of maximal isotonic contractions of the gastrocnemius of a frog. Note that in the later part of the experiment, and especially after periods of tachycardia, the up and down strokes of the volume curve meet in less acute angles than at the outset, and that the amplitude of the curve is thus diminished. Note also the general but not invariable proportionality of the pulse pressure and ventricular volume curves. These data are summarized in Fig. 6 and in Table II. The gaps in the time record marked 5 seconds, 10 seconds, etc., indicate omission of the tracings for this number of seconds. (About one sixth the original size.)

heart rates, and is derived from the volume curve of a vagus beat. The small circles are placed to indicate the heart rates, and the blood stream calculated from the tracings in Fig. 5. The numbers in the

circles and the corresponding times of the tracings are given in columns 1 and 2 of Table II. During the first hour the circles (1 to 7) lie on or near the curve. Later they fall far below it. After periods of excessive pulmonary ventilation (indicated here by tachycardia) the deficiency is greater (thus 17 after 13); after intervals

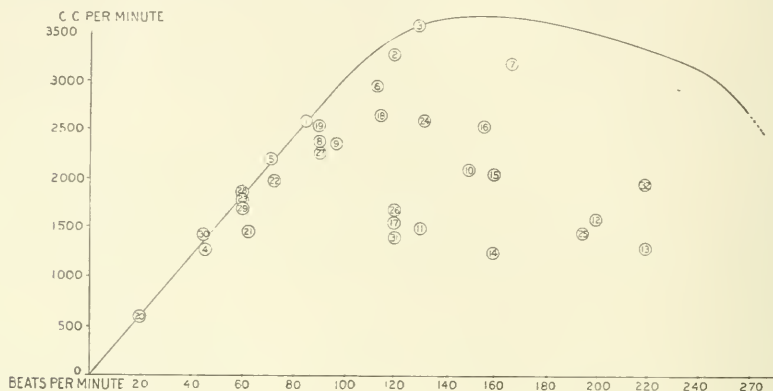


FIGURE 6. — A summary of data from Fig. 5. The curve shows the volume of the arterial blood stream at all heart rates as it would have been if the volume curve of the heart had never deviated from the form "superimposable" upon a vagus beat. The positions of the circles indicate the actual blood stream calculated from the tracings by multiplying half the amplitude of the volume curve by the heart rate. The circles are numbered so that the time of the observations can be found in Table II, and the corresponding tracings in Fig. 5 can be identified.

of diminished ventilation (indicated by bradycardia) the volume of the blood stream is partially restored (thus 24 after 20).

Hill and Flack³⁴ reject the view that the failure of the circulation under excessive ventilation is due to Acapnia. They suggest that the pulmonary circulation is obstructed. If such were the case, the pressure in the systematic veins would be raised by the damming back of the blood into the right heart. On the contrary, venous pressure falls. Another answer to the objection of Hill and Flack will be found in a recent paper by Scott.³⁵ By the expression "excessive pulmonary ventilation" in this and in our preceding paper we do not mean violent artificial respiration. A respiratory movement slightly deeper and a little more rapid than that which a dog would spontaneously maintain is sufficient ultimately to produce shock. Indeed we believe that in the large majority of the experiments in the literature, in which the thorax was opened

³⁴ HILL and FLACK: *Journal of physiology*, 1908, xxxvii, p. 86.

³⁵ SCOTT, F. H.: *Ibid.*, xxxvii, p. 323.

and artificial respiration maintained, the ventilation was excessive, without the investigators realizing it.

A part of our experiments were performed under the positive pressure respiration method of Brauer. To these experiments Hill's objection certainly is inapplicable. Brat,³⁶ on the other hand, on the basis of similar experiments rejects the idea that the variations in the heart rate and the development of shock are due to alterations in the CO₂ content of the blood. He concludes that they are induced reflexly through the pulmonary vagi by changes in the tension of the lungs. In our experiments under Brauer's method of respiration the pressure at which air was supplied to the lungs was constant (13 cm. of water) so that this explanation of the variations in the heart rate is excluded. Recently we have repeated some of Brat's experiments. In some cases, but with no regularity, an increase in the pressure of the air supplied to the lungs was followed by a slower heart rate. But whenever this occurred there was also a corresponding alteration observable in the animal's breathing. This retardation of respiration is of course the well-known reflex from the distended lungs through the afferent fibres of the vagi. Scott has demonstrated that by this reflex the excitability of the respiratory centres to CO₂ can be completely inhibited. The accumulation of CO₂ during this inhibition is, we believe, a factor in the slower heart rate observed by Brat under increased pulmonary tension, although we agree with him in the opinion that there exists an intimate and immediate sympathy between the respiratory and cardiac centres in the spinal bulb.

V. THE INFLUENCE OF THE VAGUS AND ACCELERATOR NERVES UPON THE BLOOD STREAM.

The opinion is held almost universally that the vagus may not only slow, but also may *diminish the amplitude* of the heart beat; and that the accelerator may both quicken the rate and *augment the force* of the systoles. Such a view has been most completely formulated in Engelmann's theory of the fourfold positive and negative influences of the nervous system upon the heart. To this theory the principle of the "superimposability" of the volume curve under ample venous supply is almost diametrically opposed. If the behavior of the heart is thus uniform, it is unnecessary to recognize more than one class of efferent nerve fibres in the cardiac vagus and one of opposite influence in the accelerator. The problem is of extreme practical importance, since upon it hangs the question

³⁶ BRAT: Zeitschrift für experimentelle Pathologie und Therapie, 1908, iv, p. 244.

whether a "failing heart" is not properly to be regarded and treated as due to venous stagnation.

In 20 experiments we have stimulated the peripheral end of one vagus (the other being uncut) while recording the volume changes of the ventricles. The curves obtained during the slowing and after the stoppage of the heart were in every case found to be "superimposable" upon those recorded before and after the stimulation. In other words, the vagus influence does not induce the heart to deviate from "uniformity" of behavior. Tonus, Treppe, and amplitude vary only dependently with the rate of beat. In Fig. 7 are reproduced the ventricular volume curves at several rates of beat obtained by varying the pulmonary ventilation, together with (in the lower line) the tracings recorded under vagus stimulations of varying strength and duration. The approximate "superimposability" of all the curves is manifest at a glance. Such deviations as occur are due to variations in the diastolic up-stroke, and not to primary alterations in the amplitude of systole.

In an earlier paper from this laboratory it was stated that in some experiments after double vagus section the heart for a time beat submaximally.³⁷ At first sight this fact appears to demonstrate the capacity of the heart for variability of behavior. In a recent repetition of these experiments we have found, however, that the phenomenon occurs only after venous pressure has begun to fall. Intravenous infusion of saline restores the normal efficiency of the ventricles. The correct explanation of the submaximal behavior of the heart is, therefore, that the venous stream was insufficient to supply the volume of blood needed for the full amplitude of beat during the tachycardia consequent on double vagus section.

In several experiments we have exposed the annulus of Vieussens, and stimulated the ventral branch. The operations involved in opening both the lower part of the thorax for the placing of the cardiac plethysmograph, and the upper portion for the exposure of these nerves, are very extensive. Difficulty was found in keeping the heart rate slow enough after these operations so that the volume curve before and during stimulation of the annulus could be recorded. This object was, however, accomplished in three experiments, — once on the left side and twice on the right. Tracings "superimposable" upon each other and essentially similar to the

³⁷ This journal, 1006, xvi, p. 351.

volume curves of Fig. 1 were obtained. This evidence indicates acceleration without augmentation. Investigators using non-quantitative methods have sometimes recorded an apparent increase in the force of systole under accelerator stimulation. A true "aug-

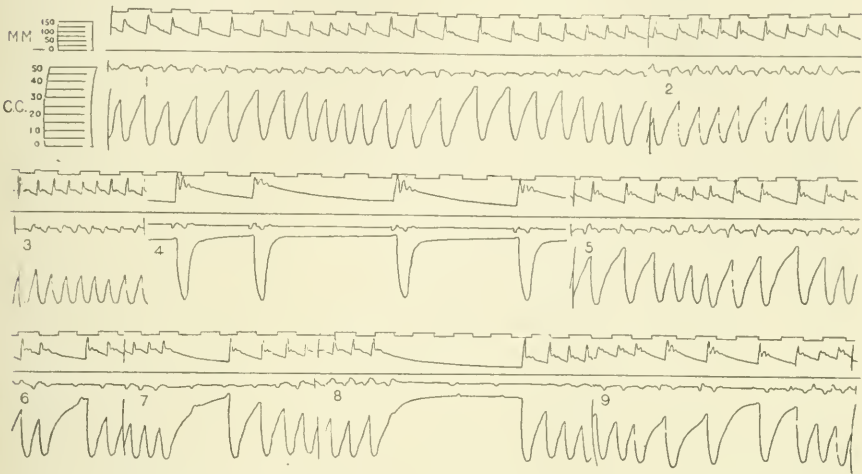


FIGURE 7. — Experiment of May 25, 1905. Dog of 9.0 kilos. Morphine and ether. Time in 0.5 second. Arterial pressure and base line recorded by Hürthle manometer. Movements of right auricular appendage recorded by tambour connected with small auricular plethysmograph. Volume curve of ventricles of heart. Natural respiration of compressed air (uniform pressure of 13 cm. water). In records 1 to 5 the heart rate was varied by adjustment of fresh-air supply. In records 1, 2, and 5 note the respiratory variations in the rate and amplitude of the volume curve. In records 6 to 9 the effects of various strengths of stimulation of the right vagus are shown. These records compared with records 1 to 5 show that vagus stimulation does not induce deviation in the form of the volume curve. Note that the three auricular systoles occurring during the standstill of the ventricles in record 8 have very little influence upon the filling of the ventricles. For a similar experiment see *This journal*, xvi, p. 349. The cardiometer was opened occasionally so as to keep the stylus of the tambour at the same level in spite of tonus and Treppe variations, which are therefore not shown. (About one fourth the original size.)

mentor " nerve to a heart beating maximally appears to us to be an impossibility. — unless indeed its action be conceived as inducing a more rapid diastolic distensibility. Some writers have described the behavior of the heart as if it were capable of discharging more blood during systoles than runs into it during diastoles.

VI. THE PROPORTIONALITY OF PULSE PRESSURE AND SYSTOLIC DISCHARGE.

One of the principal objections to the theory of the uniformity of the heart's behavior is afforded by the measurements of the pulse pressure in man. According to the views of v. Recklinghausen and of Erlanger the pulse pressure (*i. e.*, the difference between systolic and diastolic arterial pressures) is to be regarded as an index of the volume of the systolic discharge of the left ventricle. The method of estimating the minute volume of the arterial blood stream by multiplying the pulse pressure by the pulse rate is at present rapidly coming into general use for clinical purposes. There is, however, very little experimental data to support the assumption that the method is reliable. The measurements of pulse pressure show considerable differences (even as high as 50 per cent) at different times in the same individual and at the same heart rate. Therefore, either our theory of the heart's behavior is wrong, or venous pressure in man varies far more widely than has been usually supposed,³⁸ or (as we conclude) pulse-pressure measurements do not afford a reliable index of the systolic discharge.

Dawson and Gorham³⁹ have published simultaneous tracings of the volume changes of the heart of dogs, and of the pulse pressure recorded by a Hürthle manometer. They conclude that the two curves maintain a sufficiently close proportionality for one to serve as a reliable index of the other. But from the tabulated data of their experiments it is evident (as shown below) that the use of this index for the calculation of the blood stream would involve frequent errors of 50 to 100 per cent, and in one observation an error of more than 200 per cent. That there is a general proportionality in such tracings may be seen at a glance in Figs. 2, 5, and 7 of this paper. But more careful analysis of the curves shows very considerable deviations from exact proportionality. In Table II are given data derived from Fig. 5. They show that in a number of observations at different times with an amplitude of heart beat of 50 to 60 c.c. the pulse pressure was 25 to 30 mm., but that at other times

³⁸ HOOKER and EYSTER by the method of v. RECKLINGHAUSEN find very considerable variations of venous pressure in men (3 to 11 cm. water above the subcostal angle). Johns Hopkins Hospital bulletin, 1908, xix, No. 210 (also bibliography).

³⁹ DAWSON and GORHAM: Journal of experimental medicine, 1908, x, p. 484.

TABLE II.
DATA OBTAINED FROM EXPERIMENT OF JULY 3, 1906.

Observations numbered as in Fig. 6.	Times of tracings in Fig. 5.	Heart rates. Beats per minute.	Amplitude of volume curve in c.c.	Systolic pressure m.m. of Hg.	Pulse pressure m.m. of Hg.	Ratio of pulse pressure to amplitude of volume curve.
0	10.00	68	..	110	30
1	10.30	84	68	125	50	1 : 1.3
2	10.45	120	63	135	50	: 1.2
3	11.00	130	58	80	15	: 3.8
4	11.15	45	61	75	25	: 2.4
5	11.17	70	65	75	25	: 2.6
6	11.23	112	60	90	25	: 2.4
7	11.30	168	39	90	20	: 1.9
8	12.00	90	57	75	20	: 2.8
9	12.05 $\frac{1}{4}$	96	53	155	30	: 1.8
10	12.05 $\frac{1}{2}$	150	30	120	15	: 2.0
11	12.05 $\frac{3}{4}$	130	26	112	12	: 2.2
12	12.06	200	17	95	15	: 1.1
13	12.06 $\frac{1}{2}$	220	15	95	15	: 1.0
14	12.15	160	16	95	15	: 1.1
15	12.30	160	27	70	15	: 1.8
16	12.45	156	38	75	20	: 1.9
17	12.55	120	53	95	30	: 1.8
18	1.00	114	55	100	30	: 1.8
19	1.05	90	64	125	40	: 1.6
20	1.10	20	64	95	45	: 1.4
21	1.15	65	45	80	25	: 1.8
22	2.00	72	55	70	25	: 2.2
23	2.15	60	62	125	35	: 1.8
24	2.15 $\frac{1}{4}$	126	47	115	30	: 1.6
25	2.16	195	15	100	15	: 1.0
26	2.30	120	27	115	30	: 0.9
27	2.45	90	52	110	35	: 1.5

with the same amplitude of beat the pulse pressure was 45 to 50 mm. Instead of the nearly uniform proportion between pulse pressure and the volume curve which should be found if the former is an index of the latter, the ratios in the right-hand column of the table vary from 1:0.9 to 1:3.8.

If the pulse pressure is to serve as an index of systolic discharge the ratio between the two quantities must be at all times the same. But if the figures for the pulse pressures in Table I of Dawson and Gorham's paper be made the numerators in a series of fractions, and the figures for the systolic discharges be placed as denominators, we find (in observation 1) $\frac{1}{3}$, (in 2) $\frac{1}{2}$, (5) $\frac{1}{2}$, (6) $\frac{1}{5}$, (8) $\frac{1}{10}$. An even more conclusive demonstration of the unreliability of this "index" is afforded by Table II in Dawson and Gorham's paper, for in it we find (1) $\frac{1}{9}$, (16) $\frac{2}{8}$, (17) $\frac{1}{8}$, (19) $\frac{1}{5}$. These fractions, instead of working out to a nearly uniform proportion, give $\frac{1}{1^8}$, $\frac{1}{1^0}$, $\frac{2}{1^2}$, $\frac{3}{1^2}$. Evidently, if observation 16 (*i. e.*, $\frac{1}{1^0}$) were taken as the standard, the errors involved in the use of this "index" in calculating the blood streams from the pulse pressures of the other three observations would be 80, 120, and 220 per cent.

In some of our tracings variations in the form of the pulse wave (especially in respect to the primary rise) afford a possible explanation of the non-parallelism of pulse pressure and systolic discharge. It is shown in Fig. 2 (see the note at the end of the legend) by a comparison of record 4 with the inspiratory beats of record 1. Whatever be the correct explanation, this demonstration that the pulse pressure is not a reliable index of the systolic discharge removes one of the principal objections to our theory of the heart's behavior.

[From a personal conversation with Professor Dawson after this paper was in type I find that I have misunderstood him. He uses the words "a reliable index" in a merely qualitative sense. He also recognizes that the pulse pressure is not an accurate *measure* of the systolic discharge.]

CONCLUSIONS.

I. The behavior of the heart, especially the diastolic relaxation as expressed in the volume curve of the ventricles, is the principal element determining the normal variations in the volume of the arterial blood stream.

II. The heart obeys a principle of "uniformity of behavior." Providing the venous pressure and supply are ample, the volume curves of the ventricles at all rates of beat are "superimposable." Tonus, Treppe, and amplitude of beat do not vary independently of the pulse rate.

III. The maximal minute volumes of the arterial blood stream

can be estimated for all pulse rates from the volume curve of a vagus beat.

IV. Deviations from these maxima are caused by insufficient venous supply to the right heart. Failure of the circulation in shock is due primarily to abolition of venous, not arterial, tonus.

V. Stimulation of the vagus and accelerator nerves does not induce the heart to deviate from "uniformity of behavior." Therefore it is superfluous to recognize more than one class of fibres either in the cardiac vagus or in the accelerators.

VI. The pulse pressure is not a reliable index of the systolic discharge.

VII. The principle of the Uniformity of Cardiac Behavior is an extension of the All or None Law.

I am indebted to Prof. L. B. Mendel for valuable criticism of the manuscript of this paper.

ON THE INFLUENCE OF SODIUM CHLORIDE AND CALCIUM CHLORIDE IN THE POTASSIUM CONTRACTION.

BY WILLIAM D. ZOETHOUT.

[From the *Physiological Laboratory of the University of Illinois.*]

NEARLY six years ago I published a paper¹ in which it was maintained that the action of potassium in causing contraction of a skeletal muscle is antagonized by sodium and by calcium. In a subsequent paper² I made the following statement: ". . . as the muscle always contains Ca salts, the presence of these salts raises the concentration of the KCl necessary to produce an increase in tone." This explanation is not accepted by Dr. Guenther; in 1905 he remarks that "These facts seem to point to the conclusion that potassium produces a contraction by an interaction involving calcium, and not, as is implied in the publication of Zoethout, by the combined effects of an increase in the potassium content and a removal of the inhibitory influence of calcium."³ It may be said at once that Dr. Guenther has misunderstood my meaning: what I wrote was that calcium antagonizes the potassium, and that the amount of calcium normally present in the muscle was sufficient to counteract the effects of a small amount of potassium introduced into the muscle. This idea was clearly based upon the following facts: (1) The simultaneous application of potassium and calcium chloride raises the concentration of the potassium chloride necessary to produce a contraction; (2) The previous application of calcium chloride has the same effect; (3) The relaxation of the potassium contraction is more rapid when the muscle is subsequently immersed in calcium chloride than when water, glycerine, or sodium chloride is used; (4) The preliminary plunging of a muscle into a solution of sodium salt which precipitates calcium renders

¹ ZOETHOUT: This journal, 1902, vii, p. 190.

² *Ibid.*, p. 320.

³ GUENTHER: This journal, 1905, xiv, p. 73.

the muscle more irritable toward potassium; (5) If a potassium salt which causes precipitation of calcium is employed instead of potassium chloride, the amount of such a potassium salt necessary to cause a contraction is much less than that of potassium chloride. With a view of establishing these facts more conclusively and also to show that sodium has an action very similar to that of calcium, and that the amount of sodium normally present in the muscle is sufficient to counteract the introduction of a small amount of potassium, the following experiments were made.

The two gastrocnemius muscles of a frog were most carefully prepared and suspended in tubes from which the surrounding fluid could be rapidly removed. The muscles were attached by the tendon to very light levers of nearly the same weight and leverage, and, to insure accurate results, the control muscle was now placed in one tube, now in the other. These levers magnified the contractions very greatly, hence an exceedingly small difference in the contraction of the two muscles can be detected.

The chemicals employed in these experiments were of the highest purity obtainable. The water was redistilled in glass vessels.

As the muscles of frogs vary considerably in their behavior toward the salts of sodium and potassium, the results obtained with muscles taken from different frogs cannot be compared; hence it was found absolutely necessary in every case to make control test with one gastrocnemius muscle when the other was used in the experiment in hand. In describing the experiments the two gastrocnemius muscles will be indicated by *A* and *B*, these two muscles always being mates and the experiment made upon them at exactly the same time, unless otherwise stated. When two or more solutions are used in succession, this is indicated by an arrow, the number above the arrow showing the number of minutes the first-mentioned solution has acted upon the muscle.

It is impossible in these experiments to employ potassium chloride solutions isotonic with the muscle, for the $m/8$ potassium chloride is so powerful in its effects as to obscure small changes caused by various agencies. Hence we must either employ a more dilute water solution of this salt or the $m/8$ KCl must be mixed with an isotonic solution of some other substance. The first method is not permissible, because a hypertonic solution of an otherwise inert substance will produce a shortening of the muscle. In casting about for a dilutant of the potassium chloride we adopted a 6 per

cent cane-sugar solution. Overton⁴ found that in a 6 per cent cane-sugar solution a muscle gradually loses its irritability without undergoing any permanent injury, for the subsequent treatment with a sodium chloride solution restores the irritability completely. This loss of irritability does not become manifest, in the case of the gastrocnemius muscle, for several minutes.

I. THE INFLUENCE OF NaCl ON THE POTASSIUM CONTRACTION.

The gastrocnemius of a frog was placed for fifteen minutes in $m/8$ sodium chloride solution; the other gastrocnemius was suspended in moist air for the same length of time. At the end of the fifteen minutes both muscles were immersed into 10 c.c. 6 per cent cane-sugar + 1 c.c. $m/8$ potassium chloride. The muscle which had been suspended in air was immediately thrown into contraction; the muscle previously treated with sodium chloride did not respond to any extent until the lapse of two minutes.

That the mere suspension in moist air is not the determining factor is proved by the fact that a muscle thus treated for fifteen minutes behaves toward potassium chloride the same as one immediately immersed into a solution of this salt. The introduction of sodium chloride very plainly decreases the power of potassium to cause contraction.

The effect of NaCl compared with that of cane-sugar. — If, on the other hand, one of the muscles is placed in a 6 per cent cane-sugar solution while the other is suspended in moist air, the first-mentioned muscle gives a better potassium contraction than the second. This is already apparent after an immersion in the cane-sugar solution of five or six minutes, and the effect is still marked after the cane-sugar solution has acted from forty to sixty minutes. Guenther⁵ states that treating a sartorius muscle with cane-sugar solution decreases the height of the potassium contraction and lengthens the latent period. This is clearly not true for the gastrocnemius muscle, for in all experiments the height and the rapidity of the contraction are increased by a preliminary treatment with cane-sugar solution if such treatment does not last longer than sixty minutes.

⁴ OVERTON: Archiv für die gesammte Physiologie, 1902, xcii, p. 346.

⁵ GUENTHER: This journal, 1905, xiv, p. 90.

This is also demonstrated in the following experiment:

A. — $12\frac{1}{2}$ c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

B. — 6 per cent cane-sugar $\xrightarrow{25}$ $12\frac{1}{2}$ c.c. cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

The contraction in muscle *B* in one minute was equal to that of *A* in eight minutes. In another experiment the muscle treated for six minutes with a 6 per cent cane-sugar solution gave, on the transfer to potassium chloride, a higher contraction in one minute than the other muscle, not previously treated with cane-sugar, gave in ten minutes.

How greatly a muscle which has previously been treated with a sodium chloride solution differs from one which has been immersed into a cane-sugar solution is seen in Experiment 96:

A. — 6 per cent cane-sugar $\xrightarrow{12}$ 10 c.c. cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

B. — $m/8$ NaCl $\xrightarrow{12}$ 10 c.c. cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

While the muscle *A* gave a very great and rapid contraction, in *B* there was a hardly perceptible contraction. This difference becomes still more apparent if the sodium chloride is followed by a potassium chloride solution diluted with a sodium chloride instead of a cane-sugar solution, as in Experiment 130:

A. — 6 per cent cane-sugar $\xrightarrow{13}$ 11 c.c. cane-sugar + $\frac{3}{4}$ c.c. $m/8$ KCl.

B. — $m/8$ NaCl $\xrightarrow{13}$ 11 c.c. NaCl + $1\frac{3}{4}$ c.c. $m/8$ KCl.

The muscle in *A* at the end of one minute was greatly contracted; at that time the contraction in *B* was insignificant, although the amount of potassium chloride used in *B* is more than twice as great as in *A*. Even after the muscle has been bathed by the sodium chloride and the cane-sugar solution for one hour, the difference is very marked.

But it is not necessary to employ the sodium chloride in such strong concentration as an $m/8$ solution to show its inhibiting effect. Experiment 126:

A. — 6 per cent cane-sugar $\xrightarrow{10}$ 10 c.c. cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl. ¶1

B. — 8 c.c. 6 per cent cane-sugar + 2 c.c. $m/8$ NaCl $\xrightarrow{10}$ 10 c.c. cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

In *B* no contraction resulted in one minute, while in *A* there was a fairly strong contraction. In another experiment of the same nature

the contraction in *A* after five minutes' immersion in the potassium chloride solution was three times as great as in *B*.

The inhibiting influence of sodium chloride is also noticeable when the sodium chloride and potassium chloride are introduced simultaneously, as in Experiment 134:

A. — $10\frac{1}{2}$ c.c. 6 per cent cane-sugar + 1 c.c. $m/8$ KCl.

B. — $10\frac{1}{2}$ c.c. $m/8$ sodium chloride + 1 c.c. $m/8$ KCl.

The muscle in *A* after an extremely short latent period undergoes a fairly strong contraction; the muscle in *B*, on the other hand, retained its original length for nineteen minutes.

The alternate application of cane-sugar and sodium chloride. — A series of experiments in which the sodium chloride and cane-sugar solutions were alternately applied previous to the potassium was also made. Experiment 139:

A. — 6 per cent cane-sugar $\xrightarrow{15}$ 10 c.c. 6 per cent cane-sugar + 1 c.c. $m/8$ KCl.

B. — 6 per cent cane-sugar $\xrightarrow{10}$ $m/8$ NaCl $\xrightarrow{5}$ 10 c.c. 6 per cent cane-sugar + 1 c.c. $m/8$ KCl.

In *A* the potassium chloride caused an immediate large contraction; the smaller contraction in *B* was preceded by a latent period of three minutes. Even though the cane-sugar be applied for twenty minutes and the sodium chloride for but two minutes, the inhibiting effect of the sodium is very marked.

In another series of experiments the inhibiting effect of the preliminary treatment with sodium chloride was removed by a subsequent bath in cane-sugar solution, as shown in Experiment 108:

A. — $m/8$ NaCl $\xrightarrow{7}$ 10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

B. — $m/8$ NaCl $\xrightarrow{7}$ 6 per cent cane-sugar $\xrightarrow{7}$ 10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. $m/8$ KCl.

In *A* no contraction occurred in seven minutes, while in *B* the transfer to the potassium chloride solution called forth an immediate contraction which in eleven minutes was three times as great as that in *A* in eighteen minutes. That, however, the washing with cane-sugar for a length of time equal to that during which the muscle is immersed in the sodium chloride does not remove all the inhibiting effect of the sodium salt is proved in Experiment 106:

- A. — 6 per cent cane-sugar $\xrightarrow{16}$ 10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. *m*/8 KCl.
B. — *m*/8 NaCl $\xrightarrow{16}$ 6 per cent cane-sugar $\xrightarrow{16}$ 10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. *m*/8 KCl.

In this experiment the height of the contraction in *A* at the end of one minute was three times as great as in *B*.

All these experiments varied in every possible manner prove that the introduction of sodium chloride previous to, or simultaneous with, the application of potassium chloride hinders the development of the potassium contraction. Furthermore, the fact that a preliminary treatment with cane-sugar solution increases the power of the muscle to respond to potassium seems to show that the amount of sodium in the muscle is sufficient to counteract the stimulating effect of a small quantity of potassium chloride, for, as already stated, Overton⁶ found that the loss of irritability caused by a 6 per cent cane-sugar solution can be completely restored by a subsequent bath in a 0.6 or 0.7 per cent sodium chloride solution. The favorable effect of a preliminary bath of cane-sugar I therefore attribute to the removal of sodium salts. These experiments also prove that Dr. Guenther is mistaken in stating "that a one per-cent solution of potassium chloride can produce its characteristic effects only when the muscle is in normal condition."⁷

On the other hand, it can readily be shown that the entire absence of sodium is unfavorable for the potassium contraction. When, for example, the potassium contraction of a muscle previously treated for forty minutes with 6 per cent cane-sugar is compared with one treated for the same length of time with 10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. *m*/8 Na Cl, it is found that the second muscle gives a better contraction than the first. This, however, is what one might expect, for the treatment with cane-sugar solution for this length of time reduces the power of the muscle to respond to all forms of stimuli. The potassium contraction depends upon the integrity of the living muscle substance, and this, as far as we know at present, is impossible without sodium. Nevertheless, this does not contradict the previous statement that sodium antagonizes the action of potassium, and that the removal of at least a part of the sodium chloride from the muscle favors the

⁶ OVERTON: *Archiv für die gesammte Physiologie*, 1902, xcii, p. 385.

⁷ GUENTHER: *This journal*, 1905, xiv, p. 92.

potassium contraction. This is in harmony with results obtained by other investigators. Overton⁸ found that the paralyzing effect of a solution containing potassium and sodium chloride is the greater, the less the amount of sodium chloride present. Biedermann⁹ found that if one end of an uninjured and almost currentless sartorius is suspended in a 0.83 per cent potassium chloride solution, a current of from 0.03 to 0.05 volts is developed; this current disappears when the end of the muscle is placed in a Ringer or physiological salt solution. Höber,¹⁰ extending this work, showed that a preliminary immersion of the muscle into a 6 per cent cane-sugar solution increases the "potassium current," and attributes this to the removal of the lymph salts; that is, the sodium chloride, which inhibits the action of the current-producing salt.

The influence of sodium chloride and cane-sugar on the relaxation. — If the above conclusions that sodium chloride antagonizes the potassium contraction and that the beneficial action of cane-sugar solution is due to the removal of sodium salts from the muscle are correct, we would expect sodium chloride to favor relaxation, while cane-sugar should have the opposite effect. And such I found to be the fact. A preliminary bath in cane-sugar solution lessens the relaxation, as is evident from Experiment 179:

A. — 10 c.c. 6 per cent cane-sugar + 2 c.c. $m/8$ KCl $\xrightarrow{2}$ muscle removed from solution.

B. — 10 c.c. 6 per cent cane-sugar $\xrightarrow{10}$ 10 c.c. cane-sugar + 1 c.c. KCl $\xrightarrow{2}$ muscle removed from solution.

The contraction in *A*, as magnified by the lever, was 33 millimetres; that of *B*, 34 millimetres. Seven minutes after the muscles had been removed from the solution, *A* had relaxed 33 per cent of its total contraction, while the relaxation of *B* was but 13 per cent.

In another experiment of similar nature but in which the bath in cane-sugar solution lasted for twenty minutes, the relaxation was nil; the control muscle in seventeen minutes relaxed 60.7 per cent of its total contraction.

⁸ OVERTON: *Archiv für die gesammte Physiologie*, 1904, cv, p. 196.

⁹ BIEDERMANN: *Sitzungsbericht der königliche Akademie der Wissenschaften in Wien*, 1880, 81 (iii), p. 76.

¹⁰ HÖBER: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 599.

Sodium chloride, on the other hand, favors relaxation. Experiment 181:

A. — 10 c.c. 6 per cent cane-sugar + 1 c.c. $m/8$ NaCl $\xrightarrow{11}$ 10 c.c. cane-sugar + $1\frac{1}{4}$ c.c. KCl $\xrightarrow{2}$ muscle removed from solution.

B. — 10 c.c. 6 per cent cane-sugar $\xrightarrow{11}$ 10 c.c. cane-sugar + 1 c.c. KCl $\xrightarrow{2}$, muscle removed from solution.

Total contraction of A = 29 mm.; relaxation in eleven minutes = 12 mm., or 41.4 per cent.

Total contraction of B = 37 mm.; relaxation in eleven minutes = 8 mm., or 21.6 per cent.

The rate of relaxation was markedly greater in A. In Figure 1 the dotted line represents the rate of relaxation of the muscle previously treated with sodium chloride (A); the full line shows relaxation of the control muscle (B).

When the stay of the muscles in the cane-sugar solution and in the cane-sugar + sodium chloride solution is prolonged, or if the amount of sodium chloride is increased, the difference in the relaxation becomes greater. A muscle immersed in 6 per cent cane-sugar solution for twenty-three minutes relaxed but 3.5 per cent in fourteen minutes; the control muscle in 10 c.c. cane-sugar + $2\frac{1}{2}$ c.c. NaCl in the same length of time relaxed 45.7 per cent.

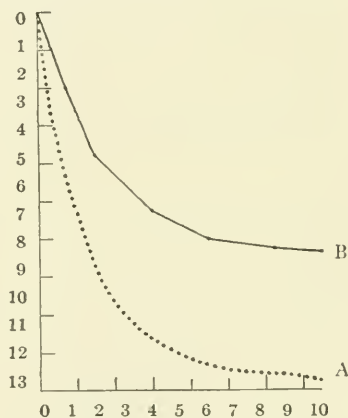


FIGURE 1.—Rate of relaxation of muscles A and B of Exp. 181 (see text). Abscissæ are minutes; ordinates are millimetres.

II. THE INFLUENCE OF $CaCl_2$ ON THE POTASSIUM CONTRACTION.

As stated in the beginning of this paper, my previous work showed that the stimulating effect of potassium is also counteracted by calcium chloride and to a far greater extent than is true of sodium chloride. However, in that work the calcium chloride was diluted either with an $m/8$ sodium chloride solution or with

water, and as both these substances have either a direct action on the muscle or interfere with the action of potassium, I thought it well to investigate this subject, using a 6 per cent cane-sugar solution as dilutant. I may briefly quote one experiment from each series. Experiment 140:

A. — 10 c.c. 6 per cent cane-sugar + $\frac{3}{4}$ c.c. *m*/8 KCl.

B. — 10 c.c. 6 per cent cane-sugar + $\frac{1}{10}$ c.c. *m*/8 CaCl₂ + $\frac{3}{4}$ c.c. *m*/8 KCl.

In *A* a fairly strong contraction was obtained; in *B* the immersion of the muscle in the solution was followed by a slight relaxation for two minutes which gave way to a very feeble contraction. Whether the calcium chloride is introduced simultaneously with or previous to the application of the potassium solution does not affect the results, as can be seen from Experiment 146:

A. — 6 per cent cane-sugar $\xrightarrow{50}$ 10 c.c. 6 per cent cane-sugar + 1 c.c. *m*/8 KCl.

B. — 10 c.c. 6 per cent cane-sugar + $\frac{1}{10}$ c.c. *m*/8 CaCl₂ $\xrightarrow{50}$ 10 c.c. 6 per cent cane-sugar + 1 c.c. *m*/8 KCl.

The contraction of the muscle in *A* one minute after its immersion in the potassium chloride solution was five times as great as that of the muscle in *B*. Experiment 144:

A. — In moist air $\xrightarrow{13}$ 10 c.c. 6 per cent cane-sugar + $\frac{3}{4}$ c.c. *m*/8 KCl.

B. — 10 c.c. *m*/8 NaCl + $\frac{1}{10}$ c.c. *m*/8 CaCl₂ $\xrightarrow{13}$ 10 c.c. 6 per cent cane-sugar + $\frac{3}{4}$ c.c. *m*/8 KCl.

While in *A* there is immediately developed a strong contraction, in *B* this occurs only after a latent period of three minutes.

These experiments leave no doubt that the introduction of calcium chloride previously to, or simultaneously with, the immersing of the muscle in potassium chloride reduces the extent of the potassium contraction and greatly lengthens the latent period, even though the concentration of the calcium chloride is but 0.01386 per cent. This would naturally lead us to conclude that the removal of calcium from the muscle favors the development of the potassium contraction. Concerning this, Guenther¹¹ states: "The application of reagents that are precipitants of calcium, like sodium

¹¹ GUENTHER: This journal, 1905, xiv, p. 93.

sulphate or sodium oxalate, destroys the ability of the potassium solution to produce contractions." In my former paper¹² I stated: "A muscle was treated for one minute with an $m/8$ solution of a sodium salt which precipitates calcium and then subjected to the action of KCl or K_2SO_4 . In this case the minimum concentration of the KCl on K_2SO_4 was much reduced and the increase in tone was more marked." To verify my previous results four series of experiments were instituted. Experiment 62:

A. — 9 c.c. 6 per cent cane-sugar + 2 c.c. $m/8$ Na_2SO_4 $\xrightarrow{7}$ 12½ c.c. 6 per cent cane-sugar + ½ c.c. $m/8$ KSCN.

B. — 11 c.c. 6 per cent cane-sugar $\xrightarrow{7}$ 12½ c.c. 6 per cent cane-sugar + ½ c.c. $m/8$ KSCN.

In *A* the height of the contraction is much greater and the maximum contraction is reached much sooner than in *B*. In the sodium sulphate we have at least two factors, the Na ion and the SO_4 ion. That this favorable action of the sodium sulphate in the development of the potassium contraction is not due primarily to the Na ion can be learned from Experiment 28:

A. — 8 c.c. cane-sugar + 2 c.c. $m/8$ Na_2SO_4 $\xrightarrow{21}$ 10 c.c. 6 per cent cane-sugar + ½ c.c. $m/8$ KSCN.

B. — 8 c.c. cane-sugar + 2 c.c. $m/8$ NaCl $\xrightarrow{21}$ 10 c.c. 6 per cent cane-sugar + ½ c.c. $m/8$ KSCN.

In this experiment a far more powerful contraction took place in *A* than in *B*. The same is evident from Experiment 27:

A. — 8 c.c. 6 per cent cane-sugar + 2 c.c. $m/8$ Na_2SO_4 $\xrightarrow{13}$ 10 c.c. 6 per cent cane-sugar + ½ c.c. $m/8$ KSCN.

B. — 8 c.c. $m/8$ NaCl + 2 c.c. $m/8$ Na_2SO_4 $\xrightarrow{13}$ 10 c.c. 6 per cent cane-sugar + ½ c.c. $m/8$ KSCN.

In this the muscle in *A* immediately produced a tremendous contraction, while that in *B* did not contract until after a latent period of four and one-half minutes.

In the last series the action of a sodium chloride solution mixed with sodium sulphate was compared with that of a pure sodium chloride solution, the results agreeing with the above.

¹² ZOETHOUT: This journal, 1902, vii, p. 321.

These results fully corroborate my former statement that sodium salts which precipitate calcium favor the production of the potassium contraction. As this effect cannot be attributed to the sodium ion, it is due either to the molecule as such or to the anion. However, whether the action is merely brought about by the precipitation of calcium or not, I am not prepared to say; certain experiments made with sodium salts that do not precipitate calcium speak against such a view.

The influence of calcium chloride on the relaxation of muscle.—A number of experiments were made to determine the effect of calcium chloride on the relaxation of a muscle in potassium contraction. In Experiment 159 one muscle (*A*) was directly treated with potassium chloride diluted with cane-sugar solution; the other muscle (*B*) received a preliminary bath with 10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. *m/8* CaCl_2 for ten minutes. After an exposure to the potassium for three minutes, both muscles were removed from the solution. The rate of relaxation is indicated in Figure 2, from which it is evident that a previous bath of dilute calcium chloride favors relaxation.

This effect of calcium can be removed by washing with cane-sugar solution, as is shown in Experiment 157:

A.—10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. *m/8* $\text{CaCl}_2 \xrightarrow{10}$ cane-sugar $\xrightarrow{10}$
10 c.c. cane-sugar + 2 c.c. *m/8* KCl.

B.—10 c.c. 6 per cent cane-sugar + $\frac{1}{2}$ c.c. *m/8* $\text{CaCl}_2 \xrightarrow{10}$ 10 c.c. cane-sugar + 2 c.c. *m/8* KCl.

After an immersion of three minutes in the potassium chloride solution, the muscles were suspended in moist air. The rate of relaxation was very much greater in *B*, which had not been washed with cane-sugar, than in *A*, as is illustrated in Figure 3. The per cent of relaxation of the total contraction of these two muscles is given in the following table:

	In <i>B</i> .	In <i>A</i> .
Relaxation in five minutes	36 per cent.	14 per cent.
Relaxation in ten minutes	58 per cent.	24 per cent.
Relaxation in twenty minutes	69 per cent.	30 per cent.
Relaxation in thirty minutes	74 per cent.	32 per cent.

III. THE INFLUENCE OF POTASSIUM CHLORIDE ON THE POTASSIUM CONTRACTION.

Concerning the influence of preliminary treatment of a muscle with potassium chloride Guenther¹³ states: “. . . it can be shown that preliminary treatment of the muscle with cane-sugar solution for varying lengths of time just previous to immersion in potas-

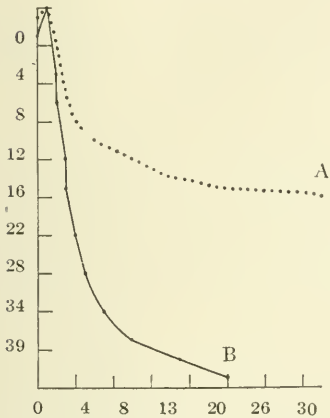


FIGURE 2.—The rate of relaxation of muscles A and B of Exp. 159 (see text). Abscissæ are minutes, ordinates are millimetres.

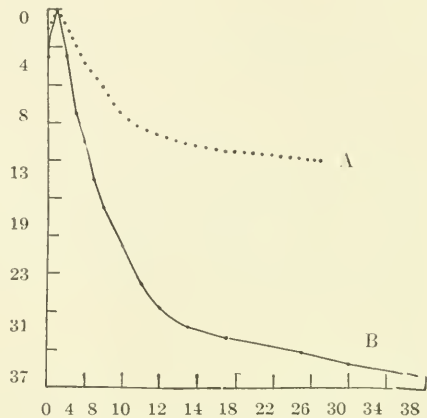


FIGURE 3.—The rate of relaxation of muscles A and B of Exp. 157 (see text). Abscissæ are minutes; ordinates are millimetres.

sium chloride alters the results. The latent period is lengthened and the height of the contraction is diminished. Preliminary treatment with potassium chloride produces the same results more rapidly.” If by preliminary treatment with potassium chloride is meant the immersion of a muscle for ten or twenty minutes in an $m/8$ solution of this salt, there is little doubt that such a treatment reduces the height of the contraction and lengthens the latent period. But such a treatment no more shows the effect of preliminary treatment with potassium chloride upon the potassium contraction than treating a muscle with $m/8$ hydrochloric acid or any other destructive agency. Potassium chloride in such concentrations is a heavy poison to the muscles; if, therefore, we wish to determine its effects on the potassium contraction, we must employ such amounts as shall not be immediately injurious to the

¹³ GUENTHER: This journal, 1905, xiv, p. 90.

muscle. Neither must the amount of potassium chloride used in the preliminary bath be so great as to cause a contraction, for this will obscure the contraction caused by the subsequent application of the potassium. The question may be put: Is this minimal concentration of potassium chloride necessary to cause a contraction decreased by the previous application of a sub-minimal amount of potassium chloride? According to our idea, we would naturally answer this in the affirmative. Experiment 188:

A. — 10 c.c. 6 per cent cane-sugar + $1/10$ c.c. $m/8$ KCl $\xrightarrow{5}$ 10 c.c. cane-sugar + $1/2$ c.c. KCl.

B. — 10 c.c. 6 per cent cane-sugar $\xrightarrow{5}$ 10 c.c. cane-sugar + $1/2$ c.c. KCl.

In one minute the contraction in *A*, as recorded on the kymograph, was 30 millimetres; that of *B*, 12 millimetres. Prolonging the stay in the diluted potassium chloride, or increasing the concentration of the salt, decreases the potassium contraction, no doubt due to its poisonous action.

IV. THE INFLUENCE OF RINGER SOLUTION ON THE POTASSIUM CONTRACTION.

A few experiments with Ringer solution were made. The modified Ringer used by Overton,¹⁴ composed of 0.65 per cent NaCl + 0.02 per cent KCl + 0.03 per cent $CaCl_2$ was first used. Such a solution always decreases the irritability of the muscle towards potassium. Experiment 56:

A. — 6 per cent cane-sugar $\xrightarrow{7}$ 10 c.c. 6 per cent cane-sugar + $1/3$ c.c. $m/8$ KSCN.

B. — Ringer solution $\xrightarrow{7}$ 10 c.c. 6 per cent cane-sugar + $1/3$ c.c. $m/8$ KSCN.

A fairly strong contraction begins almost immediately in *A*; in *B*, after a latent period of nine minutes, there is a small contraction.

In another experiment the muscle is immersed in the Ringer solution for but five minutes, yet the potassium contraction at the end of the seven minutes is only one sixth of that of the control muscle. The same result is obtained if the potassium contraction of a fresh muscle is compared with that of a muscle previously treated with Ringer. Experiment 84:

¹⁴ OVERTON: Archiv für die gesammte Physiologie, 1904, cv, p. 186.

- A.* — Ringer solution $\xrightarrow{10}$ 10 c.c. 6 per cent cane-sugar + $\frac{2}{5}$ c.c. $m/8$ KSCN.
B. — 10 c.c. 6 per cent cane-sugar + $\frac{2}{5}$ c.c. $m/8$ KSCN.

In *B* a very good contraction took place; in *A* no contraction for four minutes. The same results were obtained when KCl instead of KSCN was used.

The well-known modified Ringer composed of 0.7 per cent NaCl + 0.035 per cent KCl + 0.025 per cent CaCl_2 was also employed. In this, as in Overton's modification, the latent period is always increased and the height and the rapidity of the potassium contraction decreased.

This action of Ringer solution is of great interest and importance. Ringer solution, as also a 0.6 per cent or 0.7 per cent sodium chloride solution, is regarded as a physiological saline solution, that is, as an inert fluid as regards the frog muscle. While it is admitted that a frog muscle will eventually lose its irritability and other physiological properties in a 0.7 per cent sodium chloride and even in a Ringer solution, yet this does not take place for some hours; especially is this true for the Ringer solution. But for a short interval, say one hour, the muscle retains its irritability toward electrical stimulation. Yet such solutions cannot be regarded as physiological salt solutions, even though employed for a comparatively short length of time, for the above experiments demonstrate that a sojourn of a muscle in Ringer solution for but five or ten minutes materially reduces the irritability of the muscle toward potassium stimulation. Hence the Ringer solution, even in this short space of time, has caused some change in the muscle which, though it cannot be detected by electrical stimulation, is readily revealed by the potassium contraction.

The reaction of a muscle toward potassium and, no doubt, other chemical stimuli is a far more delicate index of the normality of a muscle than the electric current. When, therefore, an inert solution, that is, a solution which shall preserve the muscle for the greatest length of time in its normal condition, is sought for, the employment of chemical stimulation is advisable, for any solution that alters the response toward potassium or other chemical substance in so short a length of time will in the long run also alter the response to electrical or any other form of stimulation.

Why does Ringer solution fail to keep the muscle in its normal condition? We have seen both sodium and calcium chloride re-

duce the power of a muscle to give a potassium contraction; it seems, therefore, likely that the large amount of sodium chloride and, perhaps, the excessive amount of calcium chloride in the Ringer solution are responsible for its deleterious action. While it is true that the amount of calcium chloride in Ringer solution is but from 0.025 per cent to 0.03 per cent, yet it was seen in Experiment 144 that a concentration of but 0.01386 per cent calcium chloride when placed in a 6 per cent cane-sugar solution greatly diminishes the potassium contraction. This is, no doubt, offset to a certain extent by the presence of the potassium chloride, but the combined influence of the sodium and calcium chloride is far greater than that of the potassium chloride. What, then, must be the composition of a more nearly correct physiological salt solution? This problem is at present under consideration.

Still another point is brought out by these experiments. When the response to electrical stimulation is little or not at all affected by the sojourn of the muscle in Ringer solution for one or two hours while the response to potassium stimulation is greatly weakened by a stay in this solution for merely five or ten minutes, there must be a radical difference between the *modus operandi* of the electrical and the potassium stimulation.

CONCLUSIONS.

1. The amount of sodium in the muscle is sufficient to counteract the stimulating effect of a small quantity of potassium salt. The removal of this sodium by immersion in a 6 per cent cane-sugar solution renders the muscle more irritable toward potassium.
2. The same is most likely true for the calcium.
3. Both sodium and calcium favor the relaxation of a muscle in potassium contraction.
4. Ringer solution and a 0.7 per cent sodium chloride solution very speedily reduce the irritability of a muscle toward potassium. These solutions can, therefore, not be looked upon as inert fluids, even though they preserve the irritability of a muscle toward electrical stimulation for a long length of time.

THE ACTION OF THE ALKALOIDS OF THE PAPA- VERACEÆ UPON THE ISOLATED FROG'S HEART.

By WORTH HALE.

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THE amount of experimental work done in determining the action of the alkaloids of the family Papaveraceæ, and especially of the genus *Sonniferum*, is very great, and yet the subject is not completely covered. The literature, with comparatively few exceptions, takes up the few active principles, three or four in number, which have obtained wide use in medicine, notably morphine and more recently codeine and the synthetic preparation heroine. In contrast the large number of therapeutically less important alkaloids found with them in the same family have received very much less attention. This is probably as it should be, but it must be remembered that these unimportant alkaloids from the view-point of therapeutics, are present in the crude preparations of opium in a considerable percentage. Morphine, however, is recognized as the chief alkaloid of the series on account of its medicinal value, and accordingly, in investigations of the other and related active principles found in the poppy family, it commonly serves as a basis for comparisons. As a result, attempts have been made to arrange the important alkaloids in a series, classifying them according to their relative depressant or stimulant action upon the central nervous system.

Grouping the several classifications which appear in the literature into a table illustrates the results obtained by the various observers:

BERNARD.	BAXT.	SCHROEDER.	LEUBUSCHER. ¹	
Narceine.	Papaverine.	Morphine.	Morphine.	Depressant.
Morphine.	Morphine.	Papaverine.	
Codeine.	Narceine.	Papaverine.	Narcotine.	
Narcotine.	Codeine.	Codeine.	Codeine.	
Papaverine.	Narcotine.	Narcotine.	Narceine.	
Thebaine.	Thebaine.	Thebaine.	Thebaine.	Stimulant.

¹ LEUBUSCHER: Deutsche medicinische Wochenschrift, 1892, p. 179.

Two reasons may be suggested in explanation of these variable results. Bernard and Leubuscher seem to have made no attempt to determine the purity of the alkaloids used in their experiments, although the former makes special note that his drugs were the products of reputable pharmaceutical houses, — a method which could hardly be called accurate enough at the present time. On the other hand, both Schroeder and Baxt seem to have made an attempt to establish the identity of the preparations used in their experiments. It seems easily a possibility that Bernard and Leubuscher were describing effects due to the presence of impurities, and in the case of the other investigators this may also have been partly true, as the separation of the different alkaloids is not an easy matter. A second chance for error was in the end reaction, which was based upon the rather indefinite phenomena of depression and stimulation as applied to the central nervous system. The differences in the classification emphasize the fact that an end reaction upon which comparisons are to be based should be sufficiently definite that slight differences in action could be determined, — a condition that was hardly complied with in the case of the early observers.

The present research, although not intended to duplicate these investigations of the relative action of these drugs on the nervous system, grew partly out of the variations in the classifications as given above. It was early evident, in a study of the literature, that their effects upon the heart and striated muscle had been rather imperfectly worked out. Rather few reports take up the action upon these structures, most observers being more especially interested in the action upon the central nervous system, and as a result, in those cases in which the question is considered at all, it is often treated as incidental or merely as a sequence of the other effects produced. Accordingly it seemed desirable that more work be done upon this phase of the action of these drugs. Also, testing the action of these alkaloids upon the heart seemed to offer many advantages in estimating their relative activity on structures other than those of the nervous system, as slight variations in either rate or strength can be easily determined.

Frogs were used as experimental animals, and after isolation the hearts were perfused with Ringer's solution to determine a normal and then with Ringer's solution to which the alkaloids to be tested had been added. A pressure of 40 to 50 mm. of the per-

fusing fluid was used, but the pressure in an individual experiment never varied. In all cases the terminations of the vagi were paralyzed with atropine before beginning the perfusion, so that nothing but the heart muscle action of the drugs would be obtained. The heart rate was determined at five-minute intervals, the number of beats in sixty seconds being counted rather than for a fraction of a minute and then multiplying by the necessary factor. It was found that a stop watch facilitated accuracy, since often the variation was only one or two beats per minute. The amount of fluid perfused was also measured at five-minute intervals, as a method of determining the changes in the pumping capacity of the heart.

In all cases the melting-point of each alkaloid experimented with was determined, as a means of establishing its purity. If this was found to be definite, no other tests were carried out, but in certain cases the end reaction was somewhat irregular, so that chemical tests were also employed. The alkaloidal salts were changed into the alkaloidal form by the addition of ammonium hydrate and, after thorough washing and drying, the melting-point was determined in the usual manner.

Chelidonine.—In testing this alkaloid upon the intact frog Meyer² found a decrease in the heart rate in all cases and the injection of atropine was entirely devoid of effect. For this reason he concluded that the slowing was due to a direct depressant action on the heart muscle.

Chelidonine hydrochloride was used in my experiments. The melting-point of the alkaloid was found to be 136° to 137° (Schmidt,³ 136°). Taking this as a sufficient proof of the purity of the preparation, isolated and atropinized frog's hearts were perfused with various strengths of the drug in Ringer's solution with results as illustrated in Table I.

In Experiment 56 there seems to be a fairly definite increase in rate and therefore direct stimulation of the heart, being shown by the tendency of the drug to check the steady fall which preceded the introduction of the drug rather than by any marked increase in rate. Stronger solutions of the drug show a very definite and rapid decrease in rate. The output of the heart is increased by weak

² MEYER: Archiv für experimentelle Pathologie und Pharmakologie, 1892, xxix, p. 398.

³ SCHMIDT: Lehrbuch der pharmaceutischen Chemie, 1901, p. 1530.

solutions, but rapidly lessened by stronger ones. The action is a direct one upon the heart muscle.

Chelerythrine. — Meyer,⁴ after experimenting with chelerythrine, came to the conclusion that large doses caused a decrease in the heart rate in both frogs and mammals, but in one experiment upon a

TABLE I.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH
CHELIDONINE HYDROCHLORIDE IN RINGER'S SOLUTION.

EXPERIMENT 56.			EXPERIMENT 58.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
10.25	27	29	10.55	35	67
10.30	26	25	11.00	35	66
10.35	24	21	11.05	34	65
....	11.10	32	65
Chelidonine, 1/1000 per cent.			Chelidonine, 1/150 per cent.		
10.37	25	..	11.12	26	..
10.40	26	20	11.15	22	64
10.45	25	22	11.20	24	60
10.50	26	22	11.25	23	60
....	11.30	23	54

rabbit there was a suggestion of increased rate. With the slowing of the frog's heart the ventricle became weaker, and both slowing and weakening were ascribed to a direct paralysis of the heart muscle.

The chelerythrine used in my experiments was tested with regard to its chemical reactions and its melting-point. Concentrated sulphuric acid gave a yellow color which later assumed a dirty hue. Concentrated nitric acid caused a bright yellow coloration, but this very quickly changed into a dark brown with a yellowish

⁴ MEYER: Archiv für experimentelle Pathologie und Pharmakologie, 1892, xxix, p. 428.

tinge. The crystals gave a melting-point of 204° (Bruhl,⁵ 203°). In order to perfuse the frog's heart it was necessary to form the soluble sulphate by the addition of the necessary amount of dilute sulphuric acid. The results of the experiments with this salt are illustrated in Table II.

TABLE II.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH CHELERYTHRINE SULPHATE IN RINGER'S SOLUTION.

EXPERIMENT 80.			EXPERIMENT 81.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
10.20	46	65	9.20	33	27
10.25	44	64	9.25	33	27
....	9.30	32	27
CHELERYTHRINE, 1/4000 PER CENT.			CHELERYTHRINE, 1/10,000 PER CENT.		
10.27	40	..	9.32	33	..
10.30	41	60	9.35	34	24
10.35	44	57	9.40	35	26
10.40	42	55	9.45	35	26
10.45	43	53	9.50	39	26
CHELERYTHRINE, 1/2000 PER CENT.			CHELERYTHRINE REMOVED.		
10.47	42	..	9.55	35	24
10.50	40	47	10.00	34	23
10.55	39	40	10.05	33	20
11.00	32	27			

Experiment 81 shows very clearly an increased heart rate, coincident with which there is a very doubtful increase in strength as measured by the output (note the lessened output immediately following the removal of the drug). On the other hand, larger

⁵ BRUHL: Die Pflanzen-Alkaloide, 1900, p. 517.

amounts are actively depressant, decreasing both the rate and the output as illustrated by Experiment 80, especially after the introduction of the 1/2000 per cent solution.

Codeinc. — On account of its importance in therapeutics codeine has received a good deal of attention at the hands of investigators, but the tendency has been to ignore the changes in the heart in favor of the more important effects upon the nervous system. Schroeder⁶ found that when given to the intact frog the heart rate was slowed through a paralyzing action upon the heart muscle, but in warm-blooded animals he noted a possible increase in the rate. Rheiner,⁷ in experiments on patients, reported the tendency of doses of 300 mgm. to increase the heart rate. Vinci,⁸ in experimenting on warm-blooded animals, found that weak solutions increased the heart rate, but when more concentrated it was slowed.

Codeine sulphate was used in my experiments. The alkaloid was formed from a solution of the salt, and the melting-point was found to be 153° (Bruhl,⁹ 153°). Atropinized frog's hearts perfused with varying percentages of the sulphate added to Ringer's solution were acted upon in the manner illustrated by the experiments given in Table III.

As will be seen by examining the table, codeine causes a marked increase in the rate of the frog's heart when given in a relatively weak solution (1/400 per cent), but by the introduction of stronger solutions of the drug the heart rate is slowed. The strength as measured by the output rises and falls coincident with the changes in rate.

Cryptopine. — Sippel¹⁰ noted, in some experiments on frogs, an increased rate following a dose of 40 mgm., but this was quickly followed by slowing. A second frog, receiving about twice the dose per gram body weight, showed slowing from the first. In some tests upon frogs Schroeder¹¹ found a decrease in the heart rate from doses of 15 to 20 mgm.

⁶ SCHROEDER: Archiv für experimentelle Pathologie und Pharmakologie, 1883, xvii, p. 111.

⁷ RHEINER: Therapeutische Monatsschrift, 1899, iii, p. 393.

⁸ VINCI: Archives internationales de pharmacodynamie et de thérapie, 1907, xvii, p. 21.

⁹ BRUHL: Die Pflanzen-Alkaloide, 1900, p. 343.

¹⁰ SIPPEL: Beiträge zur Kenntnis der Wirkung des Cryptopine, Marburg, 1874.

¹¹ SCHROEDER: Archiv für experimentelle Pathologie und Pharmakologie, 1883, xvii, p. 140.

To test the purity of the cryptopine used in the following experiments chemical tests were employed and the melting-point determined. The addition of concentrated sulphuric acid gave a yellow

TABLE III.
PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH
CODEINE SULPHATE IN RINGER'S SOLUTION.

EXPERIMENT 12.		
Time.	Rate.	Output per 5 minutes.
3.25	47	45
3.30	45	45
3.35	45	43
CODEINE, 1/400 PER CENT.		
3.37	48	--
3.40	47	46
3.45	46	48
3.55	45	46
CODEINE, 1/20 PER CENT.		
4.00	42	43
4.05	41	40
4.10	40	38
4.15	39	35

color immediately changing to violet. The addition of concentrated nitric acid produced an orange yellow color.¹² The melting-point was not absolutely definite, but the figures, 215° to 217°, agreed closely with those (217°) given by Bruhl.¹³ The hydrochloride was formed and perfused through the isolated and atropinized

¹² SCHMIDT: Lehrbuch der pharmaceutischen Chemie, 1901, p. 1520.

¹³ BRUHL: Die Pflanzen-Alkaloide, 1900, p. 364.

frog's heart in various strengths. The results are illustrated by the following experiments:

TABLE IV.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH CRYPTOPINE HYDROCHLORIDE IN RINGER'S SOLUTION.

EXPERIMENT 79.			EXPERIMENT 78.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
3.25	46	31	10.05	22	47
3.30	47	31	10.10	22	45
3.35	45	33	10.15	22	45
3.40	44	32	10.20	22	44
CRYPTOPINE, 1/6000 PER CENT.			CRYPTOPINE, 1/1000 PER CENT.		
3.42	46	..	10.22	20	..
3.45	47	32	10.25	19	43
3.50	47	34	10.30	19	41
3.55	47	32	CRYPTOPINE REMOVED.		
4.00	45	29	10.32	23	..
....	10.35	24	51
....			

The results indicate that cryptopine has a very definite stimulant action on the heart muscle if in sufficient dilution, but a somewhat stronger solution depresses the heart, as measured by both rate and output. The stimulant action of weak solutions is not manifested in any definite increase in the amount of perfused fluid.

Heroine. — Heroine has been the subject of numerous investigations during the past few years, but these have been almost entirely limited to the action of the drug upon the respiratory system. Dresser,¹⁴ working in the laboratory of Friedrich Bayer & Co.,

¹⁴ DRESSER: Archiv für die gesammte Physiologie, 1898, lxxii, p. 516.

stated that there was little or no change in the heart rate or in blood pressure, even when the respiratory centre had become markedly depressed. Later, however, both were found to be lessened

TABLE V.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH HEROINE HYDROCHLORIDE DISSOLVED IN RINGER'S SOLUTION.

EXPERIMENT 17.			EXPERIMENT 22.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
10.05	52	55	2.50	47	53
10.10	54	60	2.55	47	54
10.15	54	55	3.00	46	55
HEROINE, 1/400 PER CENT.			HEROINE, 1/2000 PER CENT.		
10.17	48	..	3.02	48	..
10.20	43	48	3.05	47	55
10.25	42	51	3.10	47	54
HEROINE REMOVED.			3.15	46	55
			3.20	44	53
10.30	48	53	3.25	45	53
10.35	50	51

from a failure in the supply of oxygen to the heart, and when compared with codeine the ratio was six to one in favor of the former. Harnack¹⁵ observed weakening in the heart's action and stated that heroine was more deleterious than morphine. Vinci¹⁶ perfused hearts of warm-blooded animals and found the rate was increased by small amounts, but that larger quantities depressed the heart's activity by a direct depression of the ganglia and the muscle.

¹⁵ HARNACK: *Münchener medicinische Wochenschrift*, 1899, xlv, p. 881.

¹⁶ VINCI: *Archives internationales de pharmacie et de thérapie*, 1907, xvii, p. 5.

The heroine hydrochloride used in my experiments was changed into the alkaloidal state, and then the melting-point test gave 171° (Schmidt,¹⁷ 171°). The results obtained from this drug are illustrated by the experiments given in Table V.

In its action upon the heart muscle heroine is not especially stimulant to the rate even in weak solution, while the output is hardly changed. Strong solutions are very poisonous, the rate being greatly slowed and the output decreased, but not to the same degree.

Morphine. — Morphine, as the chief therapeutic alkaloid of this series, has received a great deal of investigation, but only a few of the reports will be given here. Witkowski¹⁸ found that the rate of the heart was increased at first but later slowed, due to the effects of the drug upon the central nervous system. An analysis of his work shows that large doses caused slowing from the beginning and that it was only from the smaller doses that increased rate resulted. Grumnach,¹⁹ in making observations to determine the effect of morphine upon the human heart, noted that doses of 20 to 30 mgm. produced weakening in all cases. Vinci,²⁰ perfusing the hearts of warm-blooded animals, demonstrated that weak solutions increased the activity of the heart both as to rate and amplitude, but stronger solutions lessened both, due to a direct depression of the heart muscle.

Morphine sulphate was used in the following experiments. The salt was changed to the alkaloidal form and the resulting crystals melted at 254° (U. S. P., VIII, 254°). The sulphate perfused through the isolated frog's heart gave results as found in Table VI.

These results agree in general with those obtained by other workers, but with the exception of Vinci the correct point of action was not understood. The experiments given in the table show very clearly the stimulant action of morphine upon the heart muscle if in weak solution, but if in stronger solution the depression is very marked. The pumping capacity of the heart changes with the increase or decrease of the rate.

¹⁷ SCHMIDT: Lehrbuch der pharmaceutischen Chemie, 1901, p. 1498.

¹⁸ WITKOWSKI: Archiv für experimentelle Pathologie und Pharmakologie, 1877, vii, p. 247.

¹⁹ GRUMNACH: VIRCHOW'S Archiv für Pathologie, cii, p. 577.

²⁰ VINCI: Archives internationales de pharmacodynamie et de thérapie, 1907, xvii, p. 5.

Narceine. — According to Bernard,²¹ narceine is more depressant than morphine, but, as has been pointed out, this result is open to question. Heinz²² reported, a few years later, that nar-

TABLE VI.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH MORPHINE SULPHATE DISSOLVED IN RINGER'S SOLUTION.

EXPERIMENT 7.			EXPERIMENT 8.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
8.15	50	52	1.55	48	37
8.20	49	49	2.00	47	37
8.25	48	49	2.05	46	35
8.30	49	48			
MORPHINE, 1/20 PER CENT.			MORPHINE, 1/150 PER CENT.		
8.32	44	..	2.07	49	..
8.35	29	42	2.10	48	39
8.40	20	29	2.15	52	38
8.45	17	27	2.20	51	37
8.50	16	15	MORPHINE REMOVED.		
...	2.25	47	35
...	2.30	48	35
...	2.35	46	..

ceine decreased the pulse rate from 10 to 12 beats per minute. Later still Schroeder²³ denied that the drug had any action whatever.

The disparity in these results made it especially important that the drug used in my experiments should be carefully tested to

²¹ BERNARD: Bulletin de thérapeutique, lxvii, p. 193

²² HEINZ: Neues Repertorium für Pharmacie, 1876, xxv, p. 676.

²³ SCHROEDER: Archiv für experimentelle Pathologie und Pharmakologie, 1883, xvii, p. 132.

determine its purity. The crystals formed from the salt were found to have a variable melting-point, part melting at about 165° , but the larger mass not melting until a temperature of 170° – 171° was reached (Bruhl,²⁴ 145° to 171° , depending upon the amount of water of crystallization). Chemical tests were also made. The addition of iodine in potassium iodide solution gave a blue color. Chlorine water added to a few crystals after the addition of ammonium hydrate gave a blue to red color.²⁴ The addition of concentrated nitric acid produced a rapidly fading yellow. Concentrated sulphuric acid gave a brown color dissolving to yellow and then to dark red.²⁵ The experiments found in Table VII show the character of the action of narceine upon the frog's heart.

Narceine has a rather weak action upon the heart, although the stronger solutions have a fairly marked depressant action upon both the rate and the output. The stimulant action of weak solutions is less clear, but there is probably a slight action of this sort shown.

Narcotine.—One of the earliest pharmacological observations of the action of narcotine was made by Schroff,²⁶ who observed, in experimenting upon students, that doses of 100 mgm. first increased and then decreased the heart rate. Schroeder,²⁷ and recently Crawford and Dohme,²⁸ in describing the effects of this drug upon frogs, noted no increase in the heart rate, but instead slowing and irregularity. Schroeder ascribed this effect to a direct depressant action on the motor ganglia of the heart; Crawford and Dohme, to a direct muscular action.

The melting-point of the narcotine used in these experiments was determined, the crystals formed from the salt melting at 175° – 176° (Bruhl,²⁹ 176°). Experiments illustrative of the results obtained with this drug are shown in Table VIII.

The action of narcotine upon both the rate and strength of the heart, as shown by these experiments, agrees closely with the action

²⁴ BRUHL: Die Pflanzen-Alkaloide, 1900, p. 324.

²⁵ ALLEN: Commercial Organic Analysis, 2d ed., iii, p. 302.

²⁶ SCHROFF: Pharmakologie, 1856, p. 476.

²⁷ SCHROEDER: Archiv für experimentelle Pathologie und Pharmakologie, 1883, xvii, p. 100.

²⁸ CRAWFORD and DOHME: Proceedings of the American Pharmaceutical Association, 1902, I, p. 472.

²⁹ BRUHL: Die Pflanzen-Alkaloide, 1900, p. 298.

of the rest of the series. Weak solutions increase the rate and output, and both are decreased by large amounts of the drug.

TABLE VII.
PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH
NARCEINE HYDROCHLORIDE IN RINGER'S SOLUTION.

EXPERIMENT 52.			EXPERIMENT 54.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
3.20	37	44	11.00	32	19
3.25	36	46	11.05	30	19
3.30	35	43	11.10	30	19
			11.15	30	19
NARCEINE, 1/80 PER CENT.			NARCEINE, 1/1000 PER CENT.		
3.32	32	..	11.17	31	..
3.35	28	35	11.20	31	19
3.40	26	29	11.25	31	20
3.45	24	29	11.30	32	21
NARCEINE REMOVED.			11.35	32	22
3.50	24	25	11.40	31	22
3.55	28	32
4.00	30

Papaverine. — Very little work has been done in determining the action of papaverine upon the heart. Schroeder³⁰ stated that the heart is slowed after the drug and that it has no tendency to increase the rate at any time. The application of atropine was without effect, so that the action was to depress the heart muscle directly. Pohl³¹

³⁰ SCHROEDER: Archiv für experimentelle Pathologie und Pharmakologie, 1883, xvii, p. 125.

³¹ POHL: Archives internationales de pharmacodynamie et de thérapie, 1904, p. 479.

stated that 20 mgm. injected into a lymph sac of a frog stopped the heart within an hour.

TABLE VIII.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH NARCOTINE HYDROCHLORIDE DISSOLVED IN RINGER'S SOLUTION.

EXPERIMENT 32.			EXPERIMENT 33.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes
2.55	50	54	2.40	40	53
3.00	52	55	2.45	40	60
3.05	47	53	2.50	40	55
3.10	46	54	NARCOTINE, 1/400 PER CENT.		
NARCOTINE, 1/1000 PER CENT.			2.52	29	..
3.12	49	..	2.55	30	50
3.15	50	60	3.00	31	53
3.20	45	61	3.10	31	54
3.25	44	58	NARCOTINE REMOVED.		
3.30	41	54	3.15	38	57
....	3.20	39	58
....			

The melting-point of the alkaloid formed from the salt used in my experiments was found to be 146.5° - 147° (Schmidt,³² 147°). Perfusing frog's hearts with a solution of this drug gave results as illustrated in Table IX.

The effect of papaverine when perfused is to slow the heart and weaken its action if strong solutions are used, but, in contrast to the reports of previous work, small amounts increase both rate and output quite markedly. The stimulant action is somewhat transitory as far as increasing the output is concerned.

³² SCHMIDT: Lehrbuch der pharmaceutischen Chemie, 1901, p. 1518.

Protopine. — The literature concerning the action of protopine upon the heart is very meagre. Von Engel,³³ in an investigation of the action of this alkaloid, reported that the heart was slowed,

TABLE IX.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH PAPAVERINE HYDROCHLORIDE IN RINGER'S SOLUTION.

EXPERIMENT 39.			EXPERIMENT 41.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
3.30	36	46	11.25	36	59
3.35	36	45	11.30	36	59
3.40	35	47	11.35	36	58
PAPAVERINE, 1/400 PER CENT.			PAPAVERINE, 1/4000 PER CENT.		
3.42	33	..	11.37	40	..
3.45	27	37	11.40	45	65
3.50	27	29	11.45	39	59
3.55	23	32	11.50	39	55
4.00	19	34	11.55	38	53
...	12.00	38	51
...	12.05	38	46

but that there was not any change in the contractile power of the heart muscle.

In my experiments a protopine was used which melted at 205° (Schmidt,³⁴ 207°). With concentrated sulphuric acid a blue to a violet color was produced which soon changed to a dirty blue or green. With Erdmann's reagent a characteristic play of colors was obtained.³⁵ When perfused through the isolated frog's heart, results were obtained as illustrated in Table X.

³³ VON ENGEL: *Archiv für experimentelle Pathologie und Pharmakologie*, 1890, xxvii, p. 419.

³⁴ SCHMIDT: *Lehrbuch der pharmaceutischen Chemie*, 1901, p. 1517.

³⁵ BRUHL: *Die Pflanzen-Alkaloide*, 1900, p. 515.

Protopine increases the rate of the heart, but only when perfused in extremely weak solutions. This effect, however, is rather transitory. Strong solutions decrease both the rate and the output, affecting the rate to a somewhat greater degree than the output.

TABLE X.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH PROTOPINE HYDROCHLORIDE DISSOLVED IN RINGER'S SOLUTION.

EXPERIMENT 73.			EXPERIMENT 76.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
10.25	34	43	9.30	14	28
10.30	34	43	9.35	13	28
PROTOPINE, 1/2000 PER CENT.			9.40	13	26
10.32	25	..	PROTOPINE, 1/10,000 PER CENT.		
10.35	24	40	9.42	18	..
10.40	21	37	9.45	14	31
10.50	25	39	9.50	15	32
PROTOPINE REMOVED.		
10.55	28	43
11.00	28	40

Sanguinarine. — Among the early pharmacological investigations of sanguinarine is that of Smith,³⁶ who observed, in experimenting on warm-blooded animals, that the heart rate was increased by very small doses, but that larger amounts invariably caused slowing. Meyer³⁷ noted only the decrease in the rate and ascribed it to a direct depressant action upon the motor heart ganglia.

³⁶ SMITH: American journal of medical sciences, 1876, lxxii, p. 346.

³⁷ MEYER: Archiv für experimentelle Pathologie und Pharmakologie, 1892, xxix, p. 397.

The melting-point of the alkaloid formed from sanguinarine nitrate melted at 214° (Schmidt,³⁸ 213°). The action of this drug is shown by the experiments given in Table XI.

TABLE XI.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH SANGUINARINE NITRATE DISSOLVED IN RINGER'S SOLUTION.

EXPERIMENT 63.			EXPERIMENT 64.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
2.55	36	56	3.50	33	30
3.00	33	52	3.55	33	28
SANGUINARINE, 1/2000 PER CENT.			4.00	30	30
			4.05	30	30
3.02	35	..	SANGUINARINE, 1/150 PER CENT.		
3.05	39	52			
3.10	39	37	4.07	21	..
3.20	38	16	4.10	11	16
SANGUINARINE REMOVED.			4.15	0	7
		
3.25	34	27
3.30	32	26

From the weaker solutions of sanguinarine the heart rate is markedly increased, but the output becomes gradually less. In another experiment 1/6000 per cent, although increasing the rate, lessened the output. The effect of stronger solutions is to slow and finally stop the heart in systole.

Thebaine. — Thebaine has always received considerable notice because of its strychnine-like effect upon the spinal cord. Falck³⁹ found that the frog's heart became much slower after the injection

³⁸ SCHMIDT: Lehrbuch der pharmaceutischen Chemie, 1901, p. 1532.

³⁹ FALCK: Deutsche Klinik, 1870, p. 18.

of this drug into a lymph sac. The same observation was made by Ott,⁴⁰ but he also noticed that the rate was first increased and that the slowing was secondary. Schroeder⁴¹ stated that the rate may be increased, but that this effect is the result of the tetanic

TABLE XII.

PERFUSION OF THE ISOLATED AND ATROPINIZED FROG'S HEART WITH THEBAINE HYDROCHLORIDE DISSOLVED IN RINGER'S SOLUTION.

EXPERIMENT 67.					
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
9.05	26	30	9.40	25	15
9.10	26	28	THEBAINE REMOVED.		
9.15	26	26	9.45	28	30
THEBAINE, 1/100 PER CENT.			9.50	28	32
9.17	25	..	9.55	29	30
9.20	25	18	THEBAINE, 1/20 PER CENT.		
9.25	26	13	10.00	21	19
9.30	25	13	10.05	12	15
9.35	25	15			

stage and that the heart is slowed later from a direct paralyzing action of the drug.

After changing the hydrochloride into the alkaloidal state the test of the melting-point gave 193.5° – 194° (Bruhl,⁴² 193°). When perfused through the isolated frog's heart, this salt had an effect upon the heart as illustrated in Table XII.

Thebaine is not very active upon the heart muscle. In strong solution there is a definite decrease in the rate and the output,

⁴⁰ OTT: British medical journal, 1875, p. 399.

⁴¹ SCHROEDER: Archiv für experimentelle Pathologie und Pharmakologie, 1883, xvii, p. 136.

⁴² BRUHL: Die Pflanzen-Alkaloide, 1900, p. 352.

especially emphasized in the above experiment by the effect of the removal of the drug and the substitution of Ringer's solution. Higher dilutions of the drug cause no alteration in the rate.

Contrary to my expectations, it seems rather difficult to classify the twelve alkaloids taken up in my experiments into any definite relation as based upon their poisonous action upon the heart muscle. This will be better understood when the difficulty of determining the first indications of stimulation or depression is considered. However, the following classification has been made on the basis of the depressant action on the heart rate of this series of drugs. The relative position is based upon the whole number of experiments rather than upon those given in the tables in this paper and is intended to be only approximately correct.

1. Chelerythrine . . .	1/4000 per cent.	7. Narcotine	1/200 per cent.
2. Protopine	1/4000 " "	8. Chelidonium . . .	1/150 " "
3. Cryptopine	1/2000 " "	9. Thebaine	1/150 " "
4. Sanguinarine . . .	1/700 " "	10. Narceine	1/80 " "
5. Heroine	1/400 " "	11. Codeine	1/40 " "
6. Papaverine	1/300 " "	12. Morphine	1/40 " "

The effect of the series upon the output of the heart is in general the same as it is upon the rate,—to increase both in weak solution and to depress them in stronger solution. In the case of cryptopine and sanguinarine there is no increase in the efficiency of the heart, although the rate is augmented by small amounts of these drugs. Thebaine does not seem to be stimulant in any dilution.

Because of therapeutic importance the relation in the toxicity of heroine, codeine, and morphine is interesting. Dresser's statement that heroine has no effect upon the heart, excepting a secondary one depending on the decrease in oxygenation of the blood, seems open to question, for when compared with codeine or morphine it is considerably more deleterious to the frog's heart.

THE ACTION OF THE ALKALOIDS OF THE PAPA- VERACEÆ UPON THE MOTOR NERVE ENDINGS.

BY WORTH HALE.

[From the Pharmacological Laboratory, University of Michigan.]

IT has been the observation of a number of investigators that the alkaloids of the papaveraceæ have an action upon some structure connected with the striated muscle tissues. Von Engel¹ in working with protopine seems to have been the first to notice that, after this drug had been given, stimulation of the sciatic nerve in the frog with a tetanizing current no longer produced the characteristic tetanus. Instead there appeared a series of wormlike movements, and myograms of these showed that in place of the long-continued contraction there was a series of rapid contractions and relaxations. He also observed a paralysis of the motor nerve endings, but ascribed the oscillatory muscle movements to a peculiar change in the muscle substance itself.

Meyer² found practically the same changes to occur in the muscles after poisoning frogs with chelidonine and sanguinarine.

Sollmann³ states that morphine and codeine have practically no action upon striated muscle tissues or nerve endings, but that the protopine group—protopine, cryptopine, and chelerythrine—causes a paralytic change in the motor nerve endings, so that stimulation with a tetanizing current produces a series of rapid contractions and relaxations.

To test the purity of the alkaloids used in my experiments melting-point determinations were made and in certain instances chemical tests were also carried out.⁴

In order to determine the nature of the peculiar contractions of

¹ VON ENGEL: *Archiv für experimentelle Pathologie und Pharmacologie*, 1890, xxvii, p. 419.

² MEYER: *Ibid.*, 1892, xxix, p. 397.

³ SOLLMAN: *Textbook of pharmacology*, 1906, p. 188.

⁴ For a detailed account of these tests see *This journal*, xxiii, pp. 389-407.

the muscles after these drugs had been given and also to determine how many of the series exhibited it, frogs were injected with varying amounts of the alkaloids of the group. At intervals a tetanizing current was applied to the lumbar spine and the character of the movement in the muscles thus stimulated was noted. Not only was it found possible to produce the characteristic oscillations with

protopine, cryptopine, chelidoniine, and sanguinarine, but the same changes occurred in the case of all the other members of the series, namely, papaverine, narcotine, narceine, chelerythrine, thebaine, morphine, codeine, and heroine. Immediately upon the appearance of this symptom

the frog was pithed and one of the gastrocnemii was removed as quickly as possible and attached to a muscle writing lever. Stimulation of the sciatic nerve of this preparation with a tetanizing current (the secondary coil of the Harvard inductorium set at 100) caused the appearance of the same symptoms as stimulation of the lumbar spine. Tracings of these peculiar contractions were taken, and examples of the myograms obtained are shown in Figs. 1 and 2. At a somewhat later stage in the poisoning these symptoms disappear, and it was noticed that the disappearance was always followed by a complete paralysis of the motor nerve endings, while the muscle would still contract upon direct stimulation without oscillatory movements.

From the above results it occurred to me that the peculiar series of contractions and relaxations was due to a beginning paralysis of the motor nerve connections, and that the same sort of contractions would be obtained if frogs were poisoned with any drug producing paralysis of these structures. To test this point frogs (*Rana pipiens*) were injected with varying doses of atropine, brucine, curara, gelseminine, and nicotine, and in all cases oscillatory movements appeared (Figs. 3 to 6). This phenomenon then seems not to be limited to any drug or group of drugs, as was thought at first, but to be a symptom of beginning paralysis of the nerve endings.



FIGURE 1. — One half the original size. Oscillations produced in the frog's gastrocnemius by morphine sulphate, tetanizing current.



FIGURE 2. — About one half the original size. Oscillations produced in the frog's gastrocnemius by thebaine, tetanizing current.

In spite of repeated trials it was found impossible to obtain the same degree of oscillation as a result of the poisoning with the various drugs of the group. However, it was observed that the oscillations became more pronounced as the muscle became fatigued (see Fig. 2).



FIGURE 3.—About one half the original size. Oscillations produced in the frog's gastrocnemius by atropine sulphate, tetanizing current.



FIGURE 4.—One half the original size. Oscillations produced in the frog's gastrocnemius by curara, tetanizing current.

The changes induced by fatigue of the motor nerve terminations. The difference in the degree of oscillation probably depends, then, upon a variability in the rapidity of the progress of the poisoning or upon a failure to secure a tracing at the optimum of the oncoming paralysis. Curara proved to be the most unsatisfactory of the drugs producing paralysis of the motor nerve endings in this respect, and, as will be noted, the tracing does not show very good oscillations. A large number of experiments were carried out using curara in various strengths, but no better results followed. It was noted, however, that this drug acted very quickly, so that the time before definite poisoning set in and complete paralysis was very short. Accordingly it might be suggested that the progress of the poisoning was too rapid in the case of this drug to show satisfactory oscillations.

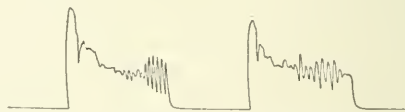


FIGURE 5.—One half the original size. Oscillations produced in the frog's gastrocnemius by brucine, tetanizing current.



FIGURE 6.—One third the original size. Oscillations produced in the frog's gastrocnemius by nicotine, tetanizing current.

For the sake of comparing the relative effects of the members

⁵ BOEHM: Archiv für experimentelle Pathologie und Pharmacologie, 1890, xxvii, p. 419.

of this series upon the motor nerve endings, 10 mgm. of each drug was dissolved in 2 c.c. of physiological salt solution and injected into a lymph sac of frogs weighing between 25 and 30 gm. The frogs were pithed (both brain and cord) to prevent fatigue with those of the series which produce a preliminary tetanus. After an interval of thirty-five minutes the lumbar spine was stimulated with a tetanizing current of constant strength, and the time required to produce complete relaxation from the combination of fatigue and paralysis was noted. The paralysis appeared so quickly after papaverine and thebaine with this dose that a shorter interval was used for them. The relative toxicity, as determined in this way, is shown in the following table, the least toxic requiring the longest stimulation to produce complete relaxation.

Drug.	Time for complete relaxation.	Drug.	Time for complete relaxation.
Narceine	90 sec.	Heroine	20 sec.
Morphine	50 "	Protopine	16 "
Chelidonium	45 "	Chelerythrine	8 "
Sanguinarine	33 "	Cryptopine	5 "
Codeine	30 "	Thebaine ⁶	3 "
Narcotine	23 "	Papaverine ⁶	0 "

Great difficulty was found in dissolving so much narceine in so little of the solvent, and accordingly it was injected partly in suspension, thus accounting, perhaps, for its relative inactivity.

In conclusion it will be noted that the whole series act as depressants of the motor nerve endings, and that the peculiar oscillations ascribed to certain of the series are dependent upon the oncoming paralysis and are present after poisoning with any of the group or after any drug producing paralysis of the motor nerve endings.

It should also be observed that morphine and codeine are clearly less toxic to the motor nerve endings than heroine, and the latter in turn is less toxic than a number of the other members of the series studied.

⁶ After fifteen minutes.

METABOLISM IN MAN WITH GREATLY DIMINISHED LUNG AREA.¹

By THORNE M. CARPENTER AND FRANCIS G. BENEDICT.

[From the Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.]

IN his recent discussion of animal factors of safety,² Dr. Meltzer has forcibly pointed out anew the liberal scale on which the animal body is constructed, particularly with reference to the functioning of the various organs. As a contribution to the interesting question to what degree does the loss of part of an organ influence the functioning of the organ, we propose to present in this paper the results of a metabolism experiment made with the respiration calorimeter at Wesleyan University upon a man who, as a result of a number of lung affections, had lost completely one lung.

While there are a number of experiments in which surgical removal of parts of the lung in animals has been made, experiments which throw light upon the metabolism of man with diminished lung area are few.

The earliest experiments with men having any particular scientific value are those made by Hannover³ in Scharling's apparatus. As a result of these experiments, which were made with a number of sick patients, some of them with phthisis, Hannover concludes that, on the basis of per kilo of body weight, there was very little variation in the amount of carbon dioxide excreted, although in phthisis it does have a tendency to sink somewhat below the normal.

¹ The experiment here reported was made in the respiration calorimeter formerly at Wesleyan University, Middletown, Conn. The preparation of the material for publication and the computation of the results were carried out subsequent to the removal of the investigations, of which this was a part, to the Nutrition Laboratory of the Carnegie Institution of Washington, located in Boston, Mass.

² S. J. MELTZER: The Harvey Lectures, 1906-1907, Philadelphia, 1908, p. 139.

³ HANNOVER: De quantitate relativa et absoluta acidi carbonici ab homine sano et aegroto exhalati. Hauniae, 1845. Cited by MÖLLER; see below.

Twenty years later, Möller,⁴ working with the Pettenkofer-Voit apparatus in Munich, made a number of experiments with patients with diseases involving the lungs, and, indeed, with one subject during empyema and again after complete recovery. As a result of these experiments, Möller concludes that there is no variation in the respiratory exchange accompanying a profound disturbance of respiration.

Working with dogs, Vaughan Harley⁵ has recently studied the influence of compression of one lung on the respiratory exchange. He concludes that when one pleural space is filled up so that the lung on one side is compressed and only the opposite lung remains active, this is usually followed by a marked increase in the rate of breathing, and there is at the same time more air breathed per minute by the active lung than was previously breathed by the two lungs together. This increase in the quantity of air breathed is accompanied by an increase in the oxygen absorbed and carbon dioxide eliminated by the animal, so that the respiratory quotient, as a rule, is not altered. He reports inability to give any satisfactory explanation of this increase in the respiratory exchange other than on the basis of Bohr's theory that carbon dioxide stimulates its own secretion, so that when one lung is compressed the other lung has to eliminate from the organism twice as much carbon dioxide as it originally did. Therefore the quantity of carbon dioxide in the alveoli at any given time would be doubled and thus act as a stimulant to the cells in the alveoli, resulting in an increased output of carbon dioxide.

More recently Hellin⁶ has made experiments with rabbits, extirpating one lung, usually the right. In the majority of instances the animal recovered, and the respiratory exchange was studied before and after the operation and indeed, in some instances, one year later. His results show that the total carbon dioxide excretion and oxygen consumption are not materially altered.

There is, thus, in experiments on animals more or less conflicting evidence, and of the experiments thus far made on man, the results obtained on those suffering from tuberculosis and other lung affections are somewhat vitiated by the fact that there was often a febrile

⁴ MÖLLER: *Zeitschrift für Biologie*, 1878, xiv, pp. 542-562.

⁵ HARLEY: *Journal of physiology*, 1899, xxv, p. 33.

⁶ DIONYS HELLIN: *Archiv für experimentelle Pathologie und Pharmakologie*, 1906, lv, p. 21.

temperature and that the exact obliterated area of the lung could not well be established. Knowing, as we do, that tuberculosis patients completely recover and live apparently normal lives with a large portion of the lung tissue destroyed, it would perhaps hardly be considered necessary to study the influence of this diminished lung area on metabolism. However, in the absence of such definite experiments on man, when our attention was called by Prof. Lafayette B. Mendel to the interesting case of H. L., we were glad to undertake a study of his metabolism. This subject had visited many hospitals and medical schools and had had many interesting observations made upon him, notably the cardiograms obtained by Dr. Erlanger.⁷

We are indebted to Dr. Horace Packard of Boston for the following description of his case:

"Mr. L., aged 36, presented at the Massachusetts Homœopathic Hospital. He had an opening, posteriorly, just above the line of the diaphragm on the left side, communicating with the interior of the thorax, from which a copious, offensive, purulent matter was exuding.

"He was emaciated and weak from septic absorption and inadequate aeration. The left lung was totally obliterated. Examination of his sputum showed freedom from tubercle bacilli.

"His previous history in brief was as follows:

"He had pneumo-hydro-thorax in January, 1900, and was repeatedly aspirated during the succeeding six months. For a year he had nothing further done, but in June, 1901, was tapped with a trocar and much pus was removed. In the same month a piece of rib was resected and permanent drainage established.

"In October, 1901, I made removal of portions of the fourth, fifth, sixth, and seventh ribs, disclosing an enormous cavity, extending from the diaphragm to the clavicle.

"The general condition of the patient was so bad that less extensive resection of the ribs was performed than would otherwise have been done. The soft parts still could not sufficiently collapse to effect obliteration. The general health, however, improved, and all through the spring and summer of 1902 the patient was out of doors and gained steadily. In October, 1902, operation was again performed and more extensive rib resection was effected, and this to the very confines of the cavity above, below, and laterally. Good recovery has been made, and although at the present writing (1903) there is still a small unobliterated area, the chances seem good for ultimate perfect repair."

⁷ JOSEPH ERLANGER: Cardiograms obtained from a case of operative defect in the chest wall. *Johns Hopkins Hospital Bulletin*, 1905, xvi.

EXPERIMENTAL DATA.

At the time of the arrival of H. L. in Middletown we were engaged in a study of the metabolism of normal men under conditions of muscular rest and after a twelve-hour fast. As both his time and ours was limited, we were able to make with H. L. but one experiment consisting of three consecutive two-hour periods. Before entering the respiration chamber he had had a lunch consisting of eggs and milk, the exact amount of which was unfortunately not known, as it was taken before he came to the laboratory. The naked body weight was 47.3 kilos, and the height 1.69 metres.

In the majority of experiments of this nature an attempt is made to obtain the rectal temperatures by means of an electrical resistance thermometer. The importance of accurate body temperature observations in experiments where a knowledge of heat production is sought can hardly be overestimated. These data are especially desirable in experiments of short duration, such as the one here described. It was, however, not thought practicable to complicate the experiment (the first experiment in which this subject had ever been inside the respiration chamber) by using this thermometer, so we had to be content with the body temperature obtained with a clinical thermometer in the mouth at the beginning and end of the experiment. Since the temperature of the air in the chamber was constant throughout the experiment, the buccal temperature was probably reasonably accurate.

During the experiment the subject remained inside the chamber, sitting quietly reading. He had been instructed beforehand to take his own pulse rate, and a number of such observations were recorded. These were obtained by counting the pulse in periods of two minutes from time to time. While, so far as we knew, he had had no previous experience in counting his pulse rate in this way, his experience in hospitals and medical schools had resulted in a very intelligent understanding of the details of similar experiments, and we believe that the pulse rates are reasonably accurate, although probably, as is often the case, somewhat higher than would be found if they had been taken by another person. It is certain that his previous experiences were such as to minimize any possible apprehension regarding the sojourn inside the respiration chamber, and hence we believe his experiment represents a reasonably normal study of metabolism so far as the psychical state is concerned.

During his sojourn in the chamber it was possible for us to determine the carbon dioxide elimination, the water vapor output, oxygen intake, and heat elimination. The measurements of temperature with the clinical thermometer indicated no change in the body temperature, and hence the heat production was practically equal to the heat elimination. This holds true obviously only for the whole six-hour period, as there may still have been fluctuations in the temperatures at the end of the first and second periods.

Unfortunately the respiration rate and blood pressure were not determined; likewise it was impossible for us to determine exactly the loss in body weight. The results of the experiment with H. L. are given in Table I herewith.

TABLE I.
RESULTS OF EXPERIMENT WITH H. L. (APRIL 4, 1905).

Period.	Water of respiration and perspiration.	Carbon dioxide exhaled.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Body temperature.	Pulse rate per minute.
	gm.	gm.	gm.		cal.	° C.	
11.14 A. M.—1.14 P. M.	70.9	48.5	40.4	.87	148.7	36.7	11.00 A. M. 70 12.10 P. M. 67
1.14 A. M.—3.14 P. M.	59.3	41.9	37.2	.82	134.6	..	1.26 P. M. 61 2.06 P. M. 58
3.14 A. M.—5.14 P. M.	57.4	44.2	37.6	.85	127.1	36.7	3.12 P. M. 58 4.38 P. M. 59 5.02 P. M. 58
Total, 6 hours.	187.6	134.6	115.2	.85	410.4		

The values obtained in three consecutive two-hour periods taken by themselves are interesting only as indicating the rate of the metabolism. It was impracticable to obtain the nitrogen excretion during this period, and hence the metabolism of protein cannot here be given. Furthermore, since we do not know the exact amount of food ingested prior to the experiment, these results could have had but little value. The carbon dioxide excretion, as well as the water of respiration and perspiration, oxygen consumption, and, indeed, the heat elimination all indicate a larger metabolism during the first two hours than during the subsequent periods. This is quite common with experiments of this type, since, no matter what has been the previous experience of the subjects with scientific experiments of varying kinds, there is in general a slight feeling of nov-

elty during the first two hours of an experiment, although the preliminary hour in many instances serves to secure fairly normal mental states. The last two periods show a reasonably uniform agreement.

Comparing the results obtained on H. L. with an ordinary individual, the results at first sight appear to indicate a very low metabolism. The heat production is also distinctly low, but with a subject of such small body weight and so poorly nourished, it is necessary for us to compare the results obtained with similar subjects with approximately the same body weight and state of nourishment. Unfortunately we have no experiments that lend themselves to an exact comparison, as *a priori* it is very difficult to compare one physical organism with another. We have selected from a number of unpublished experiments three that lend themselves to a comparison in so far as the body weight is in all cases not far from that of H. L., although the comparison, as pointed out above, is not without its limits.

A summary of the results obtained in these experiments and in that of H. L. is given in Table II.

The experiment with E. H. R., who was a vegetarian with a body weight but little over that of H. L., is of interest as the metabolism was determined twelve hours after the last meal. Another subject with small body weight but a very different general build was H. G., a college athlete. This experiment was made after a light breakfast which consisted of one shredded wheat biscuit, a glass of milk, and a little other carbohydrate food. The experiment with T. M. C. was also made two hours after a very light breakfast, consisting of coffee and a roll.

Computing the results on the basis of per kilo of body weight, which is the most satisfactory method of comparing experiments with different subjects, we find that with H. L. the carbonic acid excretion, oxygen consumption, and heat elimination were not noticeably different from those of the other subjects. According to Rubner, we would expect a comparison of heat production per square metre of body surface⁸ to be perhaps the best method of comparison. Comparing these results as given in the last column of the table, we find that H. L. produced as much heat per square

⁸ Computed according to the formula of Meeh, $S = 12.312 \sqrt[3]{G^2}$. Where S = surface and G = the body weight in kilograms.

metre of body surface as did the other subjects, with the exception of the athlete H. G., whose heat production was somewhat higher.

Before drawing final deductions from these figures we must take into consideration the fact that H. L. had immediately before en-

TABLE II.

COMPARISON OF METABOLISM OF H. L. WITH OTHER SUBJECTS OF APPROXIMATELY THE SAME BODY WEIGHT. (QUANTITIES PER HOUR.)

Subject.	Period.	Naked body weight.	Carbon dioxide.		Oxygen.		Heat.		
			Total.	Per kilo- gram.	Total.	Per kilo- gram.	Total.	Per kilo- gram.	Per square metre of body surface.
			gm.	gm.	gm.	gm.	cal.	cal.	cal.
H. L. One lung wholly oblit- erated. Eggs and milk one hour before.	1st	47.3	24.3	.51	20.2	.43	74.4	1.57	46.2
	2d	..	20.9	.44	18.6	.39	67.3	1.42	41.8
	3d	..	22.1	.47	18.8	.40	63.6	1.34	39.5
E. H. R. (vege- tarian). Last meal twelve hours before.	1st	50.4	23.2	.46	15.8	.31	75.6	1.50	45.0
	2d	..	24.6	.49	21.5	.43	69.1	1.37	41.1
	3d	..	23.8	.47	17.4	.35	67.3	1.34	40.1
H. G. (athlete). Light break- fast two hours before.	1st	49.2	30.1	.61	77.6	1.58	46.9
	2d	..	25.7	.52	19.9	.40	77.4	1.57	46.8
	4th	..	25.0	.50	23.9	.47	80.9	1.61	48.2
T. M. C. Very light break- fast two hours before.	1st	50.2	25.3	.50	17.3	.34	76.1	1.52	45.4
	2d	..	23.6	.47	20.6	.41	65.7	1.31	39.2

tering the chamber eaten considerable proteid food, which always results in a noticeable increase in the metabolism. E. H. R. had had no food for twelve hours, H. G. had had but a slight breakfast with but a small amount of protein, while T. M. C. had had practically no protein in his breakfast. Consequently, other things being equal, we would expect that the effect of the ingested food eaten by H. L. would be to increase his heat production over and above that of the other subjects made under similar conditions. While, therefore, we find that actually under the conditions of the experiments as here outlined there was no noticeable decrease in the metabolism, yet,

owing to the prior ingestion of considerable proteid food, one would expect there would be a somewhat greater metabolism. An increased metabolism might furthermore be looked for, since, owing to the emaciated condition of this subject, the proportion of active protoplasmic tissue compared with the body weight must have been relatively large. Taking the results as they stand, however, the only deduction that can be drawn is that the reduction of the area for oxygen absorption and carbonic acid elimination in the lungs by about one half has not materially altered the total metabolism.

A COMPARATIVE STUDY OF THE DIGESTIBILITY OF DIFFERENT PROTEINS IN PEPSIN-ACID SOLUTIONS.¹

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¹ A preliminary report of this investigation was published in the Proceedings of the American Society of Biological Chemists, 1908, i, p. 139; also the Journal of biological chemistry, 1908, iv, p. xlv.

This research is a continuation of the work by BERG and GIES that was recently described in the Journal of biological chemistry (1907, ii, p. 489). See also another recent paper from this laboratory for related observations that were made as an outcome of the earlier study (FOSTER and LAMBERT: Journal of experimental medicine, 1908, x, p. 830).

I. INTRODUCTION.

IN a research described in a previous paper² from this laboratory, experiments were made for the purpose of finding direct or obvious relations between the speed of peptolysis and the concentration of acid molecules, acid anions, or hydrogen kations in the solutions used. For this purpose the speed of digestion of fibrin, elästín, and edestín was determined in solutions of various acids and of various concentrations. The following quotations from the above-mentioned paper (pp. 544-546) briefly summarize the results obtained in that research, upon which the plan of the present work is based:

“Peptolysis of either fibrin, edestín, or elastin is quantitatively unequal in a series of aqueous solutions of different acids under any uniform digestive conditions. Striking disparities in the velocity, quality, and extent of digestion of these proteins occur in solutions of common acids, whether the acids are present in the solutions in equal *masses* (equipercentage), or in equal *numbers* of acid *molecules* (equimolecular), hydrogen *atoms* (equinormal), or hydrogen *ions* (equidissociated).”

“The hydrogen ion is the favorable acid factor in peptolysis. The associated anions or molecules (or both) appear to interfere (as a rule) with the peptic process, and their divergent influences seem to account, in part at least, for the quantitative disparities noted in each digestive series of these experiments.”

“In a given series of equivalent acid or basic solutions under uniform digestive conditions the degree and sequence of zymolysis of fibrin were strikingly different from those of the digestion of elastin. This fact necessitates thorough study of the zymolysis of many proteins in samples of the same equivalent acid and basic solutions before general deductions relating to peptolysis, or tryptolysis, or to influences on them, are permissible. Such a study has been inaugurated.”

The reason for this emphasis on the probable importance of the nature of the protein as a factor which influences the rate of its digestion is to be found in the rather frequent practice of earlier investigators of generalizing about the digestion of proteins from

² BERG and GIES: Journal of biological chemistry, 1907, ii, pp. 489-546.

the results obtained in experiments with very few (only too frequently with but one) of these substances. That conclusions so arrived at may be erroneous, either wholly or in part, is evident from the results of the previous experiments (of which the present work is a continuation) or from the results of the experiments herein described.

II. HISTORICAL.

The results obtained by previous investigators cannot be satisfactorily compared among themselves because of the general lack of uniformity in the digestive methods they employed. Of the number of investigations that have been carried out in this field very few have been conducted under exactly similar conditions. Large amounts of pepsin or of the various protein products in the form of dry, finely divided powders of uniform composition, probably were not prepared by the earlier investigators for their digestion experiments. Instead, an extract of the gastric mucosa of the pig or some other animal and crude protein materials were generally used. In many cases the amount of peptic extract prepared at one time was sufficient for only a very few experiments, so that while the same method of preparation was employed, it is highly probable that the amounts of pepsin varied indeterminately. That different acids could be used in the preparation of artificial gastric juice was probably first discovered by Eberle³ in 1834. The activation of pepsinogen by the various acids seems to have made no appreciable difference in the results, although the possibility of this difference probably was not recognized before Ebstein and Grützner's⁴ announcement that a proteolytically inactive glycerin extract of the pyloric lining could be made active by the addition of hydrochloric acid.

With few exceptions, fibrin and egg albumin were the only proteins used. The proportion of weight of protein to volume of digestive solution was not the same in every investigation. The methods generally used were the weighing of the undigested protein residues, or the noting of the time required for complete solution of a known amount of protein.

³ EBERLE: *Physiologie der Verdauung*, Würzburg, 1834, p. 80.

⁴ EBSTEIN and GRÜTZNER: *Archiv für die gesammte Physiologie*, 1874, viii, p. 132.

But in spite of all these variations in method, kind, and quantity of digestive materials, and objects of the various investigations, the general results are singularly concordant. Valentin,⁵ Lehmann,⁶ Donders,⁷ Meissner,⁸ Davidson and Dieterich,⁹ and Wolffhügel¹⁰ studied the digestion of fibrin and egg albumin in solutions of various acids. The results reported by them are practically the same as those obtained with the same proteins by subsequent investigators. In general, digestion was most rapid in the mineral acids, excepting sulfuric, in which peptolysis was extremely slow. In the organic acids, with the exception of lactic acid, digestion was slow; acetic acid was found to be inert. Hydrochloric and lactic acid solutions were commonly considered the best digestive media. Digestive sequences are tabulated on page 457.

The results were naturally interpreted or explained in the light of the then incomplete knowledge of the nature of the digestive process. The slow solution of a protein in hydrochloric acid, in the absence of pepsin, with the formation of peptone, was observed by several investigators, among whom may be mentioned Mulder, Von Wittich, and Wolffhügel.¹¹ According to Mulder,¹² the acid, acting on the protein molecules, caused some of them to rearrange themselves with the formation of ferment molecules. A rather early insight into the nature of peptolysis was given by Von Wittich,¹³ who concluded that the pepsin simply accelerates a reaction which the acid alone will bring about more slowly. According to Davidson and Dieterich,¹⁴ the acid caused the protein to swell, after which the ferment acted. The digestibility of a protein in an acid solution was dependent upon the ability of the acid to cause

⁵ VALENTIN: Lehrbuch der Physiologie, 2d ed., Braunschweig, 1844-1847, i, p. 320.

⁶ LEHMANN: Lehrbuch der Physiologische Chemie, Leipzig, 1850-1852, ii, p. 54.

⁷ DONDERS: Physiologie, 1st ed., Leipzig, 1856, p. 220.

⁸ MEISSNER: Zeitschrift für rationelle Medizin, 3d series, 1858, vii, p. 16.

⁹ DAVIDSON and DIETERICH: Archiv für Anatomie und Physiologie, 1860, pp. 688-703.

¹⁰ WOLFFHÜGEL: Archiv für die gesammte Physiologie, 1873, vii, p. 190.

¹¹ WOLFFHÜGEL: *Loc. cit.*

¹² MULDER: SCHMIDT'S Jahrbücher der in- und ausländischen gesammten Medizin, 1858, ci, p. 153.

¹³ VON WITTICH: Archiv für die gesammte Physiologie, 1872, v, p. 469.

¹⁴ DAVIDSON and DIETERICH: *Loc. cit.*, p. 702.

the protein to swell. The later experiments of Pfeleiderer and Wroblewski, however (pp. 431, 432), question the correctness of this theory.

Fiechter¹⁵ studied the effect of hydrocyanic acid on the digestibility of fibrin and of coagulated egg albumin in a pepsin-0.2 per cent hydrochloric acid solution. In pepsin-hydrochloric acid solutions containing 0.1 per cent to 0.25 per cent hydrocyanic acid, the speed of digestion of fibrin, as determined by suitable controls, was not diminished (p. 9). Quite different results were obtained with egg albumin. Distinct inhibition was observed in peptic solutions containing 0.04 per cent hydrocyanic acid (p. 12). In such a solution only one half as much egg albumin dissolved as in the control. In solutions containing 0.1 per cent hydrocyanic acid, only one fifth as much was dissolved. The concentration of hydrocyanic acid was varied from 0.04 per cent to 0.3 per cent, but in no case was the inhibition complete. In so far as Fiechter's conclusions are based on weighings of undigested residues obtained from 1 to 2 gm. of coagulated egg albumin, the digestive differences between the two proteins are properly emphasized by him. In its inhibitory action hydrocyanic acid is not exceptional; under similar conditions sulfuric acid¹⁶ will do the same.

Because of the extensive use of potassium bromid and iodid in medicine, Putzeys¹⁷ studied the digestibility of fibrin in pepsin-hydrochloric acid to which either of these salts or the corresponding haloid acid had been added. Because Putzeys used finely divided fibrin dried at 110° C., he was criticised by a commission¹⁸ appointed to examine his work, on the ground that while fibrin is not altered chemically by being heated to 110° C., it is altered physiologically. Putzeys' conclusions, however, were accepted by the commission. From the results obtained, Putzeys concluded that

¹⁵ FIECHTER: Ueber den Einfluss der Blausäure auf Ferment-Vorgänge, Dissertation, Basel, 1875.

¹⁶ PFLEIDERER: Archiv für die gesammte Physiologie, 1897, lvi, p. 623; also Dissertation, Tübingen, 1897; BERG and GIES, Journal of biological chemistry, 1907, ii, p. 529.

¹⁷ PUTZEYS: Bulletin de l'Académie royale de médecine de Belgique, 3d series, 1877, xi, p. 213.

¹⁸ Rapport de la commission chargée d'examiner le travail de M. le docteur PUTZEYS, Bulletin de l'Académie royale de médecine de Belgique, 3d series, 1877, xi, p. 104.

in equivalent amounts hydrochloric acid may be replaced by hydrobromic or hydriodic acids, although these latter acids are not peptolytically as efficient as hydrochloric.

Petit¹⁹ was among the earliest investigators who used dried pepsin preparations and thereby secured a uniform product which could be worked with for a long time and through a long series of experiments. While his digestion experiments were qualitative rather than quantitative, they were sufficiently accurate to show the differences between the comparatively strong organic acids, such as lactic, tartaric, citric, malic, oxalic, and formic, in which proteolysis did take place with varying speeds, and the weak organic acids, such as acetic, butyric, valeric, and succinic, which were proteolytically inactive. A grouping such as this, if it were the result of rigorously controlled quantitative work, would be very significant when the dissociation of an acid is considered as a possible factor determining its proteolytic efficiency. Petit made the following observation, since confirmed by other investigators:²⁰ Fibrin will not readily digest in a solution of pepsin-acetic acid. If to such a mixture hydrochloric acid be added, solution of the fibrin quickly takes place. From this experiment Petit concluded that acetic acid is proteolytically inert, but offered no theoretical explanation of the observed facts.

Mayer²¹ used short lengths of filaments of dried coagulated egg albumin which weighed about 8 mgm. each. These were placed in from 2 to 2½ c.c. of solutions of various acids, equivalent (equinormal) to 0.2 per cent hydrochloric acid, to which known weights, from 12 to 20 mgm., of dry pepsin were added, and the time required for the complete solution of the small fragment of egg albumin noted. The order of digestibility was the following: hydrochloric, nitric, oxalic, sulfuric, the last acid being particularly poor. Then follow lactic and tartaric acids, which in twenty-four hours digested about one half of the protein indicator; formic, succinic, and acetic acids, which showed a very slight though perceptible peptolysis; and butyric and salicylic acids, which were without action.

Mayer states very definitely (p. 360) that in general the digestibility in the various solutions varied with the strength of the acid, the mineral acids being most favorable. Then follow the organic

¹⁹ PETIT: *Journal de thérapeutique*, 1880, pp. 136, 173, 201, 288, 453, 488.

²⁰ PFLEIDERER: *Loc. cit.*, p. 623; BERG and GIES: *Loc. cit.*, p. 529.

²¹ MAYER: *Zeitschrift für Biologie*, 1881, xvii, p. 351.

acids in the order of their strength, the strong organic acids being more efficient peptolytically than the weak fatty acids.

Certain results obtained by Georges²² are not in accord with those obtained by other investigators. In his digestion experiments Georges used small cubes of coagulated egg albumin, 5 to 6 mm. to the edge. These were allowed to remain for twenty-four hours in solutions of pepsin-hydrochloric, lactic, acetic, or tartaric acids of various concentrations. The qualitative results were probably obtained by inspection. Some of the experiments were repeated with confirmatory results, using raw or boiled meat in place of egg albumin. He found that hydrochloric was the only acid in which egg albumin would digest, all the other acids in the various concentrations used were without any action whatsoever. More remarkable are the following conclusions quoted from Georges' paper (p. 45): "1. Lactic acid combined with pepsin has no peptonizing action, in any quantity or in any way that it may be used, although the egg white is slightly swelled. 2. A mixture of lactic and hydrochloric acids containing pepsin has a peptonizing power proportional to the amount of hydrochloric acid present in the mixture; this power, however, is less than the same amount of hydrochloric acid would have alone. In other words, lactic acid inhibits the digestive action of pepsin-hydrochloric acid."

Thoyer²³ doubted the correctness of Georges' conclusions and repeated a large part of Georges' work under similar conditions. Thoyer concluded (p. 9) that other acids may replace hydrochloric acid in peptolysis, but are less efficient. Hydrofluoric acid strongly inhibits digestion (see Hübner, p. 430).

An interesting phase of the subject on the condition of an acid as related to its peptolytic efficiency was touched upon by Kumagawa and Salkowski.²⁴ Their object was to determine whether the hydrochlorids of alanin, leucin, and glycocoll (typifying "combined acids") could cooperate with pepsin in the digestive process. Digestive solutions were prepared by adding the calculated amounts of the amino acids to pepsin-0.28 per cent hydrochloric acid, in

²² GEORGES: Étude chimique du contenu stomacal et de ses rapports avec le diagnostic et le traitement des maladies de l'estomac. Dissertation, Paris, 1890.

²³ THOYER: Comptes rendus, Société de Biologie, 1891, iii, 9th series (or xliii), p. 1.

²⁴ KUMAGAWA and SALKOWSKI: VIRCHOW'S Archiv für pathologische Anatomie, etc., 1890, cxxii, p. 235.

the ratio of $1\frac{1}{4}$ mols of amino acid to 1 mol of hydrochloric acid. This was done (p. 238) to make certain that all of the hydrochloric acid would be neutralized. In these mixtures, and in suitable controls containing pepsin-hydrochloric acid, fibrin digested with equal speed. From the results obtained, the following conclusion was drawn (p. 250): Pepsin-hydrochloric acid which contains an amino acid in so large an amount that the solution must be regarded as a solution of the hydrochlorid of the amino acid, contains chemically free hydrochloric acid which is physiologically active.

That such a solution contains free hydrochloric acid and unneutralized amino acid is almost a certainty. The amino acids used are such extremely weak bases,²⁵ that in all probability only a portion of the hydrochloric acid is neutralized. The digestion is due, not to combined, but to free hydrochloric acid. Anilin is 100 times as strong a base as either alanin or glycocoll, yet in a $n/32$ solution of anilin hydrochlorid 2.6 per cent of the salt is dissociated hydrolytically.²⁶

Brod²⁷ studied the swelling of fibrin in solutions of hydrochloric, hydrobromic, and hydriodic acids. The amount of liquid absorbed by the swollen fibrin was determined as follows: Portions consisting of 5 gm. of freshly washed fibrin which had not been treated with alcohol or ether were placed in beakers containing 100 c.c. of solutions of the above-named acids, in concentrations varying from 0.03 per cent to 0.2 per cent hydrochloric acid or the equivalent in one of the other two. After maintaining the mixtures at 40° C. for two hours, they were filtered through glass wool and the volume of the filtrate measured. The volume absorbed was obtained by difference.

Brod found that the swelling of fibrin was greater in dilute acid solutions than in pure water. The maximal absorption took place in solutions equivalent to 0.08 per cent and 0.09 per cent hydrochloric acid. The (average) volumes, calculated for 1 gm. of pure dry fibrin, are 40 c.c. absorbed from 100 c.c. of hydrochloric acid; 30 c.c. from a like volume of hydrobromic; and 20 c.c. from hydriodic acid (p. 13). The possibility that the amount of dis-

²⁵ ABEGG: Die Theorie der elektrolytischen Dissociation, Stuttgart, 1903, p. 37.

²⁶ ABEGG: *Loc. cit.*, p. 61.

²⁷ BROD: Beiträge zur Lehre von der Eiweissverdauung. Dissertation, Würzburg, 1892.

solved protein might be a factor which partly determines the optimal absorption concentration is not mentioned, although Brod noticed (p. 16) a slight precipitate when neutralizing some of the filtrates. By titrating the filtrates with standard alkali, the amount of acid contained in the absorbed liquids was obtained by difference. The liquid absorbed by 1 gm. of dry fibrin contained an amount of acid proportional to the concentration of the acid, and varied from 13 mgm. in 0.03 per cent to 67 mgm. in 0.2 per cent hydrochloric acid. Equivalent amounts were absorbed from the other acid solutions. One gram of dry fibrin (p. 12) absorbed an average of 23 c.c. of liquid when immersed in 100 c.c. of 0.2 per cent hydrochloric acid. This volume originally contained about 0.5 gm. of acid, and since only 67 mgm. of acid were contained in the absorbed liquid, it follows that the swelling fibrin did not absorb the acid solution; it absorbed water with about 13 per cent of the original amount of acid in it.²⁸ By pressing the swollen fibrin and titrating the expressed fluid, Brod found (p. 44) that about one third of the absorbed acid was combined with the fibrin, the rest being dissolved in the absorbed water.

Had this work been repeated with the addition of small amounts of pepsin to the acid solutions, results would undoubtedly have been obtained which would throw much light on the nature of the digestive process. A record of such a continuation has not yet been seen by the writer.

In acid solutions equimolar to 0.2 per cent hydrochloric acid, the order of digestion of ground cotton-seed meal was found by Stutzer²⁹ to be the following: hydrochloric, 77; lactic, 43; tartaric, 42; formic, 41; malic, 39; citric, 37; acetic, 15; propionic, 9; butyric, 9. The figures are the numbers of milligrams of soluble nitrogen in equal volumes of digestion filtrates. Practically the same order was obtained with similar acid solutions equimolar to 0.05 per cent and to 0.1 per cent hydrochloric acid. Stutzer calls attention to the low value of acetic acid and the unexpectedly high value of formic acid. The relatively high dissociation of formic acid as compared with that of the other fatty acids of the same series is a probable reason for its correspondingly high peptolytic

²⁸ The osmotic work done by the swelling fibrin could be calculated from such data, perhaps with suggestive results.

²⁹ STUTZER: Die Landwirtschaftlichen Versuchs-Stationen, 1891, xxxviii, p. 257.

efficiency.³⁰ Similar observations on formic acid were made by Petit³¹ and Mayer.³²

Hoffmann³³ believed that the peptolytic efficiency of an acid depended solely upon the concentration of hydrogen ions, and therefore the speed of digestion in a series of equimolar acid solutions should vary with the dissociability of the acids. The digestibility of coagulated egg albumin in *m*/10 solutions of various acids was determined. From the results the following comparative peptolytic values of the acids were obtained (assigning the value of 1000 to hydrochloric acid and 0 to acetic acid): hydrochloric, 1000; phosphoric, 670; arsenic, 550; sulfuric, 250; citric, 150; lactic, 90; acetic, 0. According to Hoffmann (p. 271), the order of digestion and the order of electrical conductivity of the solutions used were the same. Sulfuric acid was an exception, as usual.

Larin³⁴ observed that while there was no exact parallelism between peptolytic efficiency and electrical conductivity, the general relationship did exist. The digestive results were obtained with egg albumin and *n*/10 acid solutions.

Hahn³⁵ determined the total nitrogen in neutralized and filtered digestive filtrates, which contained principally proteoses and peptones, as a measure of proteolysis in various acid solutions equinormal to 0.28 per cent hydrochloric acid. The results are unusual in so far as sulfuric acid is almost as efficient as hydrochloric or nitric, when measured by this method. Similarly, some of the other acids used, such as acetic and citric, have an unexpectedly high peptolytic value. These results were possibly due to the use of comparatively large amounts of pepsin³⁶ (100 mgm. of a very

³⁰ According to ABEGG (*Loc. cit.*, p. 32) formic acid is seventy times as strong as acetic acid. According to KOHLRAUSCH and HOLBORN (*Loc. cit.*, p. 184) formic acid is twelve times as strong as acetic acid, in solutions of equimolar (or equivalent) concentration.

³¹ PETIT: *Loc. cit.*, see p. 425.

³² MAYER: *Loc. cit.*, see p. 425.

³³ HOFFMANN: SCHMIDT'S Jahrbücher der in- und ausländischen gesammten Medizin, 1892, ccxxxiii, p. 268.

³⁴ LARIN: (abstract) MALY'S Jahresbericht über die Fortschritte der Thierchemie, 1903, xxxiii, p. 543. The original paper in Arbeiten der medicinischen chemischen Laboratorium der kaiserlichen Universität zu Tomsk, not obtainable.

³⁵ HAHN: VIRCHOW'S Archiv für pathologische Anatomie, etc., 1894, cxxxvii, p. 597.

³⁶ For similarly high peptolytic values of sulfuric and acetic acids containing large

active preparation in 100 c.c. of acid solution) for the proteins used, *i. e.*, coagulated egg albumin, a neutral solution of egg albumin, moist fibrin, and air-dried fibrin. Hahn comments upon the probable dependence of digestive results upon the nature and physical condition of the protein. As the practical result of the work, he concluded (p. 604) that the hydrochloric acid in the stomach is best replaced by phosphoric acid.

Hübner³⁷ studied the digestion of dried fibrin in solutions of the haloid acids for the same reason that Putzeys (see p. 424) did, *i. e.*, because the salts of these acids were so extensively used in medicine, and because, according to these investigators, these salts are decomposed in the stomach with the liberation of the free acid. Experimental data on this point are lacking in the paper by Hübner. Putzeys³⁸ records that in the digestive mixtures of pepsin-hydrochloric acid (100 c.c.) to which potassium iodid (100–200 mgm.) had been added, iodine was liberated; sometimes in amounts large enough to coat the particles of fibrin and thus mechanically retard the digestion. The liberation of iodine is a good indication of the previous liberation of the unstable hydriodic acid. Similar observations on bromine or hydrobromic acid liberation, although not recorded by Putzeys, are implied. That the acid in the stomach is not sufficiently strong to liberate any bromine from barium bromid was shown by Welker,³⁹ in a research published from this laboratory.

Hübner used the gastric mucosa of a pig's stomach. This, after careful washing and fine subdivision, was weighed into 6 equal portions, to each of which was added 200 c.c. of the haloid acid solutions in concentrations varying from 0.1 per cent to 0.8 per cent. To 100 c.c. of this mixture 2 gm. of dry blood fibrin were added. The amount of peptone nitrogen in the digestive filtrates (after the digestive period) was determined as the measure of proteolysis. By this method hydrofluoric acid was found to be as efficient, peptolytically, as hydrochloric, then follow hydrobromic and hydriodic acids. Thoyer (see p. 426) found that hydrofluoric acid strongly inhibited the action of pepsin. In Hübner's work, as in

amounts of pepsin, see BERG and GIES: *Journal of biological chemistry*, 1907, ii, pp. 516, 535.

³⁷ HÜBNER: *Fortschritte der Medizin*, 1894, xii, p. 163.

³⁸ PUTZEYS: *Loc. cit.*, p. 219.

³⁹ BERG and WELKER: *Journal of biological chemistry*, 1906, i, p. 381.

the work of several others, the pepsinogen in the gastric mucosa (or glycerin extract thereof) was activated by different acids, with results which apparently did not differ from those which would have been obtained had pepsin been used instead. In so far as several different stomachs were used in the work, a quantitative comparison between the several series of experiments is difficult. Putzeys' work is not mentioned in Hübner's paper.

Wroblewski⁴⁰ used water-swelled carmin-stained fibrin in his digestion experiments which were made according to the method of Grützner.⁴¹ To test tubes of equal dimensions containing 10 c.c. of $n/20$ acid solutions, about 1 c.c. portions of the swelled fibrin were added. To each of these tubes 1 c.c. of a glycerin extract of the gastric mucosa (obtained from various animals for different experiments) was added. The first appearance of a rose color in the solutions was the indication of the beginning of the digestive process. The time required for the complete solution of the fibrin was noted and used as the means of comparison. This method has the advantage of showing the order of digestion throughout the entire digestive period. The order of digestion in the solutions was practically the same as that obtained by other investigators (see p. 457). The fibrin had not swollen to its maximum in water. Wroblewski observed its swelling in the various acid solutions. From his observations he concluded that the digestibility of fibrin in an acid was not dependent upon the ability of that acid to swell the fibrin. The swelling in lactic and paralactic acid (p. 10) was unusually great, in tartaric acid there was almost none. The fact that swollen fibrin digested more slowly in sulfuric than in any other acid solutions used shows that the inability of this acid to swell fibrin probably is not the cause of the slow digestion. The fact that elastin⁴² will digest with equal speed in hydrochloric or sulfuric acid without swelling in either is another indication.

Special experiments were made to ascertain whether fibrin and casein would digest more rapidly in $n/10$ oxalic than in $n/10$ hydrochloric acid. In oxalic acid digestion was more rapid (pp. 11, 12). Wroblewski concluded (p. 18) that the order of digestion was not in the order of the strength of the acids and that oxalic acid is peptolytically more efficient than hydrochloric acid.

⁴⁰ WROBLEWSKI: *Zeitschrift für physiologische Chemie*, 1895, xxi, p. 1.

⁴¹ GRÜTZNER: *Archiv für die gesammte Physiologie*, 1874, viii, p. 452.

⁴² BERG and GIES: *Journal of biological chemistry*, 1907, ii, 521.

Klug⁴³ experimented with alkali albuminate, casein, serum albumin, syntonin, serum globulin, fibrin, and legumin. These proteins were digested in solutions of the following acids: hydrochloric, lactic, phosphoric, nitric, acetic, sulfuric, and citric. Pepsin preparations obtained from various animals were used, but no material differences in their activities were observed. The order in which the proteins are mentioned above is the order of their digestibility, the first named being the most rapidly digested. The acids are mentioned in the order of their proteolytic efficiencies, hydrochloric acid being the most efficient. The amounts of the various digestive products were determined "spectrophotometrically." Klug found (p. 341) that 8 per cent lactic acid was peptolytically as efficient as 0.6 per cent hydrochloric acid. This observation is remarkably in accord with that of Meissner,⁴⁴ made almost forty years before: that to replace hydrochloric acid in the preparation of artificial gastric juice by lactic acid, ten times as much of the latter acid had to be used.⁴⁵

Pfleiderer⁴⁶ studied the swelling and the digestion of fibrin. To test tubes containing 0.5 gm. moist fibrin, 15 c.c. of the acid solutions were added and the increments in the volumes of fibrin compared at suitable intervals of time. The figures given indicate that the volume of the fibrin at the end of one or two hours is three or four times the original volume, etc. At the end of twenty-four hours the order of swelling in $n/300$ acid solutions was the following: hydrochloric, nitric, phosphoric, lactic, acetic. In $2n/15$ acid solutions the order was the following: phosphoric, lactic, acetic, hydrochloric, nitric. It is probable that the sustained maximal swelling observed in many cases was due to the more rapid solution of the fibrin in the stronger acids, *i. e.*, hydrochloric and nitric.

Pfleiderer used Grützner's method in his digestion experiments. In $n/20$ acid solutions fibrin was digested most rapidly in hydrochloric acid; then follow phosphoric, lactic, nitric, acetic, and sulfuric. The very slow digestion of swollen fibrin in sulfuric acid led Pfleiderer to study the digestion of the same protein in hydro-

⁴³ KLUG: Archiv für die gesammte Physiologie, 1897, lxy, p. 330.

⁴⁴ MEISSNER: Zeitschrift für rationelle Medizin, 3d series, 1858, vii, p. 16.

⁴⁵ The concentration (gram-atoms per 1000 litres) of hydrogen ions in 0.6 per cent hydrochloric acid is 152, in 8 per cent lactic acid, 11.

⁴⁶ PFLEIDERER: Archiv für die gesammte Physiologie, 1897, lxvi, p. 605; also Dissertation, Tübingen, 1897.

chloric acid containing sulfuric acid or a sulfate. He found that both the sulfate and the sulfuric acid inhibited the action of pepsin-hydrochloric acid.⁴⁷ That the inhibiting action of the sulfuric acid (or its salts) is not due to its inability to swell the protein has already been referred to in the work of Wroblewski (p. 431). Pfeleiderer observed that very small amounts of sulfates (p. 625, footnote) will retard the swelling of fibrin much more than equimolar solutions of other salts. The remarkable inhibiting action of $n/10,000$ sulfate solutions on the digestion of fibrin was observed. This led to the conclusion that sulfuric acid and sulfates exert a specifically harmful effect (or toxicity) on pepsin.⁴⁸ But as the observations were made on but one kind of protein, the inference need not be drawn that similar results would be obtained with every protein.

Fischer and Moore⁴⁹ studied the swelling of fibrin by the method used by Pfeleiderer. The height of the fibrin column in the test tubes was measured in millimetres. While this is an improvement upon the more approximate measurements of Pfeleiderer, the volume of liquid absorbed is not shown by the method (see Brod, p. 427). Fischer and Moore found that the amount of swelling is determined by the concentration of the acid, and varies directly with the concentration. The addition of any salt to a pure acid solution decreases the amount of water absorbed in proportion to the amount added. Non-electrolytes, up to one half mol per litre, do not inhibit the swelling of fibrin either in acid solutions or in water. Fischer and Moore did not find any relation between the dissociability of an acid and its power to swell fibrin. This may be due to the fact that frequently the heights of the fibrin columns in three or four acid solutions differed by but 3 or 4 mm., which made the order of swelling in some instances dependent upon differences of only 1 mm. If to the difficulty of making such measurements there is added, as a complicating factor, the different solubilities of fibrin in the different acid solutions, then the relation (quite perceptible in Pfeleiderer's work) is wellnigh obscured, espe-

⁴⁷ For some quantitative measurements of this inhibition, see BERG and GIES: *Journal of biological chemistry*, 1907, ii, p. 532.

⁴⁸ In this connection it seems remarkable that certain molluscs should have sulfuric instead of hydrochloric acid in the gastric juice.

⁴⁹ FISCHER and MOORE: *This journal*, 1907, xx, p. 330; also FISCHER: *Archiv für die gesammte Physiologie*, 1908, cxv, p. 99.

cially since sulfuric acid is an exception and is to be found near the last in the order of digestive and swelling powers. The following order of swelling in $n/300$ acid solutions observed at the end of twenty-four hours by Pfeleiderer is undoubtedly the order of their dissociability: hydrochloric, nitric, phosphoric, lactic, acetic. If the greater solubility of fibrin in the stronger acids be borne in mind, the reason for the following change in the order of swelling, when the strength of the acid solutions is increased to $2n/15$, is clear: phosphoric, lactic, acetic, hydrochloric, nitric.

Fischer and Moore concluded that "the general arrangement of the acids according to the way they favor proteolysis under the influence of pepsin is in large measure identical with the arrangement of the same acids according to their power of making fibrin swell." "Those salts which were found to be most effective in diminishing the amount of swelling in hydrochloric acid are those which most powerfully retard the digestion of this substance in pepsin-hydrochloric acid mixtures."

Jastrowitz⁵⁰ studied digestion in solutions of hydrochloric acid to which various amino acids had been added. He concluded that the amino acids inhibited peptolysis by combining with some of the free acid.

The work by Berg and Gies and some of its results have already been referred to on p. 421.

The historical data may be summarized as follows:

1. In many of the investigations, the immediate practical application of the digestive results to medicine was the principal item of interest. In comparatively few of the investigations was the work carried out along lines planned to elucidate the nature of the digestive process, the rôle of the acid, the enzyme, or any other part of the pure theory involved.

2. With the exception of sulfuric acid, the strong (or mineral) acids were found to be most efficient in the peptolysis of the proteins used. In solutions of sulfuric acid containing small amounts of pepsin, digestion was extremely slow. The organic acids are next in the order of efficiency, the stronger organic acids preceding the weaker. The very weak organic acids, such as acetic, propionic, etc., always were least effective.

⁵⁰ JASTROWITZ: *Biochemische Zeitschrift*, 1906, ii, p. 157; also Dissertation, Leipzig, 1906.

III. EXPERIMENTAL.

A. SOLUTIONS, PEPSIN, AND PREPARATION OF PROTEINS.

Solutions of acids. — All of the acid solutions used in the experiments were equinormal to 0.2 per cent hydrochloric acid. They contained, therefore, 0.0548 equivalent of acid in 1 litre, or 1 equivalent of acid in 18.229 litres. The following acids were employed: hydrochloric, nitric, sulfuric, phosphoric, oxalic, tartaric, citric, lactic, acetic, boric.

These were selected because (1) most of them occur, either in the free or combined state, in certain foods, (2) they are easily obtained in the pure condition, (3) with proper care standard solutions of them can be easily and accurately prepared, (4) the degree of dissociation of most of them can be easily calculated from the readily accessible conductivity figures.⁵¹

In preparing the standard acid solutions required, the calculated weight of the pure acid was dissolved in a little less than the required amount of water; the strength of the solution so obtained was ascertained by titration against standard alkali; the amount of water calculated to properly dilute the solution was then added; and the diluted solution again titrated to check the accuracy of the previous titration and dilution. In this way all of the acid solutions, excepting phosphoric and boric, were prepared. The phosphoric acid solution was standardized by weighing the magnesium pyrophosphate obtained from a known volume of the solution. The figure so obtained was used in preference to that obtained by titration against standard alkali, although both figures agreed very closely. The boric acid solution was prepared by dissolving a convenient amount of the pure dry acid in the calculated amount of water. In every case, the precautions made necessary by the individual peculiarities of the acids were taken in order to insure accuracy.

Pepsin. — The pepsin preparation used in the experiments was a uniform mixture of about equal parts of "Pepsin A," Merck's, 1:4000, purchased 1901, and "Pepsin C," Parke, Davis, & Co.'s, 1:3000, purchased 1903, both of which were used in the previous investigation.⁵² The inorganic content of the mixture was between 1.94 and 2.55 per cent, these being the figures obtained for Pepsin A and Pepsin C respectively.

Preparation of proteins. — The following proteins were used in the experiments: fibrin, egg albumin, edestin, myosin, acid albuminate, alkali albuminate, nucleoprotein, elastin, tendo-collagen, ossein, and mucoid. They are typical examples of various classes of proteins, and in the dry, finely divided state were easily handled in the experiments.

⁵¹ For this purpose KOHLRAUSCH and HOLBORN'S *Leitvermögen der Elektrolyte*, Leipzig, 1898, was used.

⁵² BERG and GIES: *Loc. cit.*, p. 495 and p. 497.

Fibrin. — “Fibrin B,” a light pulverulent product, was used, the preparation⁵³ of which from ox blood has been described in detail in the previous paper.

Coagulated egg albumin. — The whites of 24 hen eggs were strained through cheese cloth and poured in a thin stream into a large volume (about 20 litres) of boiling water. The finely flocculated coagulum was washed with water by decantation till the washings were free from chlorids, and then thoroughly dehydrated by suspension in alcohol for several days, increasing the strength of the alcohol used. Finally, the product was suspended in absolute alcohol, and freed from all moisture and traces of alcohol by washing with anhydrous ether. The product was then exposed to the air at room temperature to free it from ether, ground to a fine powder, and bottled. Portions of it were dried to constant weight at 110° C. before use.

Edestin. — Edestin was prepared from hemp seed by Osborne’s⁵⁴ method.

Myosin. — Hashed lean meat, washed with water till free from blood, was extracted for several days with 10 per cent sodium chlorid solution. The extract was then filtered and saturated with sodium chlorid to precipitate the myosin. The precipitate was washed with water, and dried by being suspended in alcohol and ether and exposed to the air. The product was then ground to a fine powder, bottled, and a portion dried to constant weight at 110° C. before use.

Acid albuminate. — Hashed lean beef, washed free from blood, was heated on a water bath for two days with a large volume of 0.4 per cent hydrochloric acid. The acid extract, freed from coarse particles by straining through cheese cloth, was neutralized with dilute sodium hydroxid solution, and the precipitated acid albuminate washed twice with large volumes of water. The precipitate was then dissolved in an equal volume of 0.8 per cent hydrochloric acid, carefully filtered through muslin, and reprecipitated by diluting the filtrate with water and neutralizing with 1 per cent sodium hydroxid solution. The precipitate was washed by decantation till free from chlorids, and then dehydrated by suspension in alcohol and ether. The finely divided, dried product, pressed between moistened litmus papers, reacted very faintly alkaline.

Alkali albuminate. — This protein was prepared exactly like the acid albuminate, with the exception that the extraction was made with dilute alkali and the precipitation with dilute acid. The dry preparation, pressed between moist litmus strips, reacted very faintly acid.

Nucleoprotein. — Bakers’ compressed yeast was macerated with sand and water to a thin paste. The thin paste was neutralized with dilute sodium

⁵³ BERG and GIES: *Loc. cit.*, p. 496.

⁵⁴ OSBORNE: American chemical journal, 1893, xiv, p. 28.

hydroxid solution, extracted for one hour with a 0.5 per cent solution of the same alkali, rapidly filtered on several large papers, and the clear filtrate slightly acidified with dilute hydrochloric acid. The precipitated nucleo-protein was washed till free from chlorids and dehydrated by suspension in alcohol and ether.

Elastin. — The preparation of elastin used in this work was a finely ground sample of one of the pure elastin products made and analyzed by Richards and Gies.⁵⁵

Tendo-collagen. — Achilles tendons (from oxen), after thorough washing to remove blood and any adherent extraneous matter, were sliced into thin sections and extracted with a half-saturated solution of calcium hydroxid for the removal of mucoid. Easily digestible protein matter was then removed by allowing the collagen to remain for several days at 40° C. in 0.25 per cent sodium carbonate containing very active trypsin.

By subsequent repeated and long-continued extractions with distilled water, all but the merest traces of mineral matter were removed. The material was thoroughly dehydrated by suspension in alcohol and in ether, and each thin section of tendon cut into several small pieces about 1 cm. in length and 1 sq. mm. in cross section. When immersed in pepsin-acid solutions, these pieces swelled rapidly to several times their original volume, and as the 1 gm. portions used contained many of the pieces, the surface exposed to the peptic action of the solution was practically the same in all of the experiments with this protein.

Ossein. — Ossein shavings from which the mucoid had been extracted were hashed and dialyzed many days in 0.1 per cent hydrochloric acid until practically free from mineral matter. The acid was removed by dialyzing in distilled water, and the water removed by washing with alcohol and with ether.

Tendo-mucoid. — A dry sample of one of Dr. Gies' typical tendo-mucoid preparations was dialyzed in 0.05 per cent hydrochloric acid until practically free from mineral matter. The acid was removed by dialyzing in distilled water, the water removed by washing with alcohol, after which the preparation was extracted with ether in a Soxhlet apparatus for several days and ground to a fine powder after the removal of adherent ether by exposure to the air. Convenient amounts of the preparation were dried to constant weight at 110° C. before use.

Inorganic contents of the proteins used. — The following table gives the amount of ash, expressed in per cent, obtained by igniting about 1 gm. of protein:

⁵⁵ RICHARDS and GIES: This journal, 1902, vii, p. 93; also GIES and collaborators: Biochemical researches, 1903, i, Reprint No. 4.

Protein.	Ash, per cent.	Protein.	Ash, per cent.
Fibrin B.	0.62	Nucleoprotein	5.83
Egg albumin	1.00	Elastin	0.08
Edestin	0.31	Tendo-collagen	0.14
Myosin	5.86	Ossein	0.29
Acid albuminate	1.67	Tendo-mucoid	0.07
Alkali albuminate	1.48		

In all probability, none of the digestive results were appreciably altered by that part of the inorganic content of the protein which dissolved in the acid solution. The order of digestion of myosin and of fibrin, in the various acid solutions used, is the same, although their ash contents are widely different (see Experiments I, II, III, p. 442, and Experiment VIII, p. 446).

Digestive procedure.— Since all of the digestion experiments were made according to the method described in detail in the previous paper,⁵⁶ only a very brief outline of the method will be given here.

Ten different acid solutions were used in each experiment. Into as many heavy, wide-mouthed, glass-stoppered bottles of 130 c.c. capacity, 100 c.c. of one of these solutions were introduced. The pepsin was then added, — in the dry state if the amount was large (*i. e.*, 300 mgm. or more) or in a small volume (not more than 0.2 c.c.) of concentrated solution when the amount was small, (*i. e.*, 50 mgm. or less). To the pepsin-acid solution, 1 gm. of the protein, dried to practically constant weight at 100°–110° C., was added.⁵⁷ The bottles containing the digestive mixtures were then placed in a water bath maintained at 40° C. throughout the experiment. At the end of the digestive period (the length of which varied in the different experiments) the bottles were removed from the water bath in the same order as they were put in, so that the digestive period was the same in all the digestive mixtures in any one experiment.

Immediately after their removal from the water bath the digestive mixtures were filtered on dry weighed papers, which, together with their contents of undigested protein, were subsequently dried and weighed. In this way the weights of undigested protein or residue were easily obtained. In Experiments I–XII aliquot portions of the filtrates were used for the determination of acid proteinate (neutralization precipitate). For this purpose the filtrates were neutralized with dilute sodium hydroxid solution, then brought to the

⁵⁶ BERG and GIES: *Loc. cit.*, p. 497.

⁵⁷ In Experiment VIII, p. 446, $\frac{1}{4}$ gm. of myosin in 25 c.c. of acid solution was used. In Experiments XIII and XIV, p. 451, and Experiments XXI and XXII, p. 455, the proportion was $\frac{1}{2}$ gm. of protein to 50 c.c. of solution. It is obvious that calculating the results so obtained to 1 gm. of protein in 100 c.c. of solution involves no error.

boiling-point, cooled and filtered on weighed papers. These, together with their contents of acid proteinate, were dried and weighed. The weight of neutralization precipitate so obtained (calculated to 100 c.c. of filtrate), plus that of the residue, was subtracted from 1 gm. to obtain the weight of combined proteoses and peptones. In Experiments XIII-XXII, in which there were no appreciable amounts of acid proteinate formed, the weights of combined proteoses and peptones are the differences between 1 gm. and the weights of the residues obtained.

B. QUANTITATIVE RESULTS OF THE EXPERIMENTS.

The experiments were made with the expectation that they would throw light on at least two questions: 1. Is the rate of peptolysis under uniform digestive conditions the same for all, or nearly all, proteins? 2. Is there any simple, obvious relation between the rate of peptolysis and the concentration of hydrogen ions?

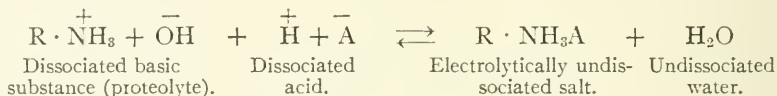
To the first of these questions a negative answer was given by the results of the previous investigation.⁵⁸ Whether any of the three proteins then used were exceptional, or represented extremes in solubility in peptolytic solutions, was now to be determined by using a larger number of widely selected proteins. This selection from groups whose physical and chemical differences are well recognized, while essential to the proper solution of the first problem, is a condition which makes the deduction of the relations sought for in the second problem all the more difficult. For it is obvious that the use of proteins having different properties, introduces a number of complicating and controlling factors into the long list of those already known to influence the speed of digestion. If the probable relation between the concentration of hydrogen ions and the speed of digestion already pointed out⁵⁹ is at all discernible in the present work, it will not be because of the selection, but rather in spite of it. Exact proportion, therefore, can hardly be expected.

All of the acid solutions used were equinormal (or equivalent) to 0.2 per cent hydrochloric acid. They contained, therefore, the same *total* amount of hydrogen in equal volumes, *i. e.*, 1 gm. atom of hydrogen in 18.229 litres. Since the acids in their aqueous solutions are dissociated to different degrees, the equinormal solutions contain different amounts of hydrogen ions in equal volumes of the

⁵⁸ BERG and GIES: *Loc. cit.*

⁵⁹ BERG and GIES: *Loc. cit.*, p. 529.

pure solutions. In any acid digestive mixture it is probable that the concentration of hydrogen ions at the end of the digestive process is not the same as it was at the beginning. As the protein is hydrolyzed into an increasing number of simpler molecules, each having one or more basic side chains or groups, hydrogen ions are removed from the solution by the union of dissociated acid with some of the dissociated basic molecules, with the formation of undissociated water, and usually a very weakly dissociated salt, according to the following equation:



When this reaction proceeds from left to right, acid molecules, if any be present, will dissociate, thus replacing the hydrogen ions just removed. It follows that the amount of undissociated acid still remaining will determine the extent to which this removal of acid from the solution will affect the speed of digestion at that time.

If it be assumed that the concentration of hydrogen ions is a controlling factor in the digestive process, then, theoretically at least, the above-mentioned changes should have very little effect upon the speed of digestion in acetic acid. The initially slow peptolysis in a solution of this acid, presumably due to the small concentration of hydrogen ions, should continue uniformly, or nearly so, for a comparatively long period of time because of the presence of a large amount of undissociated acid available for the replacement of hydrogen ions removed. On the other hand, the initially rapid peptolysis in a solution of a strong or highly dissociated acid, such as nitric or hydrochloric, should diminish rapidly in speed because the removal of hydrogen ions cannot be followed by their replacement, in so far as nearly all of the acid originally present was dissociated.⁶⁰

⁶⁰ It may be argued that the more rapid inhibition of digestion in a strongly dissociated acid solution is due to the speedier accumulation of digestion products, which, according to the law of mass action, should produce such an inhibition regardless of any change in ionic concentration. While the enzyme does not change its concentration, the acid co-operating with it does. The fact that the reversible action of proteolytic enzymes has not yet been conclusively proved is an indication that the

An exact proof of the relation between the concentration of hydrogen ions and the speed of digestion based upon experimental evidence is made extremely difficult by the necessity of limiting the digestive period to that short length of time in which the unequal diminutions in peptolytic speed described above do not take place. The many factors, beside the nature of the protein and the concentration of hydrogen ions, which control the digestive process add innumerable difficulties to such a work.

In the following tables the figures in the third column indicate the concentrations of hydrogen ions in the various solutions *before*, but not necessarily *during*, the entire digestive process.

General Results of Experiments I–XII.

Considered as a group, the proteins used in these experiments have certain common characteristics which distinguish them from the second group of proteins used in Experiments XIII–XXII. The first group consists of simple proteins; they are more rapidly digested in acid solutions of small pepsin content, and in less time, than the proteins of the second group. Furthermore, the peptolytic speed of the first group proteins is apparently more strongly influenced by the concentration of hydrogen ions than that of the second group. In general, with increasing pepsin content and lengthened digestive period, peptolysis of any protein in a series of acid solutions approaches uniformity.

It is evident from the experiments (summarized on p. 448) that the first group proteins did not digest with equal speed in any one of the acid solutions. The uniformity noticeable in some of the weak acid solutions is probably due to simple solubility. As the concentration of the acid is increased, the uniformity disappears under the strengthened digestive influence. The general order of digestion of the proteins in the acid solutions was determined as follows: The average amount of combined proteoses and peptones formed per hour in each experiment was ascertained by adding the amounts formed in each acid solution and dividing the total amount by the number of hours in the digestive period. In this way the following figures were obtained: edestin, 4258; myosin, 1980; alkali albuminate, 1116; acid albuminate, 1089; fibrin, 794; coagulated

mass action of the accumulating digestion products might not be great enough to completely mask the effect of the removal of hydrogen ions from the solution.

EXPERIMENTS I—VII.

CONDITIONS OF THE EXPERIMENTS. ALL: Pepsin — 10 mgm. (0.1 c.c. of a 10 per cent solution). TEMPERATURE, 40° C.

I—III. FIRST SERIES. **Fibrin**. DIGESTIVE PERIODS, 4 HOURS. NEUTRALIZATION, 1 HOUR AFTER FILTRATION WAS STARTED. [IN THIS AND ALL OTHER SUMMARIES "R" SIGNIFIES RESIDUE; "N. P.," NEUTRALIZATION PRECIPITATE; "P. P.," COMBINED PROTEOSES AND PEPTONES.]

Nature.	ACID.			WEIGHTS OF PRODUCTS.			RELATIVE PEPTOLYSIS.			SEQUENCE.		
	Concentration.			R.	N. P.	P. P.	R.	N. P.	P. P.	R.	N. P.	P. P.
	$\frac{\text{Mol}^1}{x}$	Per cent.	H• gram atoms per 1000 litres.	Mgm.	Mgm.	Mgm.	10	100	100	HCl product equals	100	100
Hydrochloric	18	0.20	53	64	92	844	10	100	100	1	1	1
Nitric	18	0.35	52	166	75	759	26	82	90	2	3	2
Sulfuric	36	0.27	37	694	..	306	108	108	36	5	5	5
Phosphoric	55	0.18	27	657	..	334	103	10	40	4	..	4
Oxalic	36	0.25	18	297	76	627	46	83	74	3	2	3
Tartaric	36	0.41	5	766	27	207	120	29	25	6	4	6
Citric	55	0.35	3	901	..	99	141	..	12	7	..	7
Lactic	18	0.49	3	919	..	76	144	..	5	8	7	8
Acetic	18	0.33	1	956	9	35	149	10	4	9	6	9
Boric	55	0.11	0 ²	971	..	29	152	..	3	10	..	10

EXPERIMENTS I—XII (continued).

ACID.				WEIGHTS OF PRODUCTS.			RELATIVE PEPTOLYSIS.			SEQUENCE.		
Nature.	Concentration.			R.	N. P.	P. P.	R.	N. P.	P. P.	R.	N. P.	P. P.
	Mol x	Per cent.	H* gram atoms per 1000 liters.	Mgm.	Mgm.	Mgm.	HCl product equals					
							10	100	100			
Hydrochloric	18	0.20	53	272	160	568	10	100	100	1.	1	1
Nitric	18	0.35	52	670	46	284	25	29	50	2	2	2
Sulfuric	36	0.27	37	904	10	86	33	6	15	5	5	5
Phosphoric	55	0.18	27	860	19	121	32	12	21	4	4	4
Oxalic	36	0.25	18	806	30	164	29	19	29	3	3	3
Tartaric	36	0.41	5	938	7	55	34	4	10	6	6	6
Citric	55	0.35	3	972	..	28	36	..	5	8	..	8
Lactic	18	0.49	3	964	5	31	35	3	5	7	7	7
Acetic	18	0.33	1	972	..	28	36	..	5	9	9	9
Boric	55	0.11	0	977	..	23	36	..	4	10	..	10
V. DIGESTIVE PERIOD, 5 HOURS.												
Hydrochloric	18	0.20	53	122	158	720	10	100	100	1	1	1
Nitric	18	0.35	52	473	72	435	39	46	63	2	2	2
Sulfuric	36	0.27	37	836	..	164	69	..	23	5	5	5
Phosphoric	55	0.18	27	789	22	189	65	14	26	4	4	4

IV AND V. SECOND SERIES. **Egg Albumin.** DIGESTIVE PERIODS — DIFFERENT. NEUTRALIZATION, 1 HR. AFTER FILTRATION WAS STARTED
 IV. DIGESTIVE PERIOD, 3 HOURS.

VI AND VII. THIRD SERIES. Edestin. DIGESTIVE PERIODS, 1, 1/4 HOURS. NEUTRALIZATION, 1/2 HOUR AFTER FILTRATION WAS STARTED.											
	36	0.25	18	577	55	368	47	35	51	3	3
Oxalic	36	0.41	5	892	12	96	73	8	13	6	3
Tartaric	55	0.35	3	958	..	42	79	..	6	8	5
Citric	18	0.49	3	951	..	49	78	..	7	7	..
Lactic	18	0.33	1	973	..	27	80	..	4	9	..
Acetic	55	0.11	0	980	..	20	80	..	3	10	..
Boric	10
VII.											
Hydrochloric	18	0.20	53	..	110	890	100	100	1	3
Nitric	18	0.35	52	67	54	879	49	99	3	4
Sulfuric	36	0.27	37	943	..	57	6	9	3
Phosphoric	55	0.18	27	183	47	770	43	87	5	5
Oxalic	36	0.25	18	65	42	893	38	100	2	6
Tartaric	36	0.41	5	397	22	581	20	65	7	7
Citric	55	0.35	3	718	..	282	32	8	8
Lactic	18	0.49	3	70	279	651	254	73	4	5
Acetic	18	0.33	1	204	476	320	433	36	6	1
Boric	55	0.11	0	975	..	25	3	10	..
Hydrochloric	18	0.20	53	12	97	891	10	100	100	1	2
Nitric	18	0.35	52	130	49	821	108	51	92	3	5
Sulfuric	36	0.27	37	969	..	31	807	..	3	9	3
Phosphoric	55	0.18	27	180	64	756	150	66	85	4	3
Oxalic	36	0.25	18	195	42	763	162	43	86	6	4
Tartaric	36	0.41	5	466	30	504	388	31	57	7	7
Citric	55	0.35	3	793	..	207	661	..	23	8	8
Lactic	18	0.49	3	106	56	838	661	58	94	2	2
Acetic	18	0.33	1	191	349	460	159	360	52	5	1
Boric	55	0.11	0	975	..	25	812	..	3	10	..

EXPERIMENTS I—XII (continued).

ACID.		CONCENTRATION.		WEIGHTS OF PRODUCTS.		RELATIVE PEPTOLYSIS.			SEQUENCE.			
Nature.	Mol x	Per cent.	H* gram atoms per 1000 litres.	R.	N. P.	P. P.	R.	N. P.	P. P.	R.	N. P.	P. P.
				Mgm.	Mgm.	Mgm.	HCl product equals	HCl product equals				
Hydrochloric	18	0.20	53	44	304	652	10	100	100	1	1	1
Nitric	18	0.35	52	412	196	392	94	64	60	2	2	2
Sulfuric	36	0.27	37	828	32	140	188	11	21	5	6	5
Phosphoric	55	0.18	27	752	100	148	171	33	23	4	3	4
Oxalic	36	0.25	18	684	68	248	155	22	38	3	4	3
Tartaric	36	0.41	5	872	8	120	198	3	18	6	7	6
Citric	55	0.35	3	916	..	84	208	..	13	8	..	8
Lactic	18	0.49	3	876	40	84	199	13	13	7	..	7
Acetic	18	0.33	1	920	..	80	209	..	12	9	..	9
Boric	55	0.11	0	968	..	32	220	..	5	10	..	10

ACID.		CONCENTRATION.		WEIGHTS OF PRODUCTS.		RELATIVE PEPTOLYSIS.			SEQUENCE.			
Nature.	Mol x	Per cent.	H* gram atoms per 1000 litres.	R.	N. P.	P. P.	R.	N. P.	P. P.	R.	N. P.	P. P.
				Mgm.	Mgm.	Mgm.	HCl product equals	HCl product equals				
Hydrochloric	18	0.20	53	49	318	633	10	100	100	1	1	1
Nitric	18	0.35	52	219	248	533	45	78	84	2	2	2
Sulfuric	36	0.27	37	730	14	256	149	4	4	6	6	4
Phosphoric	55	0.18	27	795	51	154	162	16	24	5	4	5
Oxalic	36	0.25	18	408	186	406	83	58	64	3	3	3
Tartaric	36	0.41	5	851	36	113	174	11	18	6	5	6
Citric	55	0.35	3	934	7	59	191	2	9	8	8	8
Lactic	18	0.49	3	921	7	72	188	2	11	7	7	7
Acetic	18	0.33	1	948	..	52	193	..	8	9	..	9
Boric	55	0.11	0	968	..	32	198	..	5	10	..	10

IX AND X. FIFTH SERIES. Acid Albuminate. DIGESTIVE PERIODS, 2 HOURS. NEUTRALIZATION, 1 HR. AFTER FILTRATION WAS STARTED.

X.

Hydrochloric	18	0.20	53	80	310	610	10	100	100	1	1	1
Nitric	18	0.35	52	295	202	503	37	82	82	2	2	2
Sulfuric	36	0.27	37	793	17	190	99	5	5	4	4	4
Phosphoric	55	0.18	27	829	35	136	104	22	22	4	4	5
Oxalic	36	0.25	18	547	125	328	68	40	54	3	3	3
Tartaric	36	0.41	5	892	26	82	111	8	13	6	5	6
Citric	55	0.35	3	957	..	43	120	..	7	9	7	9
Lactic	18	0.49	3	920	9	71	115	..	11	7	7	7
Acetic	18	0.33	1	950	..	50	119	..	8	8	..	8
Boric	55	0.11	0	968	..	32	121	..	5	10	..	10

XI AND XII. SIXTH SERIES. Alkali Albuminate. DIGESTIVE PERIODS, 2 HOURS. NEUTRALIZATION, 1 HOUR AFTER FILTRATION WAS STARTED.

Hydrochloric	18	0.20	53	74	458	468	10	100	100	1	1	1
Nitric	18	0.35	52	202	347	451	27	76	96	2	2	2
Sulfuric	36	0.27	37	830	9	161	112	2	34	6	6	6
Phosphoric	55	0.18	27	504	270	226	68	59	48	4	4	4
Oxalic	36	0.25	18	292	268	440	39	59	94	3	3	3
Tartaric	36	0.41	5	765	54	181	103	12	39	5	5	5
Citric	55	0.35	3	924	10	66	125	2	14	8	7	8
Lactic	18	0.49	3	877	22	101	119	5	22	7	6	7
Acetic	18	0.33	1	946	10	44	128	2	9	9	8	9
Boric	55	0.11	0	967	..	33	131	..	7	10	10	10

XII.

Hydrochloric	18	0.20	53	60	498	442	10	100	100	1	1	2
Nitric	18	0.35	52	188	333	479	31	67	108	2	2	1
Sulfuric	36	0.27	37	838	16	146	140	3	33	6	6	6
Phosphoric	55	0.18	27	490	202	308	82	41	70	4	3	4
Oxalic	36	0.25	18	407	177	416	68	36	94	3	4	3
Tartaric	36	0.41	5	776	36	188	129	1	43	5	5	5
Citric	55	0.35	3	906	4	90	151	1	20	8	9	8
Lactic	18	0.49	3	865	13	122	144	3	28	7	7	7
Acetic	18	0.33	1	934	8	58	156	2	13	9	8	9
Boric	55	0.11	0	957	..	43	159	..	10	10	10	10

COMPARATIVE SUMMARY OF DIGESTIVE RESULTS. — EXPERIMENTS I—XII.
WEIGHTS OF COMBINED PROTEOSES AND PEPTONES (IN MILLIGRAMS).

Acid solutions N 18.	H. gram atoms per 1000 litres.	Fibrin.			Egg, albumin.		Edeestin.		Myo- sin.	Acid albuminate.		Alkali albuminate.	
		844	783	772	568	720	890	891		633	610	468	442
Hydrochloric	53	759	626	608	284	455	879	821	392	533	503	451	479
Nitric	37	306	294	318	86	164	57	31	140	256	190	161	146
Sulfuric	27	334	333	338	121	189	770	756	148	154	136	226	308
Phosphoric	18	627	560	550	164	368	893	763	248	406	328	440	416
Oxalic	5	207	216	226	55	96	581	504	120	113	82	181	188
Tartaric	3	99	125	95	28	42	282	207	84	59	43	66	90
Citric	3	76	95	82	31	49	651	838	84	72	71	101	122
Lactic	1	35	59	51	28	27	320	460	80	52	50	44	58
Acetic	0	29	49	36	23	20	25	25	32	32	32	33	43

RELATIVE PEPTOLYSIS. — COMBINED PROTEOSES AND PEPTONES. HYDROCHLORIC ACID PRODUCT EQUALS 100.

Hydrochloric	53	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Nitric	52	90	80	79	50	63	99	92	60	84	82	96	108						
Sulfuric	37	36	38	41	15	23	6	3	21	40	31	34	33						
Phosphoric	27	40	43	44	21	26	87	85	23	24	22	48	70						
Oxalic	18	74	72	71	29	51	100	86	38	64	54	94	94						
Tartaric	5	25	28	29	10	13	65	57	18	18	13	39	43						
Citric	3	12	16	12	5	6	32	23	13	9	7	14	20						
Lactic	3	9	12	11	5	7	73	94	13	11	11	22	28						
Acetic	1	4	8	7	5	4	36	52	12	8	8	9	13						
Boric	0	3	6	5	4	3	3	3	5	5	5	7	10						
Pepsin (mgm.)		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Digestive periods (hours)		4	4	4	3	5	1½	1½	1	2	2	2	2	2	2	2	2	2	2
Number of the experiment		1	2	3	4	5	6	7	8	9	10	11	12						

egg albumin, 444. By this method the following order was obtained, the first-named protein being the most rapidly digested: edestin, myosin, alkali albuminate, acid albuminate, fibrin, coagulated egg albumin.

The order of digestion in the various acid solutions was practically the same for all the proteins. Digestion was most rapid in the first-named solution. The average order is as follows: hydrochloric, nitric, oxalic, phosphoric, sulfuric, tartaric, lactic, citric, acetic, boric.

The extent to which the concentration of hydrogen ions determined the speed of digestion may better be seen by referring to the relative peptolysis of combined proteoses and peptones (p. 449). The figures emphasize the conclusion already expressed,⁶¹ that the concentration of hydrogen ions is an important controlling factor in determining the speed of digestion.

General Results of Experiments XIII-XXII.

The proteins used in Experiments XIII-XXII were all of the complex type. They are distinguished from the first group of proteins by their greater resistance to peptolytic action.

The order of digestion of the various proteins, as determined by the method described on p. 441, beginning with the protein that was most rapidly digested, is as follows: ossein, nucleoprotein, tendocollagen, elastin, tendo-mucoid. In this connection the following complication was introduced: While the first group proteins were all digested in solutions containing the same amounts of pepsin, the second group proteins were not. But this added very little difficulty to the determination of the order of digestion. The average amounts of combined proteoses and peptones formed per hour in the experiments with ossein and with nucleoprotein, were 2100 and 550 mgm. respectively. For the ossein, 300 mgm. of pepsin were used; for the nucleoprotein, 50 mgm. Which digested the more rapidly? If the same protein had been used in both experiments, it is probable that digestion in the first would not have been more than three times as rapid as it was in the second. It is evident, on this basis, that the ossein digested more rapidly than the nucleoprotein. The other two proteins which were digested in acid solu-

⁶¹ BERG and GIES, *Loc. cit.*

EXPERIMENTS XIII-XXII.

CONDITIONS OF THE EXPERIMENTS. ALL: TEMPERATURE, 40° C.

XIII AND XIV. SEVENTH SERIES. Nucleoprotein. PEPSIN, 50 MGM. (DRY). DIGESTIVE PERIODS, 3 HOURS.									
ACID.				WEIGHTS OF PRODUCTS.		RELATIVE PEPTOLYSIS.		SEQUENCE.	
Nature.	Concentration.			R.	P. P.	R.	P. P.	R.	P. P.
	Mol x	Per cent.	H• gm- atoms per 1000 litres.	Mgm.	Mgm.	HCl product equals			
						10	100		
Hydrochloric . . .	18	0.20	53	586	414	10	100	1	1
Nitric	18	0.35	52	704	296	12	71	2	2
Sulfuric	36	0.27	37	748	252	13	61	3	3
Phosphoric	55	0.18	27	864	136	15	33	5	5
Oxalic	36	0.25	18	786	214	13	52	4	4
Tartaric	36	0.41	5	876	124	15	30	6	6
Citric	55	0.35	3	892	108	15	26	8	8
Lactic	18	0.49	3	888	112	15	27	7	7
Acetic	18	0.33	1	918	82	16	20	9	9
Boric	55	0.11	0	932	68	16	16	10	10
XIV.									
Hydrochloric . . .	18	0.20	53	608	392	10	100	1	1
Nitric	18	0.35	52	728	272	12	69	2	2
Sulfuric	36	0.27	37	818	182	13	46	3	3
Phosphoric	55	0.18	27	884	116	15	30	5	5
Oxalic	36	0.25	18	858	142	14	36	4	4
Tartaric	36	0.41	5	896	104	15	27	6	6
Citric	55	0.35	3	916	84	15	21	8	8
Lactic	18	0.49	3	910	90	15	23	7	7
Acetic	18	0.33	1	938	62	15	16	10	10
Boric	55	0.11	0	924	76	15	19	9	9

EXPERIMENTS XIII—XXII (continued).

XV AND XVI. EIGHTH SERIES. Elastin. PEPSIN, 300 MGM. (DRY). DIGESTIVE PERIODS, 6 HOURS.									
ACID.				WEIGHTS OF PRODUCTS.		RELATIVE PEPTOLYSIS.		SEQUENCE.	
Nature.	Concentration.			R.	P. P.	R.	P. P.	R.	P. P.
	Mol x	Per cent.	H ^o gm. atoms per 1000 litres.	Mgm.	Mgm.	HCl product equals			
						10	100		
Hydrochloric . . .	18	0.20	53	144	856	10	100	1	1
Nitric	18	0.35	52	206	794	14	93	3	3
Sulfuric	36	0.27	37	164	836	11	98	2	2
Phosphoric . . .	55	0.18	27	232	768	16	90	5	5
Oxalic	36	0.25	18	222	778	15	91	4	4
Tartaric	36	0.41	5	280	720	19	84	6	6
Citric	55	0.35	3	294	706	20	82	7	7
Lactic	18	0.49	3	314	686	22	80	8	8
Acetic	18	0.33	1	410	590	28	69	9	9
Boric	55	0.11	0	660	340	46	40	10	10
XVI.									
Hydrochloric . .	18	0.20	53	170	830	10	100	2	2
Nitric	18	0.35	52	189	811	11	98	3	3
Sulfuric	36	0.27	37	144	856	8	103	1	1
Phosphoric . . .	55	0.18	27	225	775	13	93	5	5
Oxalic	36	0.25	18	192	808	11	97	4	4
Tartaric	36	0.41	5	236	764	14	92	6	6
Citric	55	0.35	3	251	749	15	90	8	8
Lactic	18	0.49	3	247	753	15	91	7	7
Acetic	18	0.33	1	282	718	17	87	9	9
Boric	55	0.11	0	481	519	28	63	10	10

EXPERIMENTS XIII—XXII (continued).

XVII AND XVIII. NINTH SERIES. Tendo-collagen. PEPSIN, 300 MG. (DRY). DIGESTIVE PERIODS, 5 HOURS.									
ACID.				WEIGHTS OF PRODUCTS.		RELATIVE PEPTOLYSIS		SEQUENCE.	
Nature.	Concentration.			R.	P. P.	R.	P. P.	R.	P. P.
	Mol x	Per cent.	H· gm. atoms per 1000 litres.	Mgm.	Mgm.	HCl product equals			
						10	100		
Hydrochloric . . .	18	0.20	53	200	800	10	100	3	3
Nitric	18	0.35	52	141	859	7	107	1	1
Sulfuric	36	0.27	37	320	680	16	85	8	8
Phosphoric	55	0.18	27	247	753	12	94	5	5
Oxalic	36	0.25	18	192	808	10	101	2	2
Tartaric	36	0.41	5	246	754	12	94	4	4
Citric	55	0.35	3	299	701	15	88	6	6
Lactic	18	0.49	3	302	698	15	87	7	7
Acetic	18	0.33	1	621	379	31	47	9	9
Boric	55	0.11	0	977	23	49	3	10	10
XVIII.									
Hydrochloric . . .	18	0.20	53	297	703	10	100	7	7
Nitric	18	0.35	52	265	735	9	104	5	5
Sulfuric	36	0.27	37	362	638	12	91	9	9
Phosphoric	55	0.18	27	211	789	7	112	3	3
Oxalic	36	0.25	18	286	714	10	102	6	6
Tartaric	36	0.41	5	181	819	6	117	1	1
Citric	55	0.35	3	182	818	6	116	2	2
Lactic	18	0.49	3	212	788	7	112	4	4
Acetic	18	0.33	1	314	686	11	98	8	8
Boric	55	0.11	0	610	390	21	55	10	10

EXPERIMENTS XIII-XXII (continued).

XIX AND XX. TENTH SERIES. Ossein. Pepsin, 300 mgm. (dry). Digestive periods, 4 hours.										
ACID.				WEIGHT OF PRODUCTS.		RELATIVE PEPTOLYSIS.		SEQUENCE.		Residues obtained in solubility experiment of four hours' duration, in mgm.
Nature.	Concentration.			R.	P. P.	R.	P. P.	R.	P. P.	
	Mol x	Per cent.	H·gm. atoms per 1000 litres.	Mgm.	Mgm.	HCl product equals				
						10	100			
Hydrochloric . . .	18	0.20	53	272	728	10	100	10	10	..
Nitric	18	0.35	52	175	825	6	113	7	7	..
Sulfuric	36	0.27	37	202	798	7	110	8	8	..
Phosphoric	55	0.18	27	60	940	3	129	6	6	..
Oxalic	36	0.25	18	211	789	8	108	9	9	..
Tartaric	36	0.41	5	49	951	2	131	5	5	..
Citric	55	0.35	3	38	962	1	132	4	4	..
Lactic	18	0.49	3	32	968	1	133	1	1	..
Acetic	18	0.33	1	34	966	1	133	2	2	..
Boric	55	0.11	0	37	963	1	132	3	3	..
XX.										
Hydrochloric . . .	18	0.20	53	514	486	10	100	10	10	904
Nitric	18	0.35	52	383	617	7	127	9	9	910
Sulfuric	36	0.27	37	323	677	6	139	7	7	919
Phosphoric	55	0.18	27	157	843	3	173	6	6	943
Oxalic	36	0.25	18	336	664	7	137	8	8	948
Tartaric	36	0.41	5	102	898	2	185	4	4	930
Citric	55	0.35	3	63	937	1	193	3	3	946
Lactic	18	0.49	3	47	953	1	196	1	1	926
Acetic	18	0.33	1	63	937	1	193	2	2	930
Boric	55	0.11	0	111	889	2	183	5	5	920

EXPERIMENTS XIII—XXII (*continued*).

XXI AND XXII. ELEVENTH SERIES. Tendo-mucoid. PEPSIN, 500 MG. (DRY). DIGESTIVE PERIODS, 5 HOURS.									
ACID.				WEIGHTS OF PRODUCTS.		RELATIVE PEPTOLYSIS.		SEQUENCE.	
Nature.	Concentration.			R.	P. P.	R.	P. P.	R.	P. P.
	Mol x	Per cent.	H·gm. atoms per 1000 litres.	Mgm.	Mgm.	HCl product equals			
						10	100		
Hydrochloric	18	0.20	53	460	540	10	100	2	2
Nitric	18	0.35	52	454	546	10	101	1	1
Sulfuric	36	0.27	37	490	510	11	94	4	4
Phosphoric	55	0.18	27	552	448	12	83	5	5
Oxalic	36	0.25	18	482	518	10	96	3	3
Tartaric	36	0.41	5	572	428	12	79	6	6
Citric	55	0.35	3	612	388	13	72	8	8
Lactic	18	0.49	3	606	394	13	73	7	7
Acetic	18	0.33	1	658	342	14	63	9	9
Boric	55	0.11	0	676	324	15	60	10	10
XXII.									
Hydrochloric	18	0.20	53	424	576	10	100	1	1
Nitric	18	0.35	52	480	520	11	90	3	3
Sulfuric	36	0.27	37	456	544	11	94	2	2
Phosphoric	55	0.18	27	524	476	12	83	5	5
Oxalic	36	0.25	18	484	516	11	90	4	4
Tartaric	36	0.41	5	546	454	13	79	6	6
Citric	55	0.35	3	574	426	14	74	7	7
Lactic	18	0.49	3	584	416	14	72	8	8
Acetic	18	0.33	1	622	378	15	66	9	9
Boric	55	0.11	0	634	366	15	64	10	10

EXPERIMENTS XIII-XXII. COMPARATIVE SUMMARY OF DIGESTIVE RESULTS.

WEIGHTS OF COMBINED PROTEOSES AND PEPTONES (IN MILLIGRAMS).

Acid solutions <i>n</i> /18.	H• gm. atoms per 1000 litres.	Nucleo protein.		Elastin.		Tendo- collagen.		Ossein.		Tendo- mucoid.	
Hydrochloric	53	414	392	856	830	800	703	728	486	540	576
Nitric	52	296	272	794	811	859	735	825	617	546	520
Sulfuric	37	252	182	836	856	680	638	798	677	510	544
Phosphoric	27	136	116	768	775	753	789	940	843	448	476
Oxalic	18	214	142	778	808	808	714	789	664	518	516
Tartaric	5	124	104	720	764	754	819	951	898	428	454
Citric	3	108	84	706	749	701	818	962	937	388	426
Lactic	3	112	90	686	753	698	788	968	953	394	416
Acetic	1	82	62	590	718	379	686	966	937	342	378
Boric	0	68	76	340	519	23	390	963	889	324	366
RELATIVE PEPTOLYSIS. — COMBINED PROTEOSES AND PEPTONES. HYDROCHLORIC ACID PRODUCT EQUALS 100.											
Hydrochloric	53	100	100	100	100	100	100	100	100	100	100
Nitric	52	71	69	93	98	107	104	113	127	101	90
Sulfuric	37	61	46	98	103	85	91	110	139	94	94
Phosphoric	27	33	30	90	93	94	112	129	173	83	83
Oxalic	18	52	36	91	97	101	102	108	137	96	90
Tartaric	5	30	27	84	92	94	117	131	185	79	79
Citric	3	26	21	82	90	88	116	132	193	72	74
Lactic	3	27	23	80	91	87	112	133	196	73	72
Acetic	1	20	16	69	87	47	98	133	193	63	66
Boric	0	16	19	40	63	3	55	132	183	60	64
Pepsin (mgm.)		50	50	300	300	300	300	300	300	500	500
Digestive periods (hours)		3	3	6	6	5	5	4	4	5	5
Number of the experiment		13	14	15	16	17	18	19	20	21	22

tions containing 300 mgm. of pepsin were easily arranged after the nucleoprotein, and mucoid comes last.

The order of digestive efficiency of the various acid solutions was not so uniform for the second group proteins as it was for the first. But the average order of digestion of the second group proteins is practically the same as the order found for the first group. Beginning with the acid solution in which peptolysis was most rapid, the order is as follows: hydrochloric, nitric, oxalic, sulfuric, phosphoric, tartaric, citric, lactic, acetic, boric.

Most remarkable were the digestive results obtained with ossein. This protein digested most rapidly in a solution of a weak acid, *i. e.*, lactic; then followed acetic, citric, boric, tartaric, phosphoric, sulfuric, nitric, oxalic, hydrochloric. Digestion was slowest in hydrochloric acid. In the order of digestion of the second group proteins above mentioned, that of ossein was not included because of its exceptional nature. To explain these anomalous results is by no means easy. That they were not due to any simple solubility of the protein in the acid solutions was shown by the solubility experiment purposely made to test this point. Nor is there anything in the chemical nature of ossein to which its exceptional digestive behavior may be attributed.

The almost obvious relation between the speed of digestion and the concentration of hydrogen ions is shown by the figures for relative peptolysis on p. 456. Leaving out of consideration the exceptional case of ossein, it is evident, from the figures, that digestion was most rapid in those solutions of initially high concentration of hydrogen ions, and that digestion was slow in the solutions of initially low concentration of hydrogen ions.

IV. CONCLUSIONS.

1. Measured under uniform, or nearly uniform, digestive conditions, different proteins digest with unequal speed. The eleven proteins used in these digestion experiments divide themselves, with regard to their digestibility, into two groups: the first group consisting of 6 simple proteins which were *rapidly* digested in pepsin-acid solutions containing 10 mgm. of pepsin in 100 c.c. of solution; the second group consisting of 5 conjugate proteins which were *slowly* digested in acid solutions containing from 50 to 500 mgm. of pepsin in 100 c.c. of solution. The order of digestibility, begin-

ning with the protein which was most rapidly digested, is as follows: edestin, myosin, alkali albuminate, acid albuminate, fibrin, coagulated egg albumin, ossein, nucleoprotein, tendo-collagen, elastin, tendo-mucoid.

2. The relative digestibility of an untried protein may be predicted only with uncertainty. That its digestive behavior will be similar to that of some other protein of similar chemical or physical constitution cannot be assumed with safety. The question must be decided experimentally.

3. The relative digestive efficiency of the acid solutions used in these experiments was fairly uniform. For the first group of proteins the average order, beginning with the solution in which digestion was most rapid, is the following: hydrochloric, nitric, oxalic, phosphoric, sulfuric, tartaric, lactic, citric, acetic, boric. For the second group of proteins (ossein excepted) the average order is the following: hydrochloric, nitric, oxalic, sulfuric, phosphoric, tartaric, citric, lactic, acetic, boric.

4. In general, the greater the concentration of hydrogen ions in the digestive solutions used, other conditions being equal, the more rapid the digestion. While there was no exact proportionality, there was a general parallelism.

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CHEMICAL STUDIES ON THE EFFECTS OF CENTRIFUGAL FORCE ON THE EGGS OF THE SEA URCHIN (*ARBACIA PUNCTULATA*).

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IN a paper now in press¹ I gave the results of a chemical study of the layers into which the frog's egg is separated by centrifugal force, in order to analyze more closely the causes of the abnormal development of such eggs. It was decided that either the regional chemical or physical changes produced by the separation of substances of different specific gravity was sufficient to account for the failure of part or all of the egg to develop. Last summer at Woods Hole, Mass., I began a similar study of the sea urchin's egg for comparison. Centrifugal force has very little effect on the development of the sea urchin's egg, as shown by Morgan and Lyon,² and I wished to determine whether it had a correspondingly slight effect on the regional chemical composition of this egg.

As the egg membranes of *Arbacia* are formed in the ovary, it was impossible to obtain eggs free from them. This membrane is very thick, but is a hyaline proteid that very gradually dissolves in sea water, and its outer limit cannot be distinguished from the surrounding sea water, so that many observers do not realize that a membrane exists. In order to see it, place the egg (Boveri's method) in india ink, and the space around the egg not occupied by ink represents the thickness of the membrane. On fertilization this membrane is separated from the egg by the accumulation of a

¹ McCLENDON: "Cytological and chemical studies of centrifuged frog eggs," *Archiv für Entwicklungsmechanik*, 1909, xxvii.

² MORGAN and LYON: "The relation of the substances of the egg separated by a strong centrifugal force to the location of the embryo," *Ibid.*, xxiv.

fluid between it and the egg, and its *inner surface* can be distinctly seen and is called the "fertilization membrane." In order to obtain sufficient material for chemical methods the membranes had to be broken and the egg material centrifuged in mass. Ripe ovaries were freed from body fluids by centrifuging, then frozen and ground in a mortar kept at low temperature, to break the egg membranes. The material was strained through bolting cloth to remove the ovarian stroma and mixed with a little toluol to prevent bacterial action. It was then transferred to an electric centrifuge having special carriers holding about 20 gm. of material and making with this load about 3200 revolutions per minute at a radius of 158 mm. and centrifuged eight hours or more. The scarcity of Arbacia, the necessity of using a storage battery of 48 cells when working during the day, the fact that the frozen material absorbed quantities of air that made it froth on thawing, and the impossibility of removing the egg membranes from the material made the work very difficult. Six weeks were spent in collecting and simultaneously centrifuging the material.

The egg of Arbacia is separated by a centrifugal force of 6400 times gravity, acting for two minutes, into four layers, which considered in order from without inwards toward the centre of rotation, are as follows (as figured and described by Lyon³):

1. A layer of yolk bodies and red pigment granules extending about half-way to the equator. The pigment is heavier than the yolk and consequently, by prolonged centrifuging, this layer marked by the red pigment is decreased in volume.

2. A layer of similar yolk bodies, but without the pigment, extending a little beyond the equator.

3. A translucent fluid layer extending almost to the centrifugal pole and containing the nucleus.

4. A very dense opaque layer or cap of little volume, sitting on the centripetal pole.

The first and second layers are composed mainly of yolk bodies, but the first has in addition the pigment which is probably of little importance to the embryo. The fourth layer is of so small a volume that it may safely be considered as of less importance to our work.

When the crushed eggs are centrifuged, as described above, the

³ LYON: Archiv für Entwicklungsmechanik, 1907, xxiii, pp. 151-173.

material is separated into two layers: a jelly like centrifugal layer, corresponding to layers one and two of the entire egg, but containing in addition the egg membranes, which greatly increases its volume, and a fluid centripetal layer, corresponding to layer three of the entire egg. The material of layer four of the entire egg did not separate out in the material centrifuged in mass, but as this material is very small in amount we have in general the same separa-

TABLE I.

COMPOSITION OF THE LAYERS OF THE CENTRIFUGED ARBACIA EGG IN PER CENTS

Layer.	Water.	Solids.	Ether extract.	P. in E. E.	Alcohol extract.	P. in A. E.
Centrip. 32.5	28.6	3.9	0.308	.00154	1.6	.0434
Centrif. 67.5	53.3	14.2	1.946	.06760	3.48	.0814
Whole egg ¹ .	81.9	18.1	2.254	.06914	5.08	.1248

¹ The lower line of the table may be compared with the composition of the whole egg Water, 77.4 per cent; solids, 22.6 per cent; fat, 4.32 per cent; ash, 2.19 per cent;

tion in the crushed eggs as in the entire eggs when centrifuged. I was unable to determine the composition of the egg membrane. It is probably a mucoid, and I think it safe to say that it is a non-phosphorized proteid, poor in nitrogen, and that its presence should be taken into account in interpreting quantitative data. It is of greater volume than the egg.

On removal from the centrifuge, the layers were separated by pouring off the centripetal layer. They were then weighed, spread out thin, and desiccated rapidly to constant weight *in vacuo* over H_2SO_4 , and weighed again to determine the water content, then sealed in vials to await analysis. This dried material was pulverized, desiccated again, and 10 gm. samples treated as follows: Extracted first with boiling ether and second with hot 95 per cent alcohol for about thirty hours in each in a Soxhlet extractor; then in water acidulated with 5 per cent acetic acid. The extracts and residue were desiccated to constant weight and their weight recorded. They were then sampled for phosphorus and the residue also for nitrogen and ash determinations. They were also examined

microscopically and some qualitative tests applied. The results are shown in Table I as per cents of the weight of the whole egg mass.

The ether extract of the centripetal layer contains "myelin forms," probably lecithin (as is also indicated by the phosphorus content), mixed with red pigment, and considerable amounts of crystals resembling fat crystals, that are not easily soluble in boiling alcohol.

TABLE I.

OF THE WEIGHT OF THE WHOLE EGG MASS (P = PHOSPHORUS, N = NITROGEN).

Water extract.	P. in W. E.	Residue.	P. in R.	N. in R.	Ash in R.	P. in layer.
0.78	0.13	1.309	.0392	.1625	.0162	.21414
1.42	0.1822	7.29	.1167	.775	.0264	.4478
2.20	0.3122	8.599	.1559	.9375	.0426	.66194

and membrane of *Strongylocentrotus lividus* as computed from the analysis by WETZEL: nitrogen, 1.626 per cent; phosphorus, .268 per cent.

The ether extract of the centrifugal layer is similar to the above except that the fat crystals are very few in number.

The alcohol extract of the centripetal layer contains "myelin forms" with some pigment and large amounts of salts, chiefly sodium chloride. Except for the salts it is nearly all soluble in ether.

The alcohol extract of the centrifugal layer is similar, but possibly contains less salts.

The pigment is dissolved and finally turned brown by acids, but unaffected by alkalis. I could not distinguish absorption bands with the spectroscope, but only a general absorption of the violet end. It is probably a lipochrome.

In the alcohol and other extracts the phosphorus content is probably an index to the lecithin content. The fact that so little lecithin was found in the ether extract of the centripetal layer may be due to its being held back by proteids or to incomplete extraction, although the extraction was continued about thirty hours.

The water extracts of both layers was largely sodium chloride

and other salts, especially phosphates, as shown by the phosphorus content. I estimated the salts in the egg as about the same concentration as in sea water.

The final residues were proteid. They contained 1-2 per cent of insoluble salts. It is possible that insoluble salts are deposited in the egg and are used later in the building of the skeleton.

The proteids in the centripetal layer are in the "sol" stage, and those in the centrifugal layer in the "gel" stage, and are probably in this condition in corresponding layers of the individual egg when centrifuged. However, they may be chemically the same.

Using fresh centrifuged material, I tried to separate the proteids in each layer by salting out. The limits of salting out varied somewhat, but were in general as follows:

The centripetal layer on dilution with 10 volumes of water is partially precipitated. The filtrate becomes turbid at 20 per cent, begins to precipitate at 25 per cent, precipitates mostly between 33 and 50 per cent and completely before 100 per cent saturated ammonium sulphate. The precipitate (by dilution) completely dissolves in 10 per cent sodium chloride and is entirely precipitated by an addition of 17 per cent saturated ammonium sulphate.

The centrifugal layer partially dissolves in 20 volumes of water. This aqueous solution is considerably precipitated by 17 per cent, almost completely by 25 per cent, and completely before 33 per cent saturated ammonium sulphate. The residue after thorough washing in water is insoluble in 10 per cent sodium chloride, but partially soluble in dilute, and completely in concentrated potassium hydrate solution.

There seems to be a proteid in the centripetal layer, insoluble in water and soluble in 10 per cent salt solution, that is not found in the centrifugal layer. However, as the ranges of salting out are so wide, I did not hope to get any of the proteids in a pure state and did not attempt to analyze them, and I do not base any conclusions on the work on fresh material.

In the centrifuged frog's egg there are three layers. When centrifuged to a certain extent the centripetal and intermediate layers take part in development and the centrifugal is omitted (*i. e.*, fails to develop). The layers taking part in development are mixed more or less during early cleavage and we may consider them as one, which we will call the centripetal layer. We have then a centripetal layer capable of development and a centrifugal layer incapable of development.

In Tables II-IV the results of the study of the eggs of the frog and Arbacia are compared. Table II shows that while there is quite a difference in the water content in the layers of the centrifuged frog's egg, Arbacia exhibits very little difference in this regard. Table III shows that while there is a great difference in the rela-

TABLES II-III.
CENTRIFUGED EGGS.

Per cent of water and solids in the layers.				Per cent of extracts and residue in the solids.		
Animal.	Layer.	Water.	Solids.	Ether + Alc. ext.	Water ext.	Residue.
Frog	Centrip.	73.3	26.7	50.85	34.6	14.55
	Centrif.	48.0	52.0	30.0	10.0	60.0
Arbacia	Centrip.	88.0	12.0	49.0	20.0	31.0
	Centrif.	79.0	21.0	38.2	10.0	51.8

TABLE IV.
CENTRIFUGED EGGS.

Per cent of phosphorus nitrogen and ash in the extracts and residue.						
Animal.	Layer.	P. in E. E. — A. E.	P. in W. E.	P. in R.	N. in R.	Ash in R.
Frog	Centrip.	0.018	1.05	0.39
	Centrif.	0.54	1.2	1.33
Arbacia	Centrip.	2.36	16.66	3.24	13.45	1.24
	Centrif.	2.74	12.84	1.6	10.6	2.02

tive amounts of the extracts and residue in the layers of the centrifuged frog's egg, in Arbacia a great difference is seen only in the water extract, which is largely salts. Table IV shows that while there are great differences in the concentration of phosphorus in the extracts of layers of the centrifuged frog's egg, in Arbacia a great difference is seen only in the residues which are proteid. This difference may be accounted for by the admixture of egg membranes

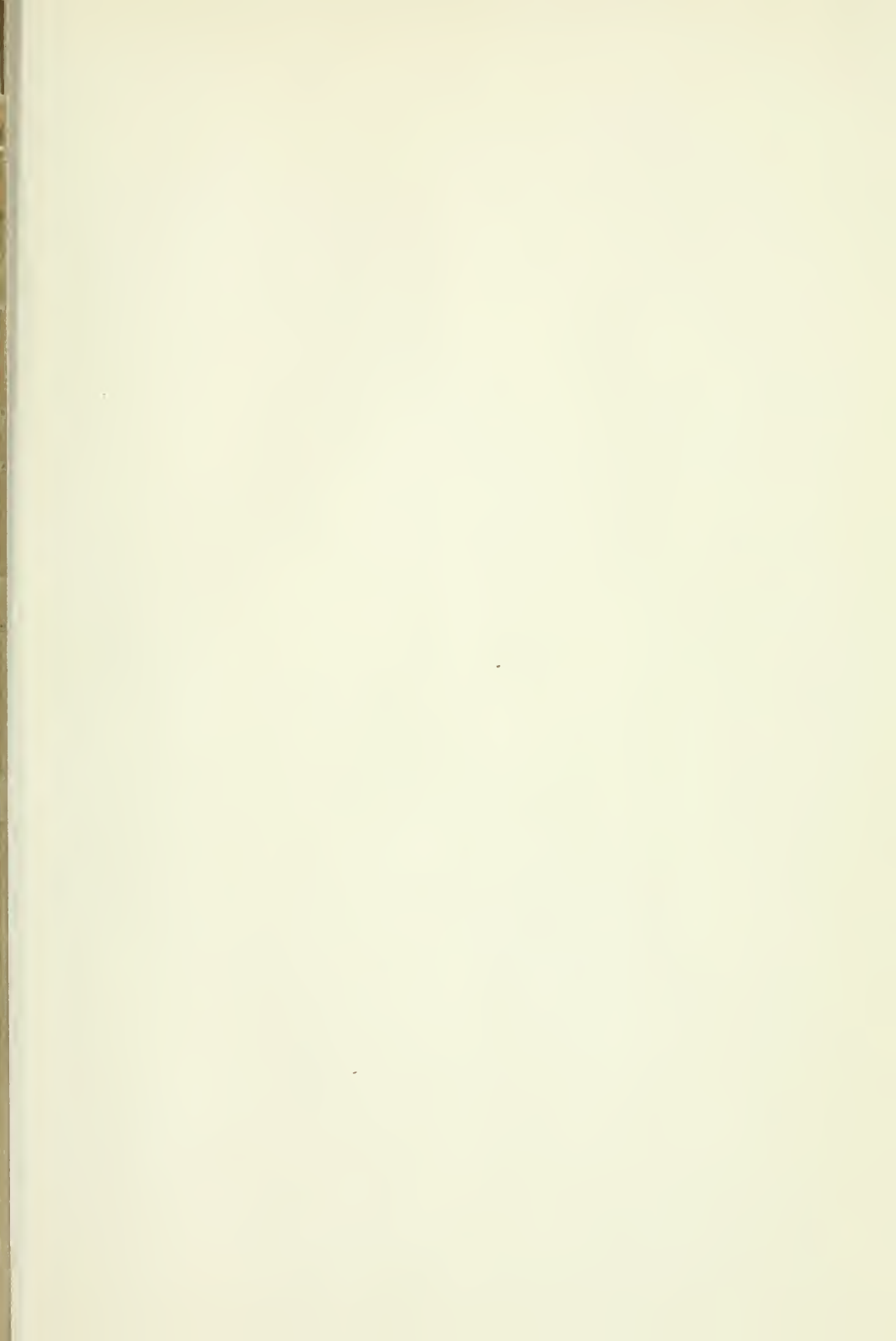
in the centrifugal layer, increasing the proteid and not the phosphorized proteid content. A less difference is shown in the proteid nitrogen, which may be accounted for on the same grounds, as the egg membranes are probably formed of a proteid poor in nitrogen. Those apparent differences are also in small part due to the difference in insoluble salts (ash) in the two layers, which was not considered in the calculations.

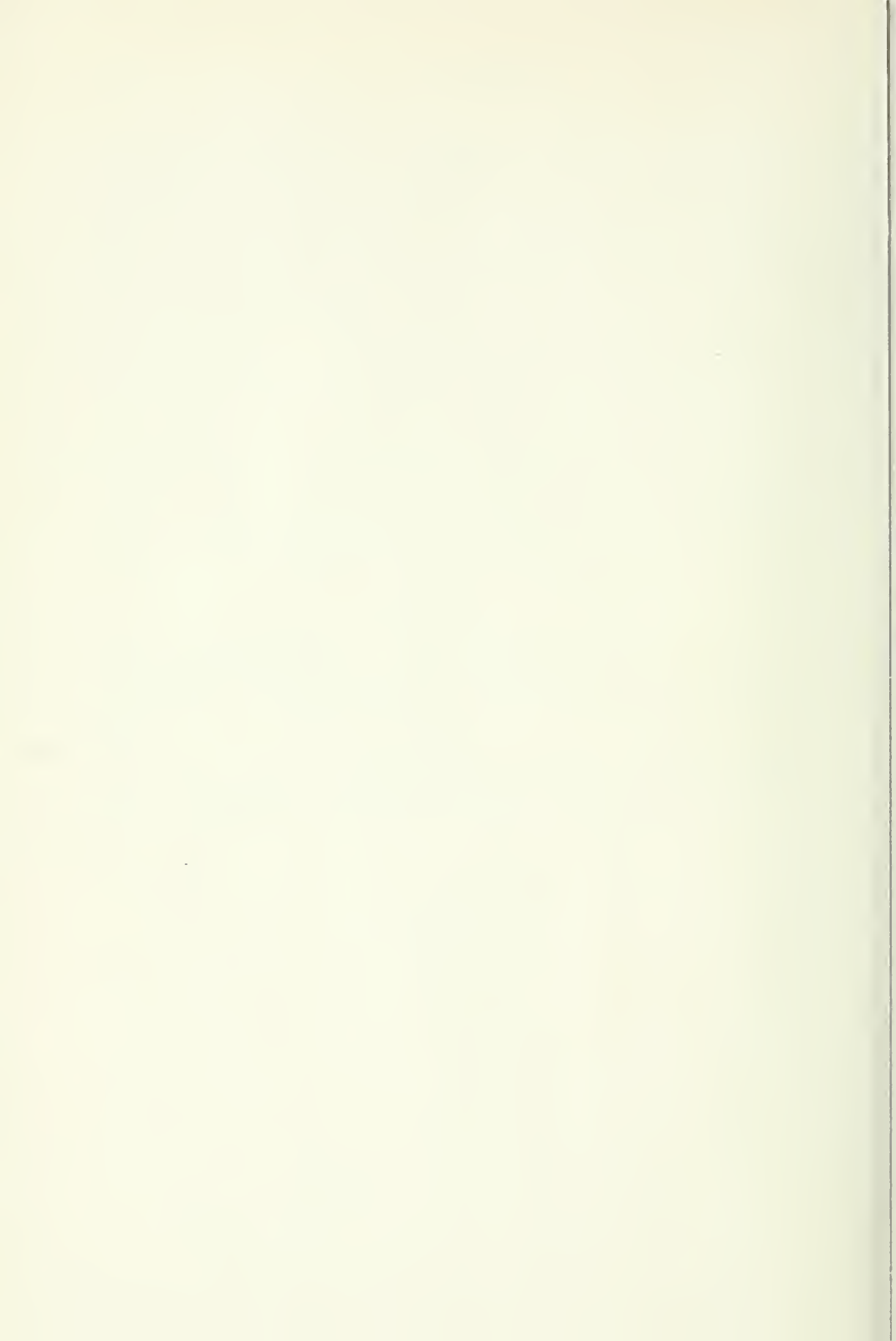
We conclude then that the reason for the failure of centrifugal force to produce abnormal development in the egg of *Arbacia*, as it did in the frog's egg, may be the fact that centrifugal force does not produce nearly so striking regional chemical differences in the egg of *Arbacia* as in the frog's egg. Moreover, the regional physical differences produced by centrifugal force in the frog's egg are greater than in *Arbacia*. Furthermore, the egg of the frog is enormously larger and harder to segment than that of *Arbacia*, and probably it is therefore much easier to inhibit or distort its cleavage.

SUMMARY.

Whereas centrifugal force causes marked regional chemical differences in the unsegmented frog's egg, it causes comparatively slight regional chemical differences in the unsegmented sea urchin's egg.

These facts are correlated with the facts that centrifugal force locally or entirely inhibits the development of the frog's egg, but affects very little the development of the sea urchin's egg.







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