THE AMERICAN JOURNAL

OF

PHYSIOLOGY.

EDITED FOR

The American Physiological Society

BY

H. P. BOWDITCH, M.D., BOSTON

R. H. CHITTENDEN, PH.D., NEW HAVEN

W. H. HOWELL, M.D., BALTIMORE

FREDERIC S. LEE, PH.D., NEW YORK

W. P. LOMBARD, M.D., ANN ARBOR

G. N. STEWART, M.D., CHICAGO

W. T. PORTER, M.D., BOSTON

THE

AMERICAN JOURNAL

OF

PHYSIOLOGY

Volume XXIV.

100712
24/2/10

BOSTON, U. S. A.

1909.

QP
1
A5
v.24
Cop.2

Copyright, 1909.
By W. T. PORTER.

University Press
John Wilson and Son, Cambridge, U.S.A.

CONTENTS.

No. I, April 1, 1909.

	Page
Heat Coagulation in Smooth Muscle: A Comparison of the Effects of Heat on Smooth and Striated Muscle. *By Edward B. Meigs*	1
On the Connection between Changes of Permeability and Stimulation and on the Significance of Changes in Permeability to Carbon Dioxide. *By R. S. Lillie*	14
Factors Regulating the Creatinin Output in Man. *By P. A. Levene and L. Kristeller*	45
Acapnia and Shock.—III. Shock after Laparotomy: Its Prevention, Production, and Relief. *By Yandell Henderson*	66
The Rôle of the Ash Constituents of Wheat Bran in the Metabolism of Herbivora. *By E. B. Hart, E. V. McCollum, and G. C. Humphrey*	86
The Effect of Smoking upon the Blood Pressures and upon the Volume of the Hand. *By James W. Bruce, James R. Miller, and Donald R. Hooker*	104
The Production by Hydrogen Peroxide of Rhythmical Contractions in the Marginless Bell of Gonionemus. *By O. P. Terry*	117
Studies in the Physiology of the Central Nervous System.—I. The General Phenomena of Spinal Shock. *By F. H. Pike*	124
Hydrolysis of Vitellin from the Hen's Egg. *By Thomas B. Osborne and D. Breese Jones*	153
Hydrolysis of the Muscle of Scallop (Pectens Irradians). *By Thomas B. Osborne and D. Breese Jones*	161
Experimental Studies on the Physiology of the Molluscs.— Fourth Paper. *By Lafayette B. Mendel and H. Gideon Wells*	170
Concerning the Supposed Connection between Protein Coagulation and the Heat Shortening of Animal Tissues. *By Edward B. Meigs*	177

No. II, May 1, 1909.

	Page
Mercurial Poisoning of Men in a Respiration Chamber. *By Thorne M. Carpenter and Francis G. Benedict*	187
Preliminary Observations on Metabolism during Fever. *By Thorne M. Carpenter and Francis G. Benedict*	203
The Variations in the Enzyme Concentration with the Variation in the Blood Supply to the Secreting Gland. *By J. G. Ryan*	234
On Rheotropism. — II. Rheotropism of Fish Blind in One Eye. *By E. P. Lyon*	244
Hydrolysis of Crystallized Albumin from Hen's Egg. *By Thomas B. Osborne, D. Breese Jones, and Charles S. Leavenworth*	252
The Effects of Chloride, Sulphate, Nitrate, and Nitrite Radicles of some Common Bases on the Frog's Heart. *By F. C. Cook*	263
A Method of Determining the Position of the Centre of Gravity in its Relation to Certain Bony Landmarks in the Erect Position. *By Edward Reynolds and Robert W. Lovett*	286
A Note on the Absorption of Fat. *By R. H. Whitehead*	294

No. III, June 1, 1909.

The Effects of Bone Ash in the Diet on the Gastro-Intestinal Conditions of Dogs. *By Alfred Peirce Lothrop*	297
An Improved Method of Desiccation, with Some Applications to Biological Problems. *By L. F. Shackell*	325
The Influence of the Temperature of the Heart on the Activity of the Vagus in the Tortoise. *By G. M. Stewart*	341
An Apparatus for Studying the Respiratory Exchange. *By Francis G. Benedict*	345

No. IV, July 1, 1909.

Can Functional Union be re-established between the Mammalian Auricles and Ventricles after Destruction of a Segment of the Auriculo-Ventricular Bundle? *By Joseph Erlanger*	375
Pseudo-Fatigue of the Spinal Cord. *By Frederic S. Lee and Sumner Everingham*	384
The Innervation of the Coronary Vessels. *By Carl J. Wiggers*	391
The Coagulation of Blood. *By L. J. Rettger*	406

No. V, August 1, 1909.

Page

Hydrolysis of Ox Muscle. *By Thomas B. Osborne and D. Breese Jones* 437

The Rate of Digestion in Cold-Blooded Vertebrates. — The Influence of Season and Temperature. *By Oscar Riddle* 447

The Relation of Ions to Contractile Processes. — IV. The Influence of Various Electrolytes in Restoring Muscular Contractility after its Loss in Solutions of Sugar and of Magnesium Chloride. *By Ralph S. Lillie* 459

The Absorption of Fats Stained with Sudan III. *By Lafayette B. Mendel* 493

INDEX 497

THE

American Journal of Physiology.

VOL. XXIV. APRIL 1, 1909. NO. I.

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

By EDWARD B. MEIGS.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

THE effects of heat on striated muscle have received a great deal of attention and are described in detail in many of the text-books of physiology. The effects of heat on smooth muscle, on the other hand, have been little studied; Vernon [1] has, however, given a more or less detailed account of the changes in length which take place in this tissue at various temperatures. Fig. 1 is a copy of two of Vernon's curves. The upper curve was obtained from the frog's gastrocnemius, and the lower one from a ring of the smooth muscle of the œsophagus of the same animal. The curves represent the changes in length which take place when the tissues are heated gradually from 20° C. to 90° C. in 0.7 per cent sodium chloride solution; the time required for the whole process was about thirty minutes. As is seen from the curves, the two tissues behave oppositely between about 40° and 50°, the striated muscle shortening in this interval and the smooth muscle lengthening almost as markedly. At about 53° both tissues shorten.

Vernon has studied the changes in length which occur under the influence of heat in the striated and smooth muscle of a number of different animals. He finds that the temperatures at which these

[1] VERNON: The journal of physiology, 1899, xxiv, p. 239.

changes make their appearance vary in different species, but the general result of his experiments is that shortening under the influence of heat begins at a considerably higher temperature in smooth muscle than in striated muscle. He evidently considers a preliminary lengthening characteristic of smooth muscle, though he states that it does not always occur. It is of course evident that such a lengthening could occur only in muscle which was in a state of more or less tone at the beginning of the experiment; a piece of muscle already in a state of extreme extension could not be made to lengthen any farther. It will appear later that there are other factors which may have occasionally masked the preliminary lengthening in Vernon's experiments.

I have repeated some of Vernon's experiments with the smooth muscle of the frog's stomach and have obtained in general the same results. Fig. 2 represents one of my curves. As may be seen by a comparison of Fig. 2 with the lower curve of Fig. 1, the chief difference between Vernon's results and mine is that he has obtained a larger and more rapid shortening from 53° onward. The probable cause of this difference will be discussed in the proper place.

It is an interesting question whether the smooth and striated muscles of the frog undergo the same chemical and coagulative changes between 40° and 50°, or whether the changes which occur in the striated muscle at these temperatures do not occur in the smooth muscle until a temperature of 53° or higher is reached. To answer this question I have compared the behavior of the two kinds of muscle between 40° and 50° in four respects: first, in regard to the changes in irritability; second, in regard to the coagulative changes; third, in regard to the production of acid; and lastly, in regard to the changes in weight. The importance of the last consideration will be seen later.

Vernon[2] states that both smooth and striated muscle lose their irritability at about 40°. In testing the irritability of the two tissues while they are being gradually heated, I have found it to persist to a somewhat higher temperature. In one experiment irritability disappeared in the frog's sartorius at 44°, and in the smooth muscle of the frog's stomach at 46°. No doubt the temperature at which irritability disappears depends on the nature and strength of the stimulus used and on the rapidity with which the temperature is raised; the same changes which occur almost instantaneously in the

[2] VERNON: *Loc. cit.*, pp. 242 and 254.

two kinds of muscle at 50° occur more slowly at 40°. It may be stated positively that irritability is permanently destroyed in both kinds of muscle by keeping them for five minutes at a temperature of 50°. It will be noted that this temperature is well below that at which the heat contraction begins in smooth muscle.

The question of protein coagulation is a more complicated one. A large amount of work has been done on the temperatures at which the proteins in the extracts of striated muscle are precipitated, and it has been found that these extracts contain proteins which are precipitated at various temperatures between 40° and 70°. Vincent and Lewis [3] have compared the behavior of extracts of striated muscle with that of similar extracts of smooth muscle. They found that the temperatures at which the proteins of the extract were precipitated depended on the nature of the salt used in the extraction and on the reaction of the extract, whether neutral, acid, or alkaline. They state (p. 452) that in extracts made from both kinds of muscle with 0.9 per cent NaCl solution the proteins undergo spontaneous precipitation at the temperature of the laboratory. Extracts made with 5 per cent $MgSO_4$ solution, however, behave differently, according as they are prepared from smooth or striated muscle. Some of the proteins in the striated muscle extracts are precipitated at about 47°, while no precipitation occurs in the smooth muscle extracts until a temperature of 55° or 60° is reached. But the authors adduce evidence to show that this difference depends partly on the reaction of the extracts. The extracts of smooth muscle, as ordinarily prepared, are neutral or faintly alkaline, while those of the striated muscle are distinctly acid. Vincent and Lewis quote

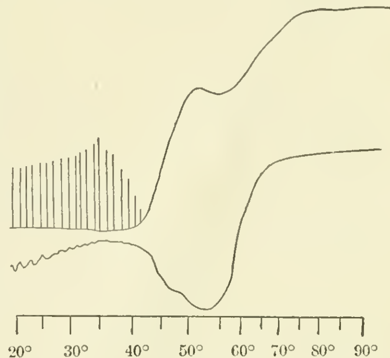


FIGURE 1. — Curves representing the effects of heat on the length of striated and smooth muscle; after Vernon. The upper curve is the heat curve of the frog's gastrocnemius; the lower one, that of the œsophagus of the same animal. In both cases the muscle was stimulated at intervals by electric shocks; the effects of this stimulation and their cessation at about 40° are represented in the curves. The heating from 20° to 90° occupied about thirty minutes. Four sevenths the original size.

[3] VINCENT and LEWIS: The journal of physiology, 1900–1901, xxvi, p. 445.

Demant,[4] who states that the proteins are not precipitated in an alkaline extract of striated muscle even after several hours at a temperature of 47°. On the other hand, the addition of 0.02 per cent lactic acid to the smooth muscle extract causes one of its proteins to be precipitated at 40°.[5]

It may be thought that the work of Vincent and Lewis does little toward answering the question whether any connection exists between protein coagulation and the heat shortening of muscle, but their experiments leave little doubt of the fact that the temperature at which the proteins in muscle extracts are precipitated depends on the reaction of the extract and on the salt used for the extraction. There is every reason to believe that the reaction of the fresh muscle fluids is neutral or very faintly alkaline. Pieces of living striated and smooth muscle pressed against litmus paper turn it blue to about the same extent, and it is improbable that the reaction of any tissue is constantly much different from that of the blood. Finally, it is perfectly easy to prepare a neutral or faintly alkaline extract of striated muscle if the precaution be taken to crush the living muscle under ice-cold alcohol according to the method of Fletcher and Hopkins.[6] Extracts of striated and smooth muscle prepared in this manner do not differ appreciably in their reaction, which is about that of the blood $[(\overset{+}{H}) = 4 \times 10^{-8}]$.

It will be shown later that, when either striated or smooth muscle is heated to a temperature between 40° and 50°, very considerable amounts of lactic acid are formed within the tissue, — much more in both cases than Vincent and Lewis found necessary to markedly alter the coagulation temperatures of the muscle proteins. It will be seen, therefore, that it is impossible to determine the temperatures at which the proteins coagulate in a gradually heated living muscle from the temperatures at which they coagulate in the muscle extracts. The rapidity with which the proteins coagulate at any given temperature in the muscle will depend on the rapidity with which the acid is formed at that temperature, and this is still an unknown quantity. A rough idea of the temperature at which the proteins coagulate in the living muscle may, however, be gained by watching pieces of muscle as they are heated at the rate used by Vernon in

[4] Vincent and Lewis: *Loc. cit.*, p. 450.

[5] Vincent and Lewis: *Loc. cit.*, p. 451.

[6] Fletcher and Hopkins: The journal of physiology, 1907, xxxv, p. 252.

his experiments. In both the smooth and striated muscle of the frog there is little change in appearance under this treatment until the temperature rises above 45°; between 45° and 50° there usually occur a marked whitening and opacity in both cases. It is quite certain, therefore, that a considerable protein coagulation occurs in both striated and smooth muscle if the tissues be kept for a few seconds at 50°.

The question of acid formation must next be considered. Fletcher and Hopkins [7] have shown that one of the most striking characteristics of heat rigor in striated muscle is the production of a large amount of lactic acid. These authors have made quantitative determinations of the amount of lactic acid in fresh muscle and in muscle which has been kept for an hour at between 40° and 50°. They find that the fresh muscle contains only from 0.015 per cent to 0.02 per cent of lactic acid, while that which has been heated contains about 0.4 per cent, — some twenty times as much. My experiments have been directed toward answering the question whether a similar production of lactic acid occurs in smooth muscle similarly heated. As material for this study I have used the muscular coat of the frog's stomach and in one case that of the cat. The stomach is dissected out, freed as much as possible from external connective tissue, and cut open along the line of the lesser curvature. The main muscular coat is then separated from the mucosa and sub-mucosa. Such a preparation of smooth muscle from an average frog weighs about 0.25 gm. In making my extracts I have uniformly employed the method described by Fletcher and Hopkins. The muscle is crushed under 96 per cent alcohol at 0°, then ground as fine as possible and allowed to stand under the alcohol for twenty-four hours. The mixture of alcohol and muscle is then filtered, the filtrate evaporated to dryness, and the residue rubbed up with distilled water at 100° and a little animal charcoal. This mixture is again filtered and the filtrate tested in the manner to be described later. As a rule, the muscle from the stomachs of 10 frogs, weighing about 2.5 gm., was used in each experiment. Enough water was used in dissolving the last residue to give a final filtrate of about 5 c.c. Extracts made in this manner from fresh muscle were compared in various ways with extracts similarly made from muscle which had been kept for an hour at between 40° and 50°.

[7] FLETCHER and HOPKINS: *Loc. cit.*, pp. 266 *et seq.*

The extracts were tested with regard to their action on litmus paper and rosolic acid, and also by Ueffelmann's test, and by a new color test for lactic acid recently devised by Fletcher and Hopkins.[8] All these tests showed that the extract of heated smooth muscle is distinctly more acid than that of the fresh muscle; and the two specific tests used indicate that the acid formed during the heating is lactic acid.

The effect of the muscle extracts on the color of rosolic acid was compared with that of a series of standard mixtures of monosodium phosphate and disodium phosphate. The results indicate that the extract of fresh muscle has an alkalinity equal to that of a solution in which $(\overset{+}{H}) = 4 \times 10^{-8}$, while the extract of heated muscle has an acidity equal to that of a solution in which $(\overset{+}{H}) = 12 \times 10^{-8}$. That is, the extract of fresh muscle is, in the physico-chemical sense, about as alkaline as an $m/8{,}333{,}333$ solution of NaOH in distilled water, while the extract of heated muscle is about as acid as an $m/15{,}000{,}000$ solution of HCl. I wish here to offer my thanks to Dr. L. J. Henderson for his kindness in suggesting this test for the reaction of the extracts and in providing me with the standard mixtures of monosodium and disodium phosphate. Dr. Henderson calculates that the results indicate a probable lactic acid production during the heating of from 0.1 per cent to 0.2 per cent of the weight of the muscle. These results are of course extremely rough, but they are in full agreement with the others, which indicate that considerable amounts of lactic acid are formed in smooth muscle when it is heated, though much less than under similar circumstances in striated muscle. With Ueffelmann's test the extracts of heated muscle give a positive reaction, while those of the fresh muscle give either a negative reaction or a positive reaction very much fainter than that obtained with the extract of heated muscle. In one case I tested the extract of heated muscle by the new color test for lactic acid recently devised by Fletcher and Hopkins. These authors believe that this test is in general more specific for lactic acid than Ueffelmann's test and quite specific in the case of physiological material. The extract of heated muscle gives a strong positive reaction with this test.

Besides testing the extracts in the manner described above, I have compared the reaction of fresh smooth muscle with that of

[8] Fletcher and Hopkins: *Loc. cit.*, p. 308.

muscle which has been heated to between 40° and 50°, by simply pressing such pieces of muscle against litmus paper. The heated muscle is always distinctly the more acid. It might be objected that in this case the increased acidity is due to CO_2, but this is unlikely, as Fletcher[9] has shown that a surviving muscle is continually giving off CO_2, which indicates of course that its fluids are always saturated with this substance.

There is every reason to believe, then, that smooth muscle heated to between 40° and 50° produces lactic acid, though in somewhat less quantity than striated muscle.

There is little tendency toward a change in weight in either smooth or striated muscle heated to 50° or less. Both kinds of muscle may be kept for half an hour in 0.7 per cent salt solution at 50° without undergoing any considerable changes in weight.

Except therefore in regard to length, smooth muscle and striated muscle undergo similar changes when heated to between 40° and 50°. Both lose their irritability, in both the proteins are coagulated, probably at about 50°; in both considerable amounts of lactic acid are formed, and neither exhibits any marked tendency to change in weight. The shortenings which occur in both tissues at 52° and above must next be considered.

It must first be pointed out that the changes in length which occur between 40° and 50° are highly characteristic of muscle, while those which occur at 52° and above are by no means so. There are very few substances which undergo a change in length of from 30 per cent to 60 per cent when heated to between 40° and 50°, but there are a great many substances which shorten quite as rapidly and quite as strongly as muscle does when heated to 52° and above. Among such substances nerve,[10] tendon,[11] and the catgut of violin strings are conspicuous. The last-named substance is, of course, prepared connective tissue, but the fact that it is still capable of undergoing such shortening after its preparation for commercial purposes indicates that the shortening in question is not a manifestation of any property peculiar to the state in which the tissues are found in the living body.

It is a fact well known to histologists that tissues "shrink" when

[9] FLETCHER: The journal of physiology, 1898, xxiii, p. 10.

[10] HALLIBURTON: Biochemistry of muscle and nerve, Philadelphia, 1904, pp. 105 and 106.

[11] JENSEN: Zeitschrift für allgemeine Physiologie, 1908, viii, pp. 309 and 310.

heated to a temperature much above 50° in the paraffin oven. This shrinkage takes place in all dimensions, though chiefly in the longitudinal direction in such tissues as nerve and tendon. It may amount to nearly half the original length of the tissue. Such shrinkage is of course accompanied by a passage of the melted paraffin out of the tissue interstices, and it seems to be a very general property of organic tissues.

The characteristic peculiarities of "shrinkage" in the histological sense are that it takes place in tissues saturated with fluid at temperatures above 50°, and that it is accompanied by a passage of fluid out of the tissue interstices and by a consequent loss of weight.

The later heat shortenings of muscle resemble shrinkage in the temperature at which they make their appearance and in the fact that they occur in a tissue saturated with fluid. It is an interesting question whether they are accompanied by a passage of fluid out of the tissue. This question may be answered at once in the affirmative. Both striated and smooth muscle exhibit a marked tendency to lose water when heated above 50°. The following experiments may serve as evidence for this statement.

A frog's sartorius was dried rapidly on filter paper and found to weigh 0.150 gm. It was then immersed for twenty-four minutes in 0.7 per cent sodium chloride solution at 50°, after which it was again dried and weighed; it still weighed 0.150 gm. It was now immersed for two minutes in 0.7 per cent sodium chloride solution at 65°, and, on being again dried and weighed, was found to have decreased in weight to 0.120 gm.

An exactly similar experiment carried out on a piece of the smooth muscle from a frog's stomach showed that this tissue suffered no considerable change in weight during twenty-four minutes' immersion in 0.7 per cent sodium chloride solution at 50°. During two minutes' immersion in the same solution at 65°, however, it decreased in weight from 0.050 gm. to 0.035 gm.

The above experiments were carried out to show that the loss of weight takes place rapidly and at a comparatively low temperature. If the tissue be kept at a higher temperature for a longer time, the loss of weight may be very much more marked. In one case a piece of smooth muscle from the frog's stomach lost one half of its original weight during about five minutes' immersion in physiological salt solution at 85°.

The shortening of catgut at about 55° is in many respects similar

to that of muscle at the same temperature. So much stress has been laid by Engelmann [12] on this subject that it seems worth while to consider it in some detail. Engelmann soaks commercial catgut in water for an hour, then heats it for two or three minutes to above 80°, after which it is ready for use. Such a piece of catgut will shorten whenever heated above 55° and lengthen on being allowed to cool again provided it be kept always saturated with water. I have followed the changes in length and the changes in weight which occur during all these processes. During the preliminary soaking the gut of course gains in weight and at the same time shortens very slightly. During the two minutes' heating above 80°, it gains still further in weight and shortens from 40 per cent to 50 per cent of its original length. At the same time it is partly gelatinized, and the previously twisted fibrils become untwisted. If it be now immersed in water at room temperature, it gains still further in weight and lengthens markedly. The absorption of water and lengthening go on for some time, — perhaps an hour, — after which an equilibrium is reached. The gut is now in the state in which Engelmann uses it for his experiments; it will shorten and lose water whenever heated to above 55°, and lengthen and absorb water on being allowed to cool again. The loss of water on heating takes place very quickly and is quite large, often more than 10 per cent of the original weight of the catgut.

The experiments which show this were carried out as follows: A piece of gut prepared for Engelmann's experiment was dried on filter paper and weighed. It was then immersed for two or three minutes in water at about 65° and again dried and weighed. Finally it was immersed for a few minutes in water at room temperature and dried and weighed as before. Sometimes these processes were repeated once or twice with the same piece of gut. The figures in a typical experiment were as follows:

A piece of catgut prepared for Engelmann's experiments weighed	0.345 gm.
The same piece after 2 minutes' immersion in water at 67° weighed	0.310 gm.
The same piece after 8 minutes' immersion in water at 20° weighed	0.350 gm.
The same piece after 2 minutes' immersion in water at 65° weighed	0.320 gm.
The same piece after 8 minutes' immersion in water at 20° weighed	0.370 gm.

It will be noticed that the catgut on being allowed to cool after the first heating gains more in weight than it lost during the heat-

[12] Engelmann: Ueber den Ursprung der Muskelkraft, Leipsic, 1893.

ing and that this tendency continues through the subsequent heating and cooling. It is practically certain that the heating of catgut in this manner causes two different changes. One of these is an irreversible tendency toward gelatinization and the capacity to absorb more water. The other is the actual driving out of water already present and is reversed on subsequent cooling. That the shortening accompanying the heating is connected with the loss of water and not with the tendency toward gelatinization is shown by the fact that it is reversed on cooling when the direction of water flow is reversed. It may also be shown by an independent experiment. If a piece of catgut prepared for Engelmann's experiment be allowed to dry, it shortens quite markedly during the drying and may be made to lengthen again by re-immersion in water.

The changes which occur during the preparation of the catgut for Engelmann's experiment seem to be of a different nature. Both the preliminary soaking and the first heating to above 80° are accompanied by a gain in weight and a decrease in length. It seems possible that the shortening in these cases is due to the fact that the catgut is much stretched during its preparation, and fixed, as it were, in a state of extension by drying. But the nature of these phenomena has no particular bearing on the questions under discussion. I only wish to show that the shortening which occurs in catgut prepared for Engelmann's experiment is accompanied by a loss of water and that the subsequent lengthening is accompanied by an absorption of water.

A remarkable peculiarity of the behavior of the catgut is the complete reversibility of the shortening and of the loss of water. These tendencies are exhibited by both striated and smooth muscle to a slight extent. Both tissues lengthen somewhat on being allowed to cool after being heated to 70° or 80°, and in both there may be demonstrated a slight tendency to reabsorb some of the water which had been lost. It must be admitted, however, that the catgut exhibits these tendencies much more markedly than does the muscle.

The differences between Vernon's results with smooth muscle and my own must now be briefly discussed. Fig. 2 represents the changes in length undergone by a piece of smooth muscle heated gradually to 100° in a solution containing 0.67 per cent NaCl and 0.06 per cent Na_2CO_3. The Na_2CO_3 was added because smooth muscle gradually loses tone in pure physiological sodium chloride solution, and it was found that this amount of alkali preserved the

particular piece of muscle used in about the state of tone it had on immersion in the solution. I have found that pieces of smooth muscle preserve their irritability in such weak alkaline solutions for a long time.

If Fig. 2 be compared with the lower curve of Fig. 1, it will be seen that in the former the preliminary lengthening begins at a

FIGURE 2. — Curve showing the effects of gradual heating on the smooth muscle of the frog's stomach; the heating was carried out at such a rate that it required twenty-four minutes for the temperature to rise from 20° to 100°. The muscle was weighted with 0.6 gm. Magnification of writing lever, 5; length of muscle between attachments at end of experiment, 6 mm.; proportional shortening, 36 per cent of greatest length. Two thirds the original size.

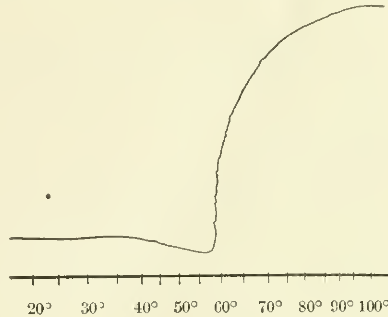


FIGURE 3. — Curve showing the effects of gradual heating on a ring of the mucous membrane of the frog's stomach; the heating was carried out at such a rate that it required twenty-six minutes for the temperature to rise from 20° to 100°. The tissue was weighted with 0.6 gm. Magnification of writing lever, 5; length of tissue between attachments at end of experiment, 8 mm.; proportional shortening, 54 per cent of original length. Two thirds the original size.

considerably lower temperature (about 25°) and that the heat shortening begins at a higher temperature (between 55° and 60°). I have obtained these results with a good deal of constancy, and do not know how they are to be explained; possibly they are due to differences between English and American frogs, possibly to differences between the tissues of the œsophagus and those of the stomach.

A more striking difference between Vernon's results and mine is the greater rapidity and height of the heat shortening which he usually obtains. It seems possible that he failed to strip the mucous membrane from his preparations. He nowhere states that he did this, and in the case of the œsophagus it is a much more difficult operation than in the case of rings from near the middle of the stomach.

If this surmise is correct, the great rapidity and height of Vernon's heat shortenings may be readily explained. Fig. 3 represents the heat shortening of the ring of mucous membrane torn from the muscle ring, of which the behavior is recorded in Fig. 2.

The facts which have just been reported are, to say the least, unfavorable to the coagulation theory as an explanation either of the contraction of heat rigor or of contraction in general. In the case of smooth muscle it seems impossible to suppose that there is any close connection between shortening and coagulation. A large proportion of the protein of this tissue is undoubtedly coagulated at or below 50°, yet the muscle lengthens markedly when heated to that temperature.

There is much evidence to show that in striated muscle also no connection exists between shortening and protein coagulation, and that the shortening which occurs in so many animal tissues at about 55° is not the result of protein coagulation in those tissues. This evidence will be presented in detail in a later article. For the present it will be enough to say that a marked shortening occurs at about 55° in striated muscle of which all the protein precipitable at that temperature has been already coagulated by the long-continued action of strong alcohol.

Whatever may be thought of the shortenings which occur in muscle at 55°, there is plainly no reason to believe that they constitute a peculiarity of muscular tissues. It is much more probable that they are of the same general nature as the shortening which occurs in catgut at the same temperature. The changes in length which occur below 50°, however, are peculiar to muscle; and for these the following explanation may be offered.

Moore and Parker,[13] Lillie,[14] and others have demonstrated that the addition of very small quantities of acid or alkali to certain colloid solutions greatly increases their osmotic pressure. It has been known for a long time that muscle also possesses this property, — it eventually swells when immersed in weak acid solutions even though the osmotic pressure of the solution be increased by the addition of crystalloids to a point much above that of the muscle. Fischer[15] has reported some interesting results on this question, and has shown that the behavior of frog's muscle immersed in

[13] Moore and Parker: This journal, 1902, vii, p. 261.

[14] Lillie: *Ibid.*, 1907, xx, p. 127.

[15] Fischer: Archiv für die gesammte Physiologie, 1908, cxxiv, p. 69.

various solutions of acids, bases, and salts, and in distilled water corresponds quite closely to the behavior of a preparation of fibrin treated in the same manner.

Fletcher and Hopkins have shown that a large production of lactic acid is a constant accompaniment of heat rigor in striated muscle. My own experiments, though far less complete and satisfactory from the chemical standpoint than those of Fletcher and Hopkins, nevertheless indicate clearly that lactic acid formation is a constant accompaniment of heat coagulation in smooth muscle.

It seems highly probable, from the observations of Moore and Parker, Lillie, and Fischer, that the presence within a muscle of acid in such quantities as are formed in both striated and smooth muscle at temperatures between 40° and 50° would cause a swelling of those parts of the tissue in which the colloids are most concentrated at the expense of the interstitial spaces. The histological evidence indicates that the colloids are most concentrated in the fibrillæ or sarcostyles of striated muscle and in the fibre cells of smooth muscle.

In a recent article[16] I have adduced evidence to show that swelling of the sarcostyles of striated muscle results in their shortening, while swelling of the fibre cells of smooth muscle results in their lengthening.

In this way, therefore, all these facts may be grouped together without the aid of a single gratuitous assumption. It is certain that the heating of either striated or smooth muscle causes the production of a considerable amount of lactic acid within the tissue. There is every reason to believe that the presence of acid in such quantities would cause the swelling of the sarcostyles in the one case and of the fibre cells in the other. Finally, there is much independent evidence to show that the swelling of the sarcostyles of striated muscle always results in their shortening, while the swelling of the fibre cells of smooth muscle results in their lengthening.

[16] MEIGS: This journal, 1908, xxii, p. 477.

ON THE CONNECTION BETWEEN CHANGES OF PERMEABILITY AND STIMULATION AND ON THE SIGNIFICANCE OF CHANGES IN PERMEABILITY TO CARBON DIOXIDE.

By R. S. LILLIE.

[*From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory Zoölogical Department, University of Pennsylvania.*]

Introductory.

A VARIETY of evidence now exists indicating that stimulation of an irritable tissue is dependent upon a temporary and readily reversible increase in the permeability of the surface layers or plasma membranes of its cells or elements.

Perhaps the most unequivocal evidence of this kind is that presented by the motile organs of such plants as Mimosa and Dionæa or the stamens of Cynareæ. In the sensitive plant (Mimosa pudica) the normal position of the leaves is maintained by the turgidity of the pulvini or cushion-like masses of parenchyma cells at the base of each leaflet and petiole. On stimulation this turgidity undergoes a sudden diminution accompanied by an escape of fluid from the turgid cells into the intercellular spaces which communicate with the vessel system of the plant. The pulvinus thus decreases in volume and the petiole falls to the drooping position characteristic of the stimulated plant. If the latter is undisturbed, a gradual recovery follows (in the light). Since turgor in plant cells is due to the osmotic pressure of the cell contents acting against the semipermeable plasma membrane (Pfeffer, de Vries), and so putting the extensible cell wall on the stretch, such a sudden collapse of the cell seems open to only two possible interpretations: (1) if the permeability of the protoplast remains unaltered, the effect can be due only to a sudden decrease in the concentration of the osmotically active substances within the cell, presumably by combination to form larger and fewer molecules; or (2) if, on the other hand, the

plasma membrane becomes suddenly permeable to the dissolved substances, the resistance to the collapse of the stretched cell wall must at once disappear, and the movement necessarily follows this disturbance of equilibrium. That the latter change is the main if not the sole determinative factor is indicated by several considerations: first, the invariable rule is that the cells regain their turgor only slowly; *i. e.*, time appears to be required for the production of osmotically active substances, indicating that these have been lost from the cell [1] and not merely rendered less active by combination to form larger molecules; in the latter case the reverse or splitting process would presumably take place with equal velocity and we should expect the curve of restoration of turgor to correspond with that of loss. The fact that the rising and falling portions of the curve are not symmetrical — as in the case (approximately) with muscular contraction — may be interpreted as indicating a mechanism not depending primarily on rapid chemical change. Second, similar changes of turgor leading to similar changes in the position of the leaves occur in practically all plants when the cells die; the loss of turgor in this case is accompanied by a demonstrable increase in the permeability of the cell and is unquestionably due to this. This change, in fact, differs from the preceding chiefly in not being reversible. Again, heat, poisons, and other destructive influences produce simultaneously increase of permeability and loss of turgor in all plant cells, and corresponding movements result in the case of motile organs. The dependence of turgor changes on changes in permeability is in fact clearly recognized to-day by the majority of plant physiologists.

The electrical change following stimulation in motile plant tissues is similar, as shown by Burdon-Sanderson,[2] to that seen in animal cells; *i. e.*, a negative variation occurs, — as also in dying tissues, where the permeability evidently undergoes an increase. This agreement indicates a fundamental similarity in the stimulation process in both classes of organisms. It is now generally agreed that the source of these potential differences in living tissues — systems con-

[1] The fluid which exudes from the pulvinus during stimulation is known to be not pure water, but a solution of considerable concentration (Pfeffer). *Cf.* Jost: Lectures on plant physiology, Oxford, 1907, p. 515.

[2] A summary of Burdon-Sanderson's work with references to his original papers is given in Biedermann's Electro-physiology, English translation, ii, ch. 6, pp. 1 *et seq.*

taining only electrolytic conductors — can be only some type of concentration cell; and since the potential differences are in many cases too great to be accounted for without assuming that certain ionic velocities must be markedly different in living tissues from those observed in homogeneous solutions, it is assumed that the *membranes* in tissues play an important *rôle* in changing the ionic velocities, depressing some and (possibly) increasing others. The theory of Bernstein,[3] based on a suggestion of Ostwald, that the semi-permeable membranes of resting cells are freely permeable to the cations of the electrolyte to which the potential difference is due, but not to its anions, affords a satisfactory explanation of the changes resulting from stimulation. If the permeability to anions increases during stimulation, the observed fall of potential between exterior and interior of the cell becomes at once intelligible. Now at death, when the permeability evidently increases, there is a fall of potential similar to that seen during stimulation, but permanent in this case. The simplest and most probable inference to be drawn from this fact is that a similar increase of permeability occurs momentarily during stimulation. This conclusion is confirmed and reinforced by the phenomena observed in motile plant cells, as we have just seen.

In the case of animal cells the evidence is in general of a less direct nature than in plants. Certain fundamental facts plainly have the above significance. There is the same increase in the permeability of the cell at death, as indicated by readier entrance of dyes, loss of susceptibility to plasmolysis, and outward diffusion of coloring matters formerly confined within the plasma membrane. This *post mortem* increase of permeability is accompanied by an electrical change of the same nature as that resulting from stimulation, and in some cases, as in muscle, by contractile changes also; the basis of these latter appears to be a coagulation of the muscle proteins (Kühne); coagulative changes in the protoplasm are in fact very generally associated with the *post mortem* increase of permeability and presumably result from it, and such changes in some instances result from excessive stimulation.[4] Again, conditions that increase the permeability of the surface layers have in general also a stimulating action: heat, mechanical influences, electricity, action of vari-

[3] J. Bernstein: Archiv für die gesammte Physiologie, 1902, xcii, p. 521.

[4] *Cf.* the discussion in my former paper on this subject: This journal, 1908, xxii, p. 75, especially pp. 81–83.

ous chemical reagents. The effect of these agencies in increasing permeability is most readily seen in the case of pigment-containing cells such as blood corpuscles, pigmented ova, and the cells of certain organisms like Arenicola larvæ, which last show this interdependence with remarkable clearness. The cells of this organism contain large quantities of a water-soluble yellow pigment; and, as I shall describe later, conditions that produce energetic and persistent muscular contractions (action of various salts and fat solvents) invariably cause a visible loss of pigment from the cells. It has long been known that the electric current, the most universal stimulating agent, increases the permeability of blood corpuscles, as shown by the laking action of induction shocks and condenser discharges.[5] Further, the view of Overton,[6] that the plasma membrane owes its specific permeability to lipoid substances, explains the stimulating action of fat solvents,[7] and its nature as colloid layer accounts for the action of electrolytes and explains why such substances both stimulate and alter permeability.[8]

Certain other well-known facts, which, so far as I am aware, have not hitherto received attention from the present point of view, strengthen the theory that stimulation is a consequence of temporary increase in permeability. Electrical stimulation almost certainly depends on localized changes in ionic concentration within the irritable tissue. Several years ago Nernst propounded the view that these changes in concentration have their seat at the various semipermeable surfaces of the tissue, *i. e., at the plasma membranes of the cells,* which the researches of de Vries and Overton have shown to be impermeable[9] in the resting cell to the electrolytes normally present in tissues. Such membranes must therefore offer a barrier to the movement of ions when a current is passed. As Nernst has

[5] *Cf.* HERMANN: Handbuch, 1880, i, pp. 14 *et seq.* for an account of these phenomena.

[6] For the application of this theory to the case of muscle, *cf.* OVERTON: Archiv für die gesammte Physiologie, 1902, xcii, p. 115.

[7] It appears also highly probable that cytolytic substances like toxines owe their stimulating action, as seen in fever or more obviously in the contraction of tetanus, to their influence on the permeability of nervous or muscular elements.

[8] *Cf.* HÖBER's interesting discussion, Physikalische Chemie der Zelle und der Gewebe, 2te Aufl., Leipzig, 1906, ch. 6, "Die Permeabilität der Plasmahaut," and ch. 8, "Wirkung reiner Elektrolytlösungen," especially pp. 270 *et seq.*

[9] The experiments demonstrate either impermeability or slow and difficult permeability to neutral salts of alkali or alkali earth metals.

shown, the observed inverse proportionality between the stimulating action of an alternating current and its rate of alternation may be theoretically accounted for on the above assumptions.[10] On the present theory these changes in ionic concentration cause stimulation by altering the colloidal consistency of the plasma membrane and thus increasing its permeability, — this last effect being the immediate condition of the stimulating action. The essential feature of Nernst's hypothesis — that stimulation is conditional on impermeability (absolute or relative) to ions — is, however, fundamental to any theory of the nature of electrical stimulation. Now it is obviously a corollary of this hypothesis that if during stimulation the plasma membrane is so changed as to become freely permeable to ions, *further stimulation should be impossible at such times.* This is in fact the case, as is shown by the persistence, for a greater or less interval after stimulation, of a period during which the tissue is non-responsive or refractory to stimulation. This condition, as all physiologists are aware, is most easily demonstrated in heart muscle; but it exists even for highly irritable tissues like nerve.[11] The refractory period, on this interpretation, is simply a period of increased permeability, during which the action of the electrical stimulus, which depends on the semi-permeability of the surface layers, is accordingly suspended. In other words, the refractory period is to be considered as evidence of a loss of semi-permeability at the height of stimulation.[12]

A further argument may be drawn from the striking analogies which the work of Bredig, Weinmayr, Wilke, and Antropoff [13] have shown to exist between the phenomena of pulsatile catalysis in the case of mercury surfaces in contact with hydrogen-peroxide solution, and the rhythmical process in organized tissues like cardiac muscle. The curves representing the variations in the velocity of

[10] NERNST: Archiv für die gesammte Physiologie, 1908, cxxii, p. 275.

[11] For the case of skeletal muscle *cf.* BAZETT: Journal of physiology, 1908, xxxvi, p. 414; of nerve, *cf.* BOYCOTT: *ibid.* 1899, xxiv, p. 144.

[12] There may be only a marked *diminution* in the normal semi-permeability, not an absolute loss; according to CARLSON the so-called *refractory* period is only relatively so (This journal, 1906, xvi, p. 67); *i. e.*, the excitability is merely *lowered*, to a degree varying in different animals.

[13] BREDIG and WEINMAYR: Zeitschrift für physikalische Chemie, 1903, xlii, p. 601; BREDIG and WILKE: Biochemische Zeitschrift, 1908, xi, p. 67; BREDIG: *Ibid.*, 1907, vi, p. 322; ANTROPOFF: Zeitschrift für physikalische Chemie, 1908, lxii, p. 513.

oxygen liberation from such surfaces show a surprising similarity to the ordinary sphygmogram or cardiogram; and parallel with the rhythm of oxygen production runs a corresponding rhythmical variation in the electrical potential difference between the peroxide solution and the mercury surface. Changes of surface tension also accompany the changes in electrical potential. The analysis of the phenomena given by Antropoff makes it highly probable that the source both of the electrical rhythm and of the oxygen evolution is a periodic electrolytic dissolution of the film of mercury peroxidate formed over the surface of the mercury by the action of the peroxide; the disappearance of this film is preceded by the appearance of a mechanically induced rupture which exposes the unaltered metallic mercury below, between which and the external surface of the film there exists a potential difference estimated at *ca.* 0.12 volt. At the margin of this fissure a circuit is thus formed by whose action the film undergoes electrolytic reduction to metallic mercury, and the change thus induced travels over the whole surface. The phenomenon is repeated in regular rhythm. The especially significant feature from the standpoint of the physiology of stimulation is that the primary occasion for this dissolution of the film is a *break in its continuity.* This corresponds to a *localized increase in permeability;* in consequence of this change the condition for the reduction of the film and the liberation of oxygen is propagated over the entire surface of the mercury.

On the theory of stimulation advocated in the present and preceding papers, the primary change in stimulation is also an increase in permeability at the point of stimulation. This results at that point in a localized depolarization which travels thence over the surface of the irritable element and is accompanied by an increased permeability. The *conduction* of this state of depolarization is an essential feature of the stimulation process, which otherwise would have an effect only at the exact point of application of the stimulus. The analogy of the mode of propagation of the surface change in the film-covered mercury drop — which is accompanied by a similar electrical disturbance — points to the possibility that in the irritable tissue *the point of stimulation acts as cathode on the adjoining portion of plasma membrane* and depolarizes this which in its turn serves as stimulating cathode for the next layer, and so on.[14] On

[14] In a more general form this view has long been held. *Cf.* Gotch's article in Schafer's Text-book, ii, 1900, pp. 458–459. The conception that permeability

this view the progressive dissolution of the surface film in the mercury drop and the similarly progressive depolarization with accompanying increase of permeability in the irritable tissue are strictly analogous and similarly conditioned phenomena.[15]

The common feature of the above two processes, otherwise so widely disparate, thus appears to lie in an automatic and periodic alteration of the surface layer in the direction of increased permeability, — in the one case the film being temporarily removed by the accompanying electrolytic process, in the other merely altered so as to acquire the increased permeability needed for depolarization and consequent stimulation. That periodic alterations in the ionic permeability of cardiac muscle accompany the rhythm of contraction is indicated by the action current; this must correspond to an ionic transfer between interior and exterior of the fibre, following a rhythm parallel with that of the stimulation process. The similarity in the surface process in the two phenomena under consideration is thus the ground of the similarity in the respective curves. If the carbon-dioxide output of the beating heart could be measured by a similar device to that used by Antropoff for the oxygen evolution from the mercury surface, it would doubtless be found to exhibit a similar rhythmicity corresponding to the rhythm of the contraction and of the potential change.

An argument from analogy or from a resemblance which a closer examination may show to be superficial may not in itself be very convincing; the present comparison may, however, serve to reinforce the preceding considerations. In brief, the situation stands thus: the automatic rhythm of cardiac muscle, and presumably of a cilium or an automatic nerve centre, is associated with an electrical disturbance which is most readily explained on the ground of a periodic increase in surface permeability. If an inorganic phenomenon showing such strikingly similar characteristics as the above is actually shown to be dependent on an automatic and periodic alteration of the surface layer, the grounds for assuming a similar

changes are the essential feature of the stimulation process is, however, purely an outcome of modern physico-chemical analysis.

[15] It has not, however, been shown that such an hypothesis can account for the high velocity with which the depolarization wave is conducted over the surface of many living tissues, particularly nerve; and until this is done the present suggestion has perhaps only the interest of a possible, though in my opinion also a highly probable, explanation of the nature of conduction in irritable tissues.

condition in the case of the organized tissue are certainly strengthened. Since the only essential distinction between the phenomena of stimulation in cardiac muscle (cilia, etc.) and in skeletal muscle or nerve is the automaticity and regular periodicity of the former under physiological conditions, any general conclusions reached from the above comparison must apply also to the phenomena of stimulation in general.

Experimental.

During the past summer at Woods Hole I have continued the study of the processes of stimulation and contraction in the larvæ of Arenicola cristata. The simple embryonic type of musculature possessed by this organism seems particularly fitted for the investigation of the more general and fundamental conditions of these processes. The following have been the chief questions under consideration: (1) the nature of the change induced by salt solutions that stimulate powerfully (isotonic sodium and potassium salts, etc.); (2) the nature of the inverse action shown by pure non-electrolyte solutions, anæsthetics like chloroform in low concentration, and certain salt solutions (*e. g.*, isotonic magnesium salts) which temporarily deprive muscles of contractility; (3) the nature and concentration of the electrolytes that restore contractility to muscles deprived of this property by solutions of non-electrolytes or magnesium salts, and the nature of this action; (4) the conditions under which lipoid solvents, like ether, chloroform, benzol, etc., produce stimulation instead of anæsthetization; (5) the influence of carbon dioxide on permeability and contraction.

These experiments are incomplete and will be continued. In the present paper I propose to describe in detail only those observations which appear to indicate clearly an increase in permeability during stimulation, or a decrease during inhibition, either by showing a visible transfer of substance between interior and exterior of the cells during strong stimulation, or, inversely, by showing that during anæsthesia (depressed irritability) the entrance of substances from outside (*e. g.*, dyes) or the exit of substances from the cells is less rapid than during muscular activity. In a later paper I shall describe experiments bearing more directly on the other problems cited above.

In an early paper [16] I have given a brief description of the appearance, organization, and behavior of Arenicola larvæ at the swarming stage. The body musculature is of a simple embryonic type, and consists at this stage exclusively of longitudinal fibres, no trace of circular muscles being visible until much later in development. The muscle cells are without definite boundaries; they consist at first of a mesenchyme which becomes applied to the ectoderm, and on the side adjoining this latter layer longitudinal fibrils early appear in the mesenchyme cells immediately beneath the cell surface. The fibrils thus show the superficial position within the cell characteristic of primitive or embryonic muscle fibres; they are unstriated and when fully formed relatively thick in comparison with the diameter of the muscle cell, indicating a progressive transformation of the sarcoplasm into the substance of the fibril.[17] The absence of circular fibres at this stage indicates that both elongation and contraction are active properties of the muscle (as Sherrington has shown for Vertebrata). The state of extension of the larva depends on the tone of the musculature, and this is variable and largely determined by the reaction of the external medium, slight acidity producing increase and alkalinity decrease of muscular tone.[18]

Another peculiarity of the larva is the presence in its cells of large quantities of a yellow pigment, which appears at this stage diffused throughout the entire organism. This pigment is derived from the egg cell which is similarly colored, and disappears in later larval stages. The individual larva as seen by transmitted light is semi-opaque and light yellowish brown in appearance; when densely massed together, as in consequence of their positive phototaxis, the larvæ exhibit a characteristic dark brown tint.

When large numbers of larvæ are treated with cytolytic substances like chloroform, or when they die, or are caused to contract strongly by the action of certain solutions (isotonic NaCl, KCl, NH_4Cl, etc.), the pigment leaves the cells and colors the water a

[16] R. S. Lillie: This journal, 1901, v, p. 56.

[17] For a fuller account of the structure and development of the musculature of Arenicola larvæ, with figures illustrating the above-described conditions, *cf.* my paper "On the structure and development of the Nephridia of Arenicola," in Mittheilungen aus der zoologischen Station zu Neapel, 1905, xvii, p. 341. The structure of the young swarming larva is somewhat fully illustrated in the figures on Plates 22 and 23.

[18] These effects will be described in a later paper.

light yellow. An increase of permeability is thus indicated. It must be assumed in the latter case that the pigment is derived from *all* of the pigment-containing cells and not merely from the muscles; *i. e.*, the increase in permeability caused by the salt is an effect exhibited by all the cells in common, although contraction is presumably confined to the muscle cells. The exit of pigment is of importance as indicating an increased permeability; that it is accompanied by strong contractions is therefore significant. In the other cells of the larva no immediate evidence of change is seen under these conditions; but later coagulative and cytolytic alterations follow, and the entire organism swells and disintegrates: the connection between death coagulation and increase of permeability is thus shown.

In general, any change that induces a rapid loss of pigment causes at the same time energetic muscular contraction. During the normal movements of the animal there is no such loss; in this case it is to be supposed that the stimulating action is confined to the neuromuscular system of the organism and that the majority of the pigment-containing cells are unaffected. It should be added that the contraction which accompanies rapid loss of pigment (as in *m*/2 KCl) is always far more energetic than any normal movement — the larva shortening to fully half its normal length and remaining thus contracted for several seconds. Conditions that produce an increase of permeability sufficient for rapid loss of pigment thus act as unusually intense stimuli. Such conditions are also highly injurious to the organism.

In the following experiments the procedure has been similar to that already described in my former papers. The larvæ, which are strongly heliotropic, are allowed to gather in a mass on the light side of the watch glass. The sea water is then poured off, and its last traces are removed by filter paper; the solution is then added.

Action of salt solutions. — Pure isotonic NaCl solutions (*m*/2 — ⅝ *m*) are rapidly destructive, as I have already described.[19] The effect may readily be observed under a magnification of 50 diameters.[20] At the instant of contact with the solution the organism contracts suddenly and violently to about half its normal length. The

[19] R. S. Lillie: *Loc. cit.*

[20] The larvæ average about one third millimetre in length.

cilia for the most part undergo rapid dissolution or liquefaction in pure solutions of Na salts, so that swarming is at once arrested. The strong initial contraction is followed by slow relaxation accompanied by feeble bending movements, and within one or two minutes the organism regains its normal length. Feeble muscular contractions may last for some minutes longer, but soon cease. The phenomena indicating increased permeability are as follows: in a few seconds after the addition of the solution the latter is observed, especially if numerous larvæ are present, to be colored by the yellow pigment from the cells; this substance leaves the cells during the initial period of strong stimulation. Under the microscope a shrinkage is seen to begin during this period, and a somewhat refractive and apparently glutinous fluid exudes from the body surface; the latter within three or four minutes exhibits a distinct separation from the thin cuticular layer which invests the entire organism. At the same time the surface consistency of the larvæ is altered so that they stick to the glass and to one another (agglutination effect).[21] The action of the pure sodium chloride solution is highly injurious, and recovery of contractility on subsequent transfer to sea water is gradual and incomplete.

Similar effects are produced by pure isotonic solutions of other neutral salts of sodium and by salts of the other alkali metals (K, Rb, Cs, Li, NH_4). Potassium salts (*e. g.*, *m*/2 KCl) produce an especially energetic initial contraction with rapid exit of pigment; the larva shortens and thickens at first to an almost spherical form; gradual relaxation then follows, and the larvæ continue to swim actively by means of the cilia, which remain unchecked in such solutions. Muscular contractions are thenceforth absent and clumping results.[22] Similar effects differing in detail — *e. g.*, NH_4 salts are more toxic than the others — are seen in solutions of the other alkali chlorides.

The above effects of pure sodium chloride solutions can be prevented by the addition of a little calcium or magnesium chloride or

[21] In Arenicola larvæ this effect almost invariably accompanies marked increase of permeability (as indicated by loss of pigment). Agglutination thus apparently depends on exit of adhesive substances from the cells through the altered plasma membrane. Hence it is very generally associated, in small cells like bacteria, spermatozoa, blood corpuscles, and ova, with the action of cytolytic substances. *Cf.* ZANGGER: Ergebnisse der Physiologie, 1908, vii, p. 138.

[22] This phenomenon is described and figured in my early paper already cited.

other favorable salt (salts of various other bivalent metals). Calcium salts are the most favorable. Thus, if a mixture consisting of 24 c.c. *m*/2 NaCl + 1 c.c. *m*/2 $CaCl_2$ is added as above, the larvæ show no initial contraction and exhibit at first and for several minutes afterwards a typical heliotropic swarming; later the movements become irregular, the cilia are checked, and death results within twenty-four to forty-eight hours. The permeability of the tissues remains at first apparently unimpaired and there is no visible loss of pigment; later, after the contractions have lost their normal character, the pigment begins slowly to diffuse from the tissues. A similar checking of the permeability change, combined with preservation of contractility for many hours, results from the addition of a little *m*/2 $MgCl_2$, though this is less favorable than $CaCl_2$. If both of these chlorides are added to NaCl solutions in favorable proportions, normal permeability and normal contractions persist for greatly prolonged periods.[23] Antitoxic action under these conditions evidently consists largely in preventing the abnormal increase of permeability induced by the pure sodium chloride.[24]

An action apparently the exact reverse of that shown by sodium or potassium chloride is seen in solutions of certain other salts, particularly those of magnesium. In these there is no initial muscular contraction with loss of pigment. Larvæ placed in *m*/2 or *m*/3 $MgCl_2$ (the same is true of $Mg(NO_3)_2$ or $MgSO_4$) undergo, on the contrary, a gradual and progressive loss of muscular contractility; during the first few seconds the body bends from side to side in an apparently normal manner and the swimming movements are heliotropic; the contractions become by degrees more and more limited and after a period of one to two minutes cease completely; the larvæ thenceforward remain entirely rigid and free from muscular contractions during their stay in the solution; the cilia, on the contrary, continue actively vibrating, if at a somewhat slower rate than normal. Muscular contractility may, however, be restored immediately and perfectly by transfer to sea water or other favorable medium. The action is thus similar to that of an anæsthetic; mixtures of *m*/2 NaCl and *m*/2 $MgCl_2$, containing magnesium in proportions of 4 parts NaCl to 1 part $MgCl_2$ and higher, induce a

[23] *Cf.* R. S. Lillie: This journal, 1902, vii, pp. 30–31.

[24] Mathews has also referred the antitoxic action of salts under certain conditions to their influence on the permeability of the cell: This journal, 1905, xii, pp. 439 *et seq.*

similar loss of muscular contractility, although more gradually. A striking difference from the action of sodium or potassium chloride solutions is also seen in the fact that there is *no loss of pigment from the cells;* solutions of magnesium chloride containing large numbers of larvæ remain absolutely clear and colorless for hours. The appearances suggest strongly that the only essential change produced by this salt is a complete cessation of interchanges (ionic and otherwise) between medium and tissues; this would result from a decided decrease in permeability. The lack of injurious action confirms this supposition; even after lying in a state of complete muscular immobility for twenty-four hours or longer in $m/2$ $MgCl_2$ larvæ promptly resume contractions on transfer to sea water; whereas even a short stay in $m/2$ KCl or NaCl is highly injurious.[25] Magnesium chloride larvæ are also peculiar in exhibiting not the slightest trace of a tendency to cohere or to stick to the glass; *i. e.*, there is no exudation from the ectoderm cells, and the organisms lie quite loosely and freely in contact with the glass and one another. The entrance of dyes like methylene blue is also decidedly retarded, though not altogether prevented.

These facts indicate that the primary action of pure NaCl or KCl solutions is to *increase,* that of $MgCl_2$ to *decrease,* the normal physiological permeability. The relations, however, appear more complex in the case of potassium salts. Thus the addition of considerable quantities of $m/2$ KCl to otherwise favorable mixtures of $m/2$ NaCl and $m/2$ $CaCl_2$ (*e. g.*, in the proportions 80 c.c. $m/2$ NaCl + 5 c.c. $m/2$ $CaCl_2$ + 15 c.c. $m/2$ KCl) results in marked muscular paralysis; such inhibiting action is in fact typical of the general pharmacological action of potassium salts[26] and on the present theory would indicate a general influence — in the presence of the other salts normally present — in *decreasing* permeability. When applied in relatively concentrated pure solution to the tissues, the effect, however, as in the case of most injurious actions, is to *increase* permeability, as just seen. This result agrees with the conclusions of Höber[27] based on the influence of salts on the demarca-

[25] R. S. Lillie: This journal, 1902, vii, p. 45.

[26] For a description of the effect on Arenicola larvæ of increasing the potassium-content of otherwise favorable solutions, *cf.* the paper just cited, pp. 28–30.

[27] Höber: *Loc. cit.* Potassium salts in isotonic solution also produce marked permanent contraction or increase of tone in frog's skeletal muscle. Stimulation thus accompanies the increased permeability which Höber's results indicate.

tion current in frogs' muscle. Why potassium salts should show in relatively low concentrations such pronounced specific action in inhibiting stimulation processes remains unexplained.

Pure solutions of calcium chloride produce on Arenicola larvæ effects somewhat resembling those of magnesium chloride, impairing muscular contractility, but without removing it completely as in the case of the latter salt. The influence of calcium on permeability is peculiar; pigment leaves the cells very slowly in pure isotonic or hypotonic $CaCl_2$, while slight and limited muscular contractions persist for some time. In combination with sodium salts calcium is highly favorable to the preservation of muscular contractility.[28]

In pure $m/2$ $SrCl_2$ and $BaCl_2$ the larvæ contract strongly and lose pigment. These salts differ from $MgCl_2$ and $CaCl_2$ in producing marked increase of permeability and stimulation in pure isotonic solutions. They may also, in association with sodium chloride, exert antitoxic action in low concentrations.[29] Here the effect is to check the increase in permeability resulting from the action of the latter salt.

An action essentially similar to that of $m/2$ $MgCl_2$ is seen in pure isotonic solutions of non-electrolytes (*m*-sugar, *m*-glycerine), and in sea water containing anæsthetics (chloroform, ether, benzol, etc.) in appropriate (not too high) concentrations. The muscular contractions become gradually less and less pronounced, without initial contraction and without loss of pigment. These solutions, it is to be inferred, also act by decreasing the permeability of the irritable elements.[30] The fact that cilia preserve their activity in these solutions is of interest as indicating that the surface permeability of these structures is less readily affected than in the case of muscle. In

[28] R. S. Lillie: *Loc. cit.*

[29] R. S. Lillie: This journal, 1904, x, p. 419.

[30] In the case of inhibition, anelectrotonus, and similar conditions it is to be assumed that a similar decrease of permeability takes place. I have already advanced this explanation, in attempting to account for the influence of calcium salts in furthering mechanical inhibition in the Ctenophore swimming plate. *Cf.* This journal, 1908, xxi, p. 200. The existence of a *positive* electrical variation accompanying vagus inhibition in heart muscle (first shown by Gaskell) indicates a permeability change in a direction the reverse of that accompanying stimulation. *Cf.* below, p. 494. Brünings found that immersion of frog's muscle in isotonic sugar solution increased the demarcation-current potential by heightening positivity at the longitudinal surface. This effect, on the present view, also indicates decreased permeability: Archiv für die gesammte Physiologie, 1907, cxvii, p. 409.

general cilia appear to be resistant and not especially irritable structures; they require relatively high concentrations of indifferent narcotics for complete anæsthesia.[31] The degree of impermeability conferred on the tissues of Arenicola larvæ by isotonic non-electrolyte solutions is, however, relatively slight as compared with that produced by magnesium chloride solutions; muscular contractions disappear more slowly and less completely, and restoration of contractility is more readily effected. The action of electrolytes in restoring contractility under such conditions will be treated in a separate paper.

The following series of experiments (Table I) will illustrate the foregoing general observations:

TABLE I

July 13, 1908. — Nearly equal quantities of larvæ were collected by heliotropism in the seven watch glasses of the series; and after removal of the sea water 10 c.c. of each solution was added. Time of addition of solutions, 11.45–11.54 A. M. The results were as follows:

1. $m/2$ LiCl. Strong initial contraction. At 11.55 the larvæ show only slight coherence, and the solution is colored a faint yellow.

2. $m/2$ NaCl. Strong initial contraction. At 11.56 the larvæ cohere and stick to the glass more strongly than in $m/2$ LiCl, and the solution is colored a deeper yellow.

3. $m/2$ KCl. Very strong initial contraction with slow relaxation. At 11.57 the larvæ cohere in clumps and the solution is colored quite bright yellow.

4. $m/2$ NH_4Cl. Strong initial contraction. At 11.58 the larvæ cohere as in $m/2$ KCl: solution a bright yellow.

5. 24 vols. $m/2$ NaCl + 1 vol. $m/2$ $CaCl_2$: solution added 11.48. No initial contraction; active heliotropic swarming for first minute or two and solution remains colorless; by 11.51 a very faint yellow tinge is imparted to the solution. At 11.58 solution is tinged faintly yellow (much less so than in Solution 1); muscular contractions continue; larvæ stick quite strongly to the glass.

6. $m/2$ $CaCl_2$: solution added 11.51. Larvæ swim actively, soon becoming rigid and collecting in clumps; solution remains colorless at first; by 11.59 a slight tinge of yellow is perceptible.

7. $m/2$ $MgCl_2$: solution added 11.53. Active swimming; no initial contraction; muscular movements soon cease and larvæ collect in clumps. Solution remains quite colorless.

[31] Overton: Studien über die Narkose, Jena, 1901, pp. 7, 185.

At 12.05 the contents of the watch glasses were transferred separately to a series of seven narrow test tubes of uniform diameter, for more accurate comparison of the color test. At 12.10 the appearances were as follows: Solutions 2, 3, and 4 are all quite deeply and almost equally tinged with pigment, presenting a light straw-yellow tint; Solution 1 has the same tint but fainter. In contrast to these solutions the series 5–7 appear practically colorless; a faint trace of pigment is present in Solution 5, and somewhat less in Solution 6; 7 is quite colorless. There has thus been a decided increase in permeability in the pure solutions of the alkali chlorides, and little or none in the others; the presence of $CaCl_2$ has prevented the action of the NaCl. A comparison of the appearances of the massed larvæ at the bottoms of the test tubes yields a confirmatory result; in Solution 7 ($m/2$ $MgCl_2$) they show the characteristic dark brown color; in Solutions 5 and 6 they are also brown but slightly lighter (5 lighter than 6); while in the first four solutions they have become light yellow, having evidently lost a large part of their pigment.

A second similar series on July 14 yielded an essentially identical result. These additional solutions were also tested: (1) 24 c.c. $m/2$ NaCl + 1 c.c. $m/2$ $MgCl_2$, (2) $m/2$ $SrCl_2$, (3) $m/2$ $BaCl_2$. In the last two solutions the effect was similar to that produced by pure $m/2$ NaCl: the larvæ showed a marked initial contraction, and the solution was instantly tinged yellow by the escaping pigment. After an hour the series of test tubes showed the following appearances: the pure $m/2$ solutions of LiCl, NaCl, KCl, NH_4Cl, $SrCl_2$, and $BaCl_2$ were all tinged light-straw yellow; $m/2$ $CaCl_2$ showed a very faint yellow tinge, while $m/2$ $MgCl_2$ was colorless. Of the two mixed solutions both were faintly colored, — the Na + Mg mixture distinctly more so than the Na + Ca, which was almost colorless; this difference corresponds to the difference in favorability, the Ca-solution being decidedly the superior of the two.

For comparison the action of the above solutions on another form of pigment-containing cell was tested, namely, the unfertilized eggs of the sea urchin Arbacia, which are deeply laden with a bright red pigment.

The eggs were found to differ from the larvæ in suffering a loss of pigment in $m/2$ $MgCl_2$, and in certain other points of detail, but, on the whole, the effects were similar to the above. NaCl shows greater toxicity than KCl;[32] a little $m/2$ $CaCl_2$ prevents the action

[32] J. Loeb found this true for the fertilized eggs of both Fundulus and Arbacia; *cf.* This journal, 1900, iii, p. 439.

of $m/2$ NaCl in increasing permeability, as in Arenicola. While there is thus a general agreement, the results indicate that considerable variety in the conditions influencing the permeability of cells exists in different organisms.

Action of solutions containing lipoid solvents. — The stimulating or inhibiting action of electrolytes is to be referred to their action in changing the aggregation state of the colloids composing the plasma membrane and thus changing the permeability of the latter. Another class of substances produce similar alterations in the permeability of the plasma membrane by virtue of their solvent action on its lipoid constituents; these are the organic fat solvents (ethers, esters, alcohols, normal and substituted hydrocarbons), which, as Overton has shown, act as narcotics or anæsthetics in low concentrations. In higher concentrations, on the other hand, these substances first stimulate the cell, and their further action rapidly produces irreversible, partly coagulative alterations and death (cytolytic action). In the former case, therefore, such substances, on the present theory, decrease, in the latter, increase, permeability.

I have accordingly begun experiments with this class of compounds. In general the result has appeared that such substances as ether, chloroform, benzol, etc., inhibit muscular contractions, *i. e.*, act as anæsthetics, in low concentrations; if, however, the concentration is increased above a certain maximum, the effect is to produce a strong contraction or increase of tone accompanied by exudation of pigment and adhesion to the glass of the vessel. Thus, marked increase of permeability induced by this class of substances is also associated with strong stimulation. The action of solutions sufficiently concentrated to produce these effects is largely irreversible and hence highly injurious.

The following experiments illustrate the action of relatively concentrated solutions of ether and chloroform in producing contractions with associated loss of pigment. These solutions were made in $m/2$ $MgCl_2$; larvæ that have lain for a few minutes in pure solutions of this salt exhibit absolutely no trace of muscular movement; the stimulating action of anæsthetics dissolved in this solution is thus rendered the more evident and unmistakable. It should be understood that these substances have a similar powerfully stimulating and destructive action when dissolved in sea water or other indifferent medium.

The following table describes the results of a typical series of experiments:

TABLE II.

July 8, 1908. — Larvæ were transferred to $m/2$ $MgCl_2$ in the usual manner at 9.52 P. M. Loss of muscular contractility follows in a few seconds, as usual, while the cilia remain active. Larvæ were transferred to the following solutions at the times designated:

1. Saturated solution of ethyl ether in $m/2$ $MgCl_2$. 10.26. Larvæ immediately contract to about half normal length; cilia cease at once. Gradual relaxation follows, which is almost complete in two minutes. Larvæ adhere to the glass, and yellow pigment soon diffuses from the cells. At 11.40 the protoplasm has an opaque and coarse (coagulated) appearance. Addition of fresh sea water at 11.45 produces no contractions: larvæ are dead.

2. $m/2$ $MgCl_2$ two thirds saturated with ether (saturated solution + one half its volume $m/2$ $MgCl_2$). 10.31. Action is less decided than in Solution 1; no immediate result is evident, but gradual and incomplete contractions soon appear in a relatively small proportion of larvæ; cilia continue activity in a fair proportion. Larvæ show some adhesion to glass and slight separation of pigment. By 11.40 there is little change in appearance, and transfer to sea water produces contractions in a fair proportion.

3. $m/2$ $MgCl_2$ one half saturated with ether. 10.36. Cilia continue actively, and no muscular contractions are seen. No noticeable adhesion to glass or separation of pigment. On return to sea water at 11.50 well-marked contractions result.

[In another experiment on July 6 larvæ showed well-marked contractions with loss of pigment and adhesion in one half saturated ether solution after one hour forty-eight minutes in pure $m/2$ $MgCl_2$. The exact conditions of stimulation probably vary with temperature and length of stay in $m/2$ $MgCl_2$, etc.]

4. $m/2$ $MgCl_2$ saturated with chloroform. 10.43. Larvæ all contract immediately to half normal length by a quick, steady, uniform contraction. Cilia cease at once. Gradual relaxation follows. Yellow pigment diffuses from larvæ, and latter adhere to glass. In an hour larvæ are dead and coagulated.

5. $m/2$ $MgCl_2$ two thirds saturated with chloroform. 10.49. Cilia cease, and larvæ all contract vigorously to half length as in Solution 4, but somewhat less promptly. Larvæ lose pigment and stick to glass. Relaxation as before. In an hour all are dead and coagulated.

6. $m/2$ $MgCl_2$ one half saturated with chloroform. 11.05. Cilia cease.

Muscular contraction begins after several seconds and is less rapid than in Solutions 4 and 5; the contracted state lasts longer (one minute or more) and the shortening is less extreme; pigment diffuses from larvæ, and latter stick to glass. Larvæ show coagulated appearance in thirty-five minutes.

7. *m*/2 $MgCl_2$ one third saturated with chloroform. 11.14. Cilia cease at once. Muscular contractions begin after an interval of *ca.* fifteen seconds and have a more normal character, are slower and less energetic than in Solutions 4–6 and last longer (four to five minutes). Loss of pigment is more gradual; is not evident for the first few minutes. The adhesion to the glass is relatively slight, and the larvæ preserve normal appearance for some time.

8. *m*/2 $MgCl_2$ one fourth saturated with chloroform. 11.29. Cilia mostly cease. No muscular movement is seen until after two minutes, when well-marked contractions appear in a few. These contractions are less energetic than in the above solutions and cease in two or three minutes; many show no contractions. There is only slight loss of pigment and adhesion in this solution.

Saturated solutions of xylol, benzol, and toluol in *m*/2 $MgCl_2$ were found to exhibit relatively slight stimulating action. Xylol produces no apparent effect on either ciliary or muscular movement; toluol has a somewhat more pronounced action, and slow bending movements appear in some few instances (not usually), while cilia are gradually checked; benzol solutions check cilia rapidly and usually cause slow muscular contractions to appear after an interval of a minute or more. The order of increasing action, in saturated solution in *m*/2 $MgCl_2$, is thus xylol $<$ toluol $<$ benzol. Permeability is changed slowly and slightly in these solutions as compared with those of ether or chloroform; benzol, however, produces well-marked adhesion within a few minutes, toluol has a similar though less effect, while xylol has no such action. The influence on pigment exit is inconspicuous. In their relative toxicity or production of irreversible coagulative changes a similar order is seen. The solutions of these substances thus act like weak solutions of chloroform or ether.

The above lipoid solvents in appropriate concentrations thus produce well-marked stimulation accompanied by increase of permeability. Such increase of permeability, if too pronounced, appears to be irreversible and is accordingly rapidly destructive, as shown by failure on the part of the larvæ to recover contractility on return to sea water. Larvæ may, however, recover after comparatively prolonged immersion in one third or one fourth saturated

chloroform or one half saturated ether solutions in $m/2$ $MgCl_2$. The poisonous influence of these substances is thus seen only in concentrations that produce marked and obvious *increase* in permeability; the effects of prolonged decrease of permeability appear only slightly if at all injurious; perfect recovery follows transfer to sea water after many hours of complete muscular anæsthesia in more dilute solutions.

Recovery of contractility after anæsthetization indicates a restoration of at least approximately normal permeability. This implies a change in the physical consistency of the plasma membrane. Such changes are most naturally referred to changes in the state of subdivision of the constituents of the membrane; the *texture* of the latter may be thus modified, with corresponding alterations in permeability. Thus the theory of Overton and Meyer refers the narcotic action of ether or chloroform or other lipoid solvent to its specific action on the lipoids. The anæsthesia induced by electrolytes like magnesium salts must, however, be due to an action not confined to the lipoids but influencing the aggregation state of *all* of the colloids composing the membrane.

Now, for stimulation, on the theory here advocated, all that is necessary is that the *permeability of the plasma membrane should be sufficiently increased,* whatever the means employed. Solution of the lipoids, coagulative or other changes in the colloids, mechanically induced breaches in the continuity of the membrane, might all conceivably produce this effect, and so result in stimulation. The results just cited show that a lipoid solvent may produce stimulating effects in the presence of a strongly anæsthetizing electrolyte like magnesium chloride; in this instance the effect depends apparently on a lipolytic action. The reverse is also true, that an appropriate change of electrolyte may stimulate in the presence of anæsthetizing concentrations of a lipoid solvent like chloroform; here the effect is to be ascribed to a general change in the combined colloids of the plasma membrane. Such a change would not necessarily be prevented by the presence of the lipoid solvent. The fact that the presence of lipoid solvents in low concentrations hinders stimulation by electrolytes seems, however, to indicate that such changes in colloidal consistency are less readily produced under conditions that increase the solubility of the lipoids or induce a fine state of subdivision of these bodies. It seems not unlikely that the lipoids exercise on the other colloids of the membrane an action comparable

to the "protective action" often seen where the presence of one colloid prevents or retards aggregation changes in another.

Whatever the parts played by the different constituents of the membrane may be, the following experiments show that changing the electrolyte, while keeping unchanged the concentration of the organic anæsthetic, may produce marked though temporary stimulation.

July 8, 1908, 2.40 P. M. — Larvæ were placed in pure *m*/2 $MgCl_2$. At 3.43 they were transferred to *m*/2 $MgCl_2$ one fourth saturated with chloroform (Solution A). The cilia cease movement; no immediate muscular contractions result; at 3.44–5 a few slight contractions are seen.

Experiment 1. — At 3.47 part of the larvæ were transferred from Solution A to sea water one fourth saturated with chloroform. Well-marked contractions appear instantly in practically all larvæ; by 3.50 all have become again inactive.

Experiment 2. — At 3.48 part of the larvæ from Solution A were transferred to normal sea water. Instant contractions result, which are more pronounced and vigorous than in Experiment 1 and continue actively.

The contact of the sea water thus produces well-marked contractions in the presence of the chloroform, though less so than in its absence. In the first experiment the chloroform soon restores the anæsthesia; in the second the contractions naturally continue.

That the immediate stimulation on transfer to sea water is an electrolyte action is shown also by the following simple experiment:

July 9, 1908, 3.14 P. M. — Two lots of larvæ were placed respectively in *m*/2 $MgCl_2$ one fourth saturated with chloroform (Solution A) and sea water one fourth saturated with chloroform (Solution B).

Experiment 1. 3.31 P. M. — Larvæ are transferred from chloroform sea water to fresh sea water. No immediate effect results; larvæ are *gradually* de-anæsthetized; by 3.32 contractions are well marked, by 3.35 vigorous.

Experiment 2. 3.33 P. M. — Larvæ are transferred from chloroform-magnesium chloride solution (A) to sea water. Contractions begin *instantly* in all; at 3.36 all show vigorous contractions.

Thus in the first transfer, where there is no change of electrolyte, contractions appear only gradually as the anæsthetic diffuses from the tissues; while in the second the change of electrolyte causes instant stimulation. These experiments were repeated with similar results using *m*/2 $MgCl_2$ and sea water, with chloroform in the con-

centrations one fourth, one fifth, one sixth, one eighth, and one twelfth saturated. Detailed description of the observed results is unnecessary. The larvæ were placed in small corked flasks containing the solutions, and after varying intervals were transferred to the fresh sea water and to chloroform sea water (with the same chloroform content as the solution). In solutions containing chloroform to one fourth and one fifth saturation a slight extraction of pigment was observable, but not in the other solutions.

It was noted that the anæsthetic action of chloroform solution in sea water tended to become less pronounced after the larvæ had been subjected to prolonged immersion. After several hours in sea water containing even so much chloroform as one fifth and one sixth saturated, the larvæ were observed to have recovered a certain though limited power of muscular contraction. Since observation was made *through the wall* of the tightly corked flask (in which the odor of chloroform was strong), this change cannot be attributed to evaporation of the anæsthetic. It appears, rather, that the plasma membrane tends partially to recover its normal permeability, even in the presence of a concentration of anæsthetic which at first completely checks muscular contraction. The prolonged action of low concentrations of the lipoid solvent apparently results in increasing permeability — possibly through a gradual and progressive solvent action.[33] Ciliary movement continues actively in sea water containing the above concentrations of chloroform.

The experiments just cited appear to throw some light on the fundamental properties of the plasma membrane. Indications point to a considerable complexity in its physico-chemical constitution;

[33] One curious effect was noted in larvæ treated with one fourth and one fifth saturated chloroform sea water; when the larvæ, after about two hours in corked flasks containing these solutions, were poured out into watch glasses, the great majority were found, after three or four hours (when the chloroform had evaporated) to exhibit a pronounced *negative* phototaxis in place of the usual positive, — gathering on the side of the watch glass away from the window. I have observed a similar reversal of the normal phototaxis in larvæ treated with weakly acidulated sea water (*e. g.*, 2 c.c. $n/10$ HCl + 100 c.c. sea water), with sea water charged with carbon dioxide, and, to a certain extent, with both hypotonic and hypertonic sea water. The change of reaction apparently depends on an alteration of the normal properties of the plasma membrane. Phototaxis would thus seem due to the direct influence of light on the plasma membranes of the contractile or irritable (nerve) elements. Possibly the pigment acts as a photodynamic substance in these larvæ; this, however, is mere conjecture as yet.

this is shown by its susceptibility to the action of both electrolytes and fat solvents, and by the mutual interference between the respective actions of both classes of substances. One important incidental consequence of such complexity would naturally be to increase the number of influences that would affect its permeability and so act as stimuli or the reverse. The above experiments suggest that normal stimulation by electrolytes depends on aggregation changes in the *protein* portion of its colloids rather than in the lipoids; the latter, however, apparently influence to a greater or less degree, according to their state of subdivision, the extent and character of the aggregation changes and thus may determine the readiness with which stimulation is effected. Such inferences are obviously tentative, and further experimentation is needed. The actions of different electrolytes on larvæ deprived of contractility by sugar solutions furnish certain other indications of the nature of the changes in the plasma membrane during stimulation, and experiments of this character will be described in a future paper.

The General Nature of the Processes of Stimulation and Inhibition.

The most evident chemical effect of stimulation upon muscle is an increase in the output of carbon dioxide; at the same time the reaction of the protoplasm, at least after prolonged stimulation, becomes acid to litmus, indicating the production of other acids. The nature of the reaction by which these substances are produced thus becomes a question of primary importance for the theory of stimulation. On the view presented in my preceding paper, the end product of oxidation, which is at the same time the electrolyte to which the physiological polarization is due, must be assumed to diffuse from the cell during stimulation. The removal of this final oxidation product through the now permeable plasma membrane is regarded as the determining condition of the disturbance of chemical equilibrium which occurs at the time of stimulation. The only oxidation product which leaves the cell in any quantity at such times appears to be carbon dioxide. We are thus led to consider the possibility that this substance may play the above rôle in stimulation.

It has been assumed by the upholders of the membrane theory — Bernstein, Brünings, and Höber — that the plasma membrane is

impermeable, at rest, to the polarizing electrolyte. This would seem to remove carbonic acid from consideration, since it can evidently traverse the plasma membrane at all times, as shown by its being continually evolved from the resting cell. The *degree* of the permeability to carbon dioxide, as to other substances, may, however, be subject to wide variation, and there seems little doubt that this is the case. On this view the carbon dioxide output during rest is slight because of the relatively slight permeability of the plasma membrane at this time; this would furnish the necessary condition for the physiological polarization, since the assumed impermeability to the anion of the polarizing electrolyte need not be absolute, but only relatively great in comparison with that to the cation. During stimulation the general permeability undergoes a marked increase, as we have seen; and this change is to be regarded as the primary ground of the increased evolution of carbon dioxide at that time. This interpretation, it will be observed, is the inverse of the prevalent, somewhat ill-defined view that stimulation in some way furthers intracellular oxidation, thus leading to an increase in the internal concentration of carbon dioxide, which accordingly escapes in increased quantity; it is, however, as I hope to be able to show, more acceptable on physico-chemical grounds, since it explains as a simple instance of disturbance of chemical equilibrium the marked acceleration in the oxidative breakdown of energy-yielding substances during stimulation.[34]

Chemical equilibrium, according to the prevailing theory, depends on equality in the velocity of the oppositely directed pairs of processes. Removal of the reaction products of the process represented

[34] It is important to note that even in resting muscle there is continual chemical activity with evolution of carbon dioxide and maintenance of a certain state of contraction or tonus. The following paragraph from Halliburton defines this condition in a clear and satisfactory manner: "While a muscle is at rest, we do not mean it is absolutely inactive; we know, for instance, that it possesses that small amount of contraction which is technically known as 'tonus.' There is also what we may call 'chemical tonus'; the evidence of this is that the blood leaving muscles which are not contracting is nevertheless venous, and heat production is occurring in muscles which are in repose. In all probability the chemical changes that occur during contraction are similar in kind to those which occur during so-called rest; there is a sudden exaggeration of the normal chemical tonus of the tissue and an explosive liberation of energy." W. D. Halliburton: Lectures on biochemistry of muscle and nerve, 1904, pp. 49–50. Supposedly the normal chemical tonus is *diminished* during inhibition in consequence of the decreased permeability to carbon dioxide.

by one half of the equation (*i. e.*, of the interacting bodies of the reciprocal process) thus produces relative acceleration of that process and the equilibrium tends to be restored. The conditions are not essentially altered if the system is in only approximate equilibrium, *i. e.*, if one set of reaction products is being continuously though slowly removed from the system; the relative velocity of the process by which these bodies are produced will then naturally depend on their *rate of removal.* In other words, any increase in the rate of removal of a reaction product from a system of chemically interacting substances in an approximate equilibrium must produce a corresponding acceleration of the process in the direction of the production of the removed substance. Carbon dioxide — on the view which I wish to present — is the reaction product whose *rate of removal from the cell* determines the velocity of the chemical process concerned in stimulation and contraction. Normally this rate of removal is controlled by the degree of permeability of the plasma membrane; it may also be controlled artificially by the simple expedient of increasing the carbon dioxide in the external medium; under these conditions stimulation is found to be prevented at an appropriate concentration. This is intelligible if stimulation depends on an *escape* of the substance from the irritable element; otherwise it is difficult to understand why so inert a gas should differ so radically in its action from similarly inert gases like hydrogen and nitrogen.[35]

What is the nature of the reaction by which carbon dioxide is produced in active muscle? It is agreed that the main source of muscular energy in Vertebrata is the oxidation of carbohydrates. This oxidation is not an immediate process, — as is shown by the lack of direct dependence between the oxygen intake and carbon-dioxide output, — but apparently takes place by a series of stages. If each of these successive stages is a reversible reaction, it is evident that a sufficient increase in the concentration of the final oxidation products must reverse the general course of the entire chain of reactions; and inversely a rapid removal of end products must promote the progress of the series of reactions in the direction of the further production of these end products. It is immaterial, from the standpoint of the present general theory of stimulation, what view is adopted of the exact course of the glycolytic action. Un-

[35] Carbon dioxide, to be sure, is a weak acid, but its action is different from that of corresponding solutions of other weak acids.

doubtedly this is highly complex, with the velocity of the successive stages influenced in all probability by specific enzymes (Oppenheimer). Schade has shown that various hypothetical steps in the process — transformation of dextrose to lactic acid, of lactic acid to acetaldehyde and formic acid, of a mixture of the latter two to carbon dioxide and alcohol and the oxidation of the latter — may take place under the influence of inorganic catalysers.[36] It seems more probable, however, that the oxidative glycolysis in muscle proceeds by a more gradual series of stages. The view has recently been emphasized by Walther Löb [37] that the oxidation of sugar in cells follows a course similar — only in the reverse direction — to that of its synthesis in green plants from carbon dioxide and water. There is on this view a progressive depolymerization by dissolution of the aldol linkage with production of formaldehyde remainders which interact to produce carbon dioxide and alcohol. Nef's studies of the oxidation of sugars by Fehling's solution indicate that the sugar molecule splits or dissociates into formaldehyde remainders; whether the splitting is partial or complete depends on the experimental conditions; Nef also considers it probable that equilibria exist between the sugar and its dissociation products, so that the successive steps would be reversible.[38]

Assuming, therefore, that the oxidation of sugar occurs in a series of reversible stages of which the final product is the carbon dioxide which escapes through the plasma membrane, the chemical conditions of stimulation must be conceived somewhat as follows: When the plasma membrane becomes freely permeable during stimulation, the carbon dioxide is rapidly lost from the cell; [39] the above chain of interdependent reactions thus undergoes acceleration in the direction of further oxidation. This continues until by the restoration of relative impermeability at the close of stimulation, the carbon dioxide concentration within the cell undergoes increase and the action is checked.[40] The high velocity of the glycolytic process in

[36] Schade: Biochemische Zeitschrift, 1908, vii, p. 299; also "Die Bedeutung der Katalyse für die Medizin," Kiel, 1907, ch. 3, pp. 86 *et seq.*

[37] Walther Löb: Biochemische Zeitschrift, 1908, xii, pp. 78, 466.

[38] Nef: Annalen der Chemie, 1907, ccclvii, p. 215; *cf.* p. 253.

[39] The chemical as well as the physical conditions in higher animals favor a very rapid removal of the escaping carbon dioxide; *i. e.*, by combination with the bicarbonates of the blood plasma, and possibly with certain of its proteins.

[40] *Cf.* my preceding paper; This journal, 1908, xxii, p. 87.

active muscle is its remarkable feature; presumably this depends on the presence of appropriate combinations of ferments and coferments; and also, in all probability, in some manner difficult to explain, on certain structural peculiarities of the tissue. The importance of the normal structure is indicated by the comparatively low velocity of enzymatic reactions induced by even concentrated tissue extracts.[41] In any case the reactions concerned must be equally accelerated in both directions;[42] hence the accumulation of the reaction products and the resultant cessation of the reaction follow with equal rapidity when stimulation ceases and the normal resting impermeability is restored. The curves of contraction and of relaxation are accordingly symmetrical in fresh muscle.

In the preceding paper I have put forward a definite theory of the manner in which the chemical energy is partly transformed into the mechanical energy of contraction. On this view the effect depends on the coagulative action of the hydrogen ions derived from the acids produced in oxidation upon the colloids composing the fibrillæ. This action is reversible, and in its normal reversal after stimulation the characteristic electrical polarization at the surface of the muscle cell is assumed to play an important part. This hypothesis would seem to require a marked sensitivity on the part of the fibrillar colloids to variations in the hydrogen-ion concentration. The facts, however, so far as known, appear to correspond to this requirement; muscle plasma according to the recently reported results of J. Mellanby[43] is rapidly coagulated by a trace of acid (0.4 c.c. $n/10$ H_2SO_4 to 6 c.c. muscle plasma), and the effect is reversible. Whether the slight rise in acidity resulting from stimulation is sufficient to produce such marked effects in the transformation of colloidal surface energy may naturally be questioned; it is perhaps not necessary to assume that carbonic is the only acid concerned; quite possibly formic and other acids may serve as sources of hydrogen ions. I hope to consider this question more fully at some future time. Certain definite experimental facts seem, however, to support this view of the *rôle* of acids in contraction; thus slight acidulation of the medium invariably causes a permanent increase

[41] *Cf.* Fletcher and Hopkins: Journal of physiology, 1907, xxxv, p. 287.

[42] A general law of catalytic action.

[43] J. Mellanby: Journal of physiology, 1908, xxxvii, Proceedings of the Physiological Society, p. xxxiv.

in the tone or state of muscular contraction in Arenicola larvæ;[44] and sea water charged with carbon dioxide also produces a marked shortening which is permanent during the stay in this solution and disappears on removal of the dissolved gas.[45] The presence of acids in the medium would tend to increase the concentration of hydrogen ions within the cell, and such a result would be expected on the above hypothesis; the fact that a weak acid like carbonic produces decided muscular shortening favors the view that the normal contraction is due to acids formed in metabolism.[46]

Inhibition or anæsthetization is a change of the reverse nature from stimulation, and its conditions require brief consideration. If stimulation depends on an increase of permeability, inhibition is presumably due to a *decrease* in this property; the plasma membrane is thus rendered less susceptible to the action of stimulating agencies.

I have already presented experimental evidence that such a decrease of permeability accompanies anæsthetization in Arenicola larvæ. The indirect evidence from the associated electrical change points in the same direction. Gaskell first discovered that cardiac inhibition was accompanied by a *positive* electrical variation; F. Buchanan has recently demonstrated a similar change during reflex

[44] I shall describe the action of acids more fully in a later paper.

[45] Ringer's solution slightly acidulated or charged with carbon dioxide produces similar contractions in frog's muscle.

[46] The above hypothesis conflicts with the view that protoplasm is always neutral in reaction and possesses marked powers of preserving neutrality under disturbing conditions. This view, however, seems to me to be a scarcely warranted deduction from the observed fact of the neutrality of blood plasma. There is a decided increase in the acidity of the muscle substance after energetic contraction—exactly how great seems not yet to have been determined.

Bernstein's theory that contraction is due to an increase in the surface tension of the contractile elements (which he conceives of as small elongated cylindrical or ellipsoidal structures which are arranged in longitudinal rows and compose the fibrillæ) becomes closely similar to the above view if his hypothetical elements are identified with colloidal particles. *Cf.* Bernstein: Archiv fur die gesammte Physiologie, 1908, cxxii, p. 166. On Bredig's theory, coagulation of colloids depends on a capillary electric increase in the surface tension of the colloidal particles; on this view the primary effect of addition of a coagulating electrolyte to a colloidal solution is to increase the surface tension of the particles; coagulation is a secondary and largely incidental consequence of the change in surface tension. *Cf.* Bredig: Anorganische Fermente, Engelmann, Leipzig, 1901, p. 15.

inhibition in skeletal muscle.[47] Now it can readily be shown on the membrane theory, if certain not improbable assumptions are made, that this change must accompany a decrease in the permeability of the plasma membrane. The chief of these assumptions is that the electrolyte to which the physiological polarization is due is an acid. The penetrating cation is thus the hydrogen ion; the nature of the anion (or anions if more than one acid is concerned) may be inferred from the character of the acid that leaves the cell during stimulation (*i. e.*, increased permeability); this acid is chiefly carbonic acid, with possibly others in smaller quantity; the anions would thus be mainly HCO_3^- and to a smaller degree $CO_3^{=}$. The chief difficulties of this assumption are the weakness of carbonic acid and its comparatively low concentration; just what this latter may be within the cell is difficult to estimate, but it may be presumed to be equivalent to that of a saturated aqueous solution, if not higher.[48] However, it is not necessary to assume that carbonic acid is the sole source of the hydrogen ions; other acids are formed in the oxidation of sugar, and these may contribute. If HCO_3 and CO_3 are the only anions that can pass the plasma membrane with any readiness during stimulation, the electrolyte actually leaving the cell will be mainly if not exclusively carbonic acid. It is assumed, therefore, that the electrolyte concerned in the production of the physiological polarization and action current is an acid, and largely but not exclusively carbonic acid.

The muscle thus represents a concentration cell with the above electrolyte. The potential difference at the boundary between two unequally concentrated solutions of the same electrolyte may be calculated from Nernst's formula:[49]

$$E = \frac{RT}{q} \frac{u - v}{u + v} ln \frac{C_2}{C_1}$$

The potential difference thus varies, at constant temperature, directly with the difference between the two ionic velocities and

[47] F. Buchanan: Journal of physiology, 1907, xxxv, Proceedings of the Physiological Society, p. xliii.

[48] Actively growing yeast cells may evolve CO_2 at pressures considerably above atmospheric.

[49] E is potential difference in volts; R, gas constant; T, absolute temperature; u, velocity of cation; v, that of anion; C_1 and C_2, molecular concentrations of dissociated part of electrolyte; q, number of coulombs of electricity associated with a monovalent gram ion, and ln the natural logarithm.

with the logarithm of the ratio of the concentrations on opposite sides of the boundary surface. This boundary surface, on the Ostwald-Bernstein theory, is the surface film or plasma membrane of the cell, which is supposed to be freely penetrable to the cation under all conditions, but to offer greater or less resistance to the passage of the anion. If the membrane is completely impenetrable to the anion, the velocity of the latter in the direction from interior to exterior of the cell is reduced to zero at the membrane and the potential difference is then at its maximum; under these conditions the electrolyte is naturally unable to diffuse from the cell; when the membrane becomes as readily penetrable to the anion as to the cation, the potential difference reaches its minimum, and the electrolyte freely leaves the cell, with resulting disturbance in the chemical equilibrium of the latter; this condition is approximated during stimulation. Now, it is clear that the normal physiological condition in the unstimulated cell cannot be one of *complete* impermeability to anions, for carbon dioxide is always being evolved from the cell even at rest. The maximum potential difference is thus never attained; it may, however, be approached *if the plasma membrane becomes still less permeable to anions* (*i. e.*, to carbon dioxide) than normally. This is presumably what occurs in inhibition; activity is checked, since the escape of oxidation products is retarded and energy production is diminished; stimulation becomes more difficult because of the greater impermeability of the plasma membrane; and the potential difference between interior and exterior of the cell increases because of the increased difference between the migration velocities of anion and cation within the membrane; the difference between the intracellular and extracellular concentrations of the electrolyte is also evidently increased at this time.

Summary.

The chief special observations and conclusions presented in this paper are as follows:

1. Arenicola larvæ are stimulated intensely by pure isotonic solutions of various salts ($NaCl$, KCl, NH_4Cl, $LiCl$, $SrCl_2$, $BaCl_2$), contracting to half their length for several seconds when first introduced into the solution. At the same time a yellow pigment contained in the cells of the organism diffuses freely to the exterior and colors the solution.

2. Solutions which do not produce this strong initial contraction do not cause such loss of pigment. Isotonic $CaCl_2$ and $MgCl_2$ (especially the latter) are instances. In these solutions the muscles lose the power of contraction and the organism becomes stiff and motionless (though still propelled by the cilia which remain active). Addition of small quantities of $CaCl_2$ to a NaCl solution prevents the strong initial contraction and loss of pigment.

3. $MgCl_2$ and similarly acting solutions appear to *decrease* the permeability of the tissues, and so prevent the ionic transfer on which stimulation depends. The general action of anæsthetics consists in *decreasing the normal permeability;* stimulating agencies, on the other hand, have the reverse effect.

4. Strong solutions of fat solvents (chloroform, ether, benzol, etc.) produce a contraction of the muscles accompanied by loss of pigment, even in $m/2$ $MgCl_2$. This effect is to be referred to an alteration of the lipoid substances in the plasma membrane. Such alteration, if slight, *decreases* permeability (anæsthetic action in low concentrations); if extreme, produces the reverse effect, with resulting stimulation.

5. The hypothesis is presented that the chemical effect of the above changes in permeability depends essentially on their influence in varying the rate at which carbon dioxide leaves the cell. The velocity of the oxidative energy-yielding processes whose end product is CO_2 is thus varied with the rate of removal of this latter substance from the system; this velocity is accordingly increased during the increased permeability of stimulation, and is decreased during anæsthesia or inhibition. This view is supported by a consideration of the electrical changes accompanying inhibition and stimulation respectively ("positive" and "negative variations").

FACTORS REGULATING THE CREATININ OUTPUT IN MAN.

By P. A. LEVENE and L. KRISTELLER.

[*From the Chemical Laboratory of the Montefiore Home for Chronic Invalids, New York, N. Y.*]

FOLIN [1] made the remarkable observation that the creatinin output in normal men remains constant regardless of the quantity or the quality of food. He also noted that normally urine contains no creatin, and even after ingestion of this substance only minimal quantities may be detected in the urine. On the basis of these observations Folin formulated the hypothesis that creatinin takes its origin not in the exogenous protein, but is formed in the process of catabolism of the cellular elements. The observations of Folin were confirmed by many investigators. His conclusions were accepted completely by some; by other investigators [2] with slight modification. Shaffer [3] alone disagreed on the question of the origin of urinary creatinin.

The work of Folin contained no analysis of the factors which regulate creatinin metabolism in a manner that its output remains constant. To this problem were devoted the efforts of the more recent workers. Their observations and experiments were made both on normal individuals and animals, and on patients with different forms of disease. The conditions which the authors attempted to obtain for their observations were, first, those which are associated with a cellular activity of a very high intensity; second, in which cellular activity is depressed, and third, those in which the deficiency in the function of one individual organ predominates. As conditions of the first order, were chosen muscular activity in mild degree and in nature to produce fatigue, convulsions produced by cinchonin poisoning, exaltation produced by the use of alcohol, forms of insanity associated with maniacal conditions, fever, exophthalmic goitre and acromegaly. Conditions of the second order which were made the subject of observation were paralysis, fast-

ing, administration of potassium bromid, hydrogen poisoning, and leukemia. Of the individual organs, the liver and the kidney received special attention.

Observations on the effect of muscular activity on the creatinin output were made by Weber,[4] Mellanby,[5] and Von Hoagenhuyze and Verploegh.[6] Weber made his experiments on the excised heart. He found, that when the organ contracted actively, the perfusion liquid contained a higher proportion of creatinin. During rest the output of creatinin by the heart muscle was insignificant. However, the muscle itself contained but little creatinin even after prolonged contractions. Previous to Weber, Mellanby had already demonstrated that muscle tissue contained no preformed creatinin. Von Hoagenhuyze and Verploegh studied the influence of muscular exercise on the elimination of creatinin by the urine in normal men and in a professional starver. Only in the latter instance did exercise cause an increase in the creatinin output. Weber found that normal exercise was followed by a fall in the creatinin output, while convulsions caused by cinchonin poisoning produced a rise in creatinin elimination.

A rise of creatinin output under the influence of alcohol and maniacal conditions was observed by Von Hoagenhuyze and Verploegh,[6] while Benedict and Meyers [7] found, in their studies on the composition of the urine of twenty-five insane women, that the form of insanity had no marked influence on the creatinin elimination.

The effect of fever on creatinin elimination was studied by Leathes,[8] Von Hoagenhuyze and Verploegh,[6] and by Shaffer.[3] Generally the rise of body temperature was associated with an increase in creatinin output. After prolonged fever, when the organism reached a state of inanition, there was observed at times a fall in the creatinin content of the urine. More noteworthy was the appearance of creatin in the urine.

In the tables compiled by Hoagenhuyze and Verploegh one finds frequently the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 1$. In the experience of Shaffer there was a moderate rise in the creatinin elimination, apparently no attention being paid to creatin.

Observations on the creatinin output in exophthalmic goitre were made by Shaffer [3] and by Froschbach,[9] and both report a low creatinin output. In contrast to these observations stand those on conditions in which the activity of an organ or of the entire organ-

ism is depressed. Closson[10] produced paralysis of the muscles of a limb by excising a part of the sciatic nerve, and noted a diminution in the creatinin content of the muscular tissue. In man muscular inactivity produced by administration of potassium bromid resulted in a diminished creatinin elimination (Hoagenhuyze and Verploegh). Shaffer,[11] on the other hand, failed to detect any change in the creatinin output during complete muscular rest.

The effect of fasting was studied by Hoagenhuyze and Verploegh,[12] by Benedict,[13] and by Benedict and Diefendorf.[14] They all record a diminution in the creatinin output with the progress of fasting. Benedict noted also an increase in the creatin elimination. In leukemia Froschbach[9] and Shaffer[3] noted a low output of creatinin.

Diseases of the muscular system were studied by Spriggs,[15] who made use of more recent methods of analysis. He noted a low creatinin elimination in all forms associated with diminution of the volume of muscular tissue, and a similar observation was made by Froschbach.

The influence of the liver on creatinin elimination was recognized first by Mellanby:[5] according to this writer the liver is the principal seat of creatinin formation, whereas the muscle is the place of its transformation into creatinin. In harmony with this hypothesis he finds a low creatinin output in individuals subject to diseases of the liver. Hoagenhuyze and Verploegh[6] also noted on some patients with diseases of the liver a low creatinin output, but also a normal creatinin content of the urine was observed by them, and not infrequently the urine showed an excessive creatinin output. The ratio of creatinin to creatin was found low.

Very similar to the latter observations are those of Underhill and Kleiner,[16] of Richards and Wallace,[17] and of Leffman.[18] The last-named author induced organic disease of the liver on a dog by poisoning with amylalcohol and with phosphorus. He recorded a high creatinin and low creatin output, so long as the diseased condition caused an increased nitrogen elimination. However, as soon as the nitrogen output fell, the creatinin elimination was also diminished and the output of creatin rose in proportion. According to this author creatinin is formed in the liver in the process of protein disintegration, and as soon as the power of the liver to catabolize protein becomes inadequate, the rate of creatinin formation is lowered. Generally at that stage of the disease the liver also fails in its power to transform creatin into creatinin, hence the rise of the

proportion of that substance in the urine. In harmony with this assumption is the observation of Mellanby, who noted a high creatin output in a patient with carcinoma of the liver.

In order to study the rôle of the kidneys on creatinin output Leffman produced lesions of these organs in dogs by poisoning them with potassium chromate. The output of creatinin in these animals was continually lowered with the progress of the lesions, but, in proportion with the fall in creatinin, the output of creatin rose so that ultimately the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 1:2$. A still greater increase in the creatin output was observed after introvascular injection of creatin or after a beef diet. On the basis of these proportional changes the author arrived at the conclusion that creatinin and creatin formation have to be regarded as two phases in the metabolism of one substance. The author failed, however, to give a detailed interpretation for the deviation in the ratio of creatinin to creatin output during the course of acute nephritis.

A review of the results of all the enumerated observations brings to light the fact that in disease the creatinin output is not so constant as in health, that there is an apparent regularity between the decrease in creatinin and the increase of creatin output. Furthermore, it appears that the rate of creatinin elimination is not alone determined by the intensity of cellular catabolism, for, in conditions of exophthalmic goitre and other conditions associated with increased catabolism, the creatinin output remains below normal. In this connection it is important to bear in mind the experiment of Weber on the excised heart, in which the perfusion liquid contained a larger proportion of creatinin when the heart was in action. On the other hand, muscular exercise did not cause a rise in the creatinin output. It thus follows that the creatinin formed in the muscle during exercise and removed from that tissue by the blood is further oxidized by the organism.

Furthermore, the observations of Froschbach contain evidence that in a diseased organism the rate of creatinin oxidation may exceed the rate in health. Thus, after administration of 30 gm. of beef extract,

A healthy man removed through the urine	1.082 gm. creatin	in excess over his normal output.
A man with muscular atrophy	0.852 " "	
A man with leukemia	0.577 " "	
A man with exophthalmic goitre . . .	0.368 " "	
The same patient after a partial removal of the thyroid	0.600 c.c. "	

The co-operation of these two factors in regulating the creatinin output had already been pointed out by Gottlieb and Stangassinger[19] and by Hoagenhuyze and Verploegh.[20] According to these workers the power to oxidize creatinin is inherent in all organs. But their conclusions are based principally on observations made on autolyzing organs, and it still remains to be established whether or not the chemical work performed by an organ during life is identical with that of one done in the process of dissolution.

The rôle of individual organs in regulating the creatinin output still remains to be established. Since the difficulties in producing permanent injuries to internal organs at present offer insurmountable difficulties, one naturally is compelled to resort to clinical observations for the analysis of the influence of these factors.

The present work was undertaken with a view to gain information regarding the rôle of the muscular system in regulating the creatinin output. Observations were made on twenty-four patients affected with diseases in which the muscular system was involved. The conditions chosen for observation were those of muscular atrophy, caused either by anatomical lesions in the central nervous system or by causes as yet obscure, but not associated with organic disease of either the brain or of the spinal cord; further, conditions of continuous tremor or of spasmodic contraction of the muscular system.

Only those patients whose urine showed an abnormal proportion of either creatinin or the presence of creatin were placed under observation. Whenever the creatinin output was found abnormal the influence of the diet was studied. Three forms of diet were generally employed in course of the observations: one of low protein content, but containing a sufficient number of calories, the nitrogen intake not exceeding 6 gm., and the sum of calories reaching about 3000 per day. The second diet of approximately the same calorific value contained milk and eggs, with a nitrogen content of about 10 gm., and the third, a beef diet, with a nitrogen content of 20 gm. per day. In health these diets should not markedly influence the creatinin output.

Methods of Analysis.

Total nitrogen was estimated by the Kjeldahl-Gunning method, ammonia by the Folin-Schaffer method, urea and creatinin by the

methods of Folin, and creatin by Benedict's modification of Folin's method.

The following clinical forms were under observation: first, forms associated with diminished muscular activity and with a diminution of the mass of muscular tissue. In this condition the muscular efficiency in the sense of Shaffer is low. Of this group several forms were not associated with lesions in the central nervous system, namely, juvenile muscular dystrophy (5 patients) and progressive muscular atrophy (2 patients). The forms associated with cerebrospinal lesions were, poliomyelitis anterior (1 patient), amyotrophic lateral sclerosis (2 patients), and spinal tumor (1 patient). Observations were also made on several patients in whom muscular inactivity was brought about by extraneous causes, namely, by diseases of the joints, arthritis deformans (2 patients), and by gout (1 patient). Finally, the results were compared with those obtained on a boy in prolonged fasting. Of the group of conditions associated with accentuated muscular activity, the following forms were under observation: tremor (2 patients), paralysis agitans (1 patient), spastic paralysis (1 patient), and locomotor ataxia (5 patients).

Analysis of Results of Observations.

We shall begin the discussion with a review of the results obtained on the patients of the second group, since the creatinin output in these conditions showed the least deviation from normal. The creatinin elimination was most nearly normal in locomotor ataxia (Nos. 1 to 5) and in paralysis agitans (No. 6). This stands in harmony with the observations of the other workers.

In conditions of tremor (Nos. 7 and 8) the output of creatinin and creatin was normal; however, on a mixed diet, the urine of all these patients contained creatin, the highest ratio of $\frac{\text{creatinin}}{\text{creatin}} = 5$. The creatin in all these conditions is of exogenous origin. A patient (No. 9) with spastic paralysis presented similar features.

We wish to call special attention to the significance of the appearance in the urine of exogenous and of endogenous creatin. We also wish to call attention to the fact that the elimination of endogenous creatin, stored up in the organism, is accomplished very slowly. In our patients of the second group, whose urine

showed the presence of creatin, it was possible to bring about a disappearance of this substance from the urine by means of a prolonged creatin-free diet. It was necessary for that purpose to continue this diet for about two weeks. However, this is not peculiar to pathological conditions only. In normal men, whose urine showed the presence of creatin, it requires the same length of time to cause its disappearance. W. Koch had noted some time ago that if the creatin output in dogs was increased by the administration of lecithin, the high output persisted several days after the last administration of this substance. The ingestion of an unusually high beef diet caused marked increase in the creatin output and a slight rise of creatinin, thus changing the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 2$; about 70 per cent of the creatinin contained in the beef was removed from the organism, and of this about 80 per cent in form of creatin.

More complicated and instructive are the results obtained on the first group of patients: we shall begin with the analysis of the results of the observations on one patient with extreme emaciation, caused by fasting (No. 10). The subject, a boy fourteen years of age, for several weeks refused nourishment, and his body weight was reduced to 44 pounds. In course of the observation it was discovered that his distress was caused by intestinal parasites, and after proper treatment he began taking nourishment. The nitrogen intake at the beginning of the experiment was 2.21 gm. per day and the output 2.15 gm. The creatinin output was normal, the creatinin coefficient being about six.

In a patient with extreme muscular atrophy (No. 11), without apparent lesions in the central nervous system, there was no change in the creatinin output. In this instance the reduction in the mass of the muscle, especially in the upper part of the body, was more pronounced than on any of the other patients under our observation. The muscle showed the reaction of degeneration. The creatinin output on a mixed diet remained normal. In a second patient with progressive muscular atrophy (No. 12) the creatinin and creatin output deviated considerably from the normal, on one day the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 1:1.5$ and on another day $= 1:2.5$. But in this patient the condition was complicated by disease of the kidneys.

The output of creatinin and creatin by a patient with paralysis,

caused by a tumor of the spinal cord (No. 13), varied markedly with the variation in the character of diet. On a mixed diet his urine contained a very appreciable proportion of creatin. By means of a prolonged creatin-free diet creatin was caused to disappear from the urine; it again appeared as soon as the patient was placed on a beef diet. It is noteworthy that during the first two days of the beef-diet period the creatin content of the urine was very slight, but in five days about 76 per cent of the total ingested creatin was removed through the urine. Of the portion reappearing in the urine 65 per cent was in form of creatin and 35 per cent in form of creatinin. In conditions of muscular inactivity, brought about by diseases of the joints, the output of creatinin on a mixed diet varied little from the normal, and the creatin content of the urine was slight (Nos. 14, 15, 16).

The output of the two substances in amyotrophic lateral sclerosis (No. 17) could not be studied in detail, for the reason that the condition of the patient was too grave to permit the obtaining of the twenty-four-hour samples of the excreta accurately. On a mixed diet the urine of this patient always contained an appreciable proportion of creatin, the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 1$.

The remaining two forms, namely, poliomyelitis anterior (Nos. 18, 19) and juvenile muscular dystrophy, furnish the more valuable suggestion for the interpretation of the place of creatinin and creatin in the course of protein catabolism. In poliomyelitis anterior the following peculiarities were observed: in both patients under observations there was noted the presence of endogenous creatin in the urine. In patient No. 19, on a creatin-free diet, the ratio of $\frac{\text{creatinin}}{\text{creatin}}$ fell to 2:3, having reached an approximately constant proportion after forty-eight hours. The coefficient of creatinin was somewhat low, but the sum of creatin and creatinin coefficients corresponds to a normal creatinin output.

On the other patient (No. 18) experiments were performed with the three forms of diet: it was noted that on a low protein diet the sum of eliminated creatinin and creatin was the lowest, the ratio $\frac{\text{creatinin}}{\text{creatin}} = 3:2$; on a creatin-free, but protein-rich diet the output of these two substances rose, but principally that of creatinin, the ratio of $\frac{\text{creatinin}}{\text{creatin}}$ increasing to 2. Finally, on a mixed diet

containing beef, the output of both substances rose, but principally of creatin, the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 5:6$. The coefficient of creatinin output was below normal on any one of the three diets. On a low protein diet the coefficient of the sum of creatin and creatinin was below normal. On a high protein and likewise creatin-free diet it reached a low normal value. On a high protein beef-diet the coefficient did not exceed the highest normal figure.

Of the five patients with muscular dystrophy, three represented typical forms, in one the symptoms of the disease appeared in very early infancy (No. 23), another patient (No. 24) showed rather late development of a mild and stationary form of the disease. In all patients of this group there was noted an output of endogenous creatin. On a cereal diet the urine of all, with the exception of patient No. 24, showed a ratio of $\frac{\text{creatinin}}{\text{creatin}} = 1:2$ approximately; on a high protein and creatin-free diet the ratio gradually diminished in two patients to 1 : 4, remaining, however, practically unaltered in one patient (No. 23). On a beef diet there was noted a rise in the elimination of both creatin and creatinin, but principally of creatin. The ratio of creatinin to creatin in these three cases reached 1 : 4, 1 : 5, and 1 : 6 respectively. The coefficient of creatin was below normal on either one of the three diets, and the sum of the two coefficients reaching the normal coefficient for creatinin in all experiments but one. The elimination of exogenous creatin was very high in all experiments except one, when only 52 per cent was removed through the body; in other experiments the output of exogenous creatin fluctuated between 88 and 99 per cent of the intake. The output was so distributed that about 80 per cent of it was in form of creatin and about 20 per cent in form of creatinin.

Summary.

A review of the results obtained on all of the patients brings to light the following facts: in all pathological conditions involving the muscular system, the rate of catabolism of ingested creatin is lowered and part of the ingested substance is removed in form of creatinin. In forms associated with exaggerated muscular activity

the catabolism of endogenous creatin generally preserves its normal course. In forms associated with dissolution of muscular tissue and with diminution of muscular activity, there were observed conditions where not only the exogenous but also the endogenous creatin followed an abnormal course of catabolism. In those conditions the output of creatinin was low and that of creatin high. In some forms the quantity of eliminated creatin and creatinin was influenced by the protein content of the food. In such forms a high protein content of the food caused an increase in the output of both creatin and creatinin. Finally, there occurred forms associated with an extreme degree of dissolution of muscular tissue, which preserved a normal creatinin output.

All of these observations cannot be interpreted adequately on the basis of any one of the existing views on the mechanism of creatin catabolism. The hypothesis formulated by Shaffer, which postulates that the extent of creatinin output is determined by muscular efficiency, does not harmonize with the observations on progressive muscular atrophy, in course of which an extreme degree of dissolution of muscular tissue was not associated with any marked alteration of the creatinin output. The theory that regards the intensity of cellular catabolism as the principal factor influencing the creatinin output is also not in harmony with our observation. It is likewise little in harmony with the observations made by others on exophthalmic goitre. The theory of Mellanby is not sufficient to interpret our observations. According to Mellanby creatinin is formed in the liver and transformed into creatin in the muscle. So long as the function of the liver remains normal the creatinin formation continues to be normal. On the other hand, a deficiency in the function of the muscle should lead to a diminution in the rate of conversion of creatinin into creatin. Hence, in diseases of the muscle, one should find principally a rise in the creatinin output. In the majority of our patients the creatinin output was low. This was not caused by an insufficient formation of the substance in the liver, since no disturbance of the function of this organ could be detected.

Thus one receives the impression that more than one factor is concerned in regulating the creatinin output, and one is bound to accept at least two: first, the formation of the substance, very probably from protein, and, second, its further oxidation. Any disturbance of either one of the two factors may lead to an abnormal creatinin output. The deficiency in the second function may be

partial, so that only the ingested creatin fails to be further oxidized. Whether or not the two functions are performed by one organ or by several still remains to be established, but there is little doubt that the muscular system takes some part in the regulation of the creatinin output.

From the results of our experiments one also receives the impression that the formation of creatin and creatinin represents two phases in the catabolism of but one substance, as in most observations a fall in the creatinin output was associated with an increased creatin elimination, and a high protein diet (creatin-free), in some patients, caused a rise in the output of both substances.

The constant value of the creatinin output in normal men is conditioned by the high velocity of creatin combustion in health. Thus, the creatinin of the urine normally represents only a small fraction of the creatin formed in the organism. The condition might be analogous to the uric acid output in the dog, in which the power of oxidation of purin derivatives is exceedingly high. The uric acid content of the dog's urine is minimal, and, being so, appears to be practically constant. However, as soon as the liver is excluded from circulation and the intensity of purin oxidation is diminished, the uric acid output in the dog begins to show marked variations influenced by the character of the food. In a similar manner the normal creatinin output in conditions of high muscular activity may be explained by the assumption of a higher intensity in the power of the organism to oxidize creatin, although the creatin production in these conditions probably exceeds the normal limits.

In harmony with this view is the observation on one of our patients with continuous tremor. In the urine of this patient only 48 per cent of the ingested creatin reappeared, while in conditions of atrophy or dystrophy practically 90 per cent reappeared in the urine. On the other hand, in the condition of muscular dystrophy, both the formation of creatin and the rate of its further combustion are lowered.

We wish to express our gratitude to Dr. Joseph Fraenkel, physician of the hospital, for his interest in the work, and to the members of the staff, Drs. S. Wachsmann and D. Felberbaum, for their constant assistance.

TABLE I.

Diagnosis.	Date. 1908	Wt. Kg. kg.	Diet.	Intake. Cal.	Intake. N. gm.	Urine. Amount.
Locomotor ataxia. No. 1. Mr. M. G. Age 48.	Aug. 4	...	Mixed	....	....	....
Locomotor ataxia. No. 2. Mrs. C. R. Age 51.	Sept. 16	...	Mixed	....	....	....
Locomotor ataxia. No. 3. Mr. E. W. Age 62.	Sept. 19	52.2 [1]	Mixed	....	....	24 hrs. 485 c.c. incomplete
Locomotor ataxia. No. 4. Mrs. R. M. Age 51.	Sept. 16	43.5 [2]	Mixed	....	....	24 hrs. 900 c.c.
Locomotor ataxia. No. 5. Mr. M. L. Age 60.	July 9	60.4	Mixed	....	....	24 hrs. 1130
	July 10	60.4	Mixed	....	....	1330
Paralysis agitans. No. 6. Mr. H. R. Age 65.	July 25	68	Mixed	....	....	755
	July 26	68	Mixed	....	....	840
Tremor. No. 7. Mrs. L. J. Age 42.	Sept. 7	55	Mixed	....	....	1440
	Sept. 8	...	Mixed	....	....	1410
	Sept. 9	...	Mixed	....	....	650
	Sept. 15–Oct. 3	55	Milk and egg	2974	14.84	1321
Tremor. No. 8. Mr. R. M. Age 50.	Avs. of June 5 and 6	54 [3]	Milk and egg	3470	18.31	1305
	June 1 and 2	54 [5]	Beef	2829	18.22	1390
	June 10 and 11	54 [6]	Cereal diet	3270	9.06	1085
Spastic paralysis. No. 9. Mr. W. H. Age 35.	May 12	50.5	Mixed	....	....	540
	May 14	...	Mixed	....	....	660
	Avs. of May 26 and 27	...	Milk and egg	2342	14.70	455
Starvation on account of intestinal worms. No. 10. Mr. M. G. Age 12.	June 30	21.0 [7]	Milk and egg	426	2.21	745
	July 1	...	" "	573	3.65	835
	July 2	...	" "	822	4.81	1235
	July 3	...	" "	925	4.59	1780
	July 4	...	" "	800	5.41	790
	July 26	...	Mixed	....	....	690
	July 27	...	Mixed	....	....	1120
	July 28	...	Mixed	....	....	710
	July 29	...	Mixed	....	....	1440

[1] Evidently incomplete. [2] Incomplete.
[3] Two preliminary days on the same diet.
[4] Of creatinin intake compared with the milk and egg period.

TABLE I.

Urine.	Output.				Coefficient.			Ratio of creatinin to creatin.
Specific gravity.	Total. N gm.	Creatinin and creatin N. mg.	Creatinin N. mg.	Creatin N. mg.	Creatinin and creatin.	Creatinin.	Creatin	
1.014	....	...	...	absent	...	...	...	....
....	....	...	...	trace	...	...	...	....
1.017	....	82	76	6	1.6	1.5	0.1	....
1.015	....	292	274	18	6.7	6.3	0.4	....
1.015	10.46	386	386	absent	6.4	6.4	...	....
1.014	11.16	416	416	trace	6.9	6.9	...	....
....	....	410	410	trace	6.0	6.0	...	....
....	....	354	354	trace	5.2	5.2	...	....
....	....	384	321	63	7.0	5.8	1.2	1 : 0.20
....	....	375	310	65	6.8	5.6	1.2	1 : 0.20
....	....	347	295	52	6.3	5.4	0.9	1 : 0.17
....	....	287	265.5	21.5	5.2	4.8	0.4	....
1.017	15.53	375	340	35	7.0	6.3	0.7	1 : 0.10
1.017	16.77	555	370	185	10.3	6.9	3.4	1 : 0.50
		+ 48 %[4]						
1.016	7.38	...	330	...	...	6.1	...	....
1.028	7.23	335	275	60	6.7	5.6	1.1	1 : 0.25
1.025	....	349	297	52	6.9	5.9	1.0	1 : 0.20
1.033	10.90	303	272	31	6.0	5.4	0.6	1 : 0.11
....	2.15	129	123	6	...	5.7	...	....
....	3.55	123	123	...	...	5.7	...	....
....	4.02	126	126	...	...	5.8	...	....
....	3.74	121	121	...	...	5.7	...	....
....	3.88	111	111	...	...	5.0	...	....
....	3.55	131	117	14	5.9	5.3	0.6	....
....	3.84	131	131	trace	5.9	5.9	...	....
....	4.23	135	125	10	6.2	5.7	0.5	....
....	4.39	119	119	trace	5.4	5.4	...	....

[5] Daily 420 gm. beef (378 mg. creatin N.). Two preliminary days on the same diet.
[6] Three preliminary days on the same diet.
[7] Table shows only the first five and the last four days of thirty days' observation.

TABLE I (*continued*).

Diagnosis.	Date. 1908	Wt. kg.	Diet.	Intake. Cal.	Intake. N. gm.	Urine. Amount.
Atrophy. No. 11. Mr. J. H. Age 48.	July 1	40.3	Mixed	....	....	735
	July 6	40.25[8]	Milk and egg	2346	11.3	730
Progressive muscular atrophy. No. 12. Mr. M. M. Age 65.	May 6	...	Mixed	....	....	1050
	May 8	...		....	....	840
Paralysis due to tumor. No. 13. Mr. A. Ba. Age 52.	Sept. 10	...	Mixed	....	....	....
	Sept. 15	...	Mixed	....	....	1425
	Sept. 16	46.3	Milk	....	....	850
	Sept. 17	...	and egg	....	....	1180
	Sept. 18	...	" "	....	....	630
	Sept. 19	...	" "	....	....	550
	Sept. 20	...	" "	....	....	820
	Sept. 21	...	" "	....	....	810
	Sept. 22	...	" "	....	....	1230
	Sept. 23	...	Beef	....	....	765
	Sept. 24	...	Beef	....	....	1445
	Av. of Sept. 25 to 29	46.5[11]	Beef	....	....	1450
Arthritis deformans. No. 14. Mrs. D. O. Age 32.	Sept. 16	45.9	Mixed	....	....	1340
Arthritis deformans. No. 15. Mrs. J. J. Age 60.	Sept. 22	42.6	Mixed	....	....	1065
Chronic gout. No. 16. Mr. N. M. Age 51.	Sept. 18	53.5	Mixed	....	....	1450
Amyotrophic lateral sclerosis. No. 17. Mr. J. L. Age 68.	Av. of Aug. 15 to 26	...	Mixed	....	....	ca. 450
Anterior poliomyelitis. No. 18. Mr. M. Sch. Age 31.	Avs. of May 14 to 16	44.5[13]	Cereal	2600	5.56	853
	May 19 to 21	...	Milk and egg	2162	9.43	823
	May 25 to 26	...	Beef	2273	10.60	995
Anterior poliomyelitis. No. 19. A. B. Age 16.	Av. of Oct. 2 to 11	30.4	Milk and egg	2315	11.1	1713
	Oct. 13 to 16	30.4[16]	Beef	2310	11.5	1440

[8] After four preliminary days on the same diet.
[9] Weight of patient could not be obtained.
[10] Table shows the slow disappearance of creatin from the urine on a milk and egg diet.
[11] Daily 5 gm. beef N. and from Sept. 25–29, 170 c.c. soup.
[12] Weight of patient could not be obtained. Twenty-four-hour quantities not always complete.

TABLE I (*continued*).

Urine.	Output.				Coefficient.			Ratio of creatinin to creatin.
Specific gravity.	Total N. gm.	Creatinin and creatin N. mg.	Creatinin N. mg.	Creatin N. mg.	Creatinin and creatin.	Creatinin.	Creatin.	
1.016	....	256	256	trace	6.3	6.3	...	....
1.016	7.24	250	250	trace	6.2	6.2	...	....
....	....	363	145	218	... [9]	...	...	1 : 1.5
....	....	426	121	305	...	...	...	1 : 2.7
....	....	346	297	49	7.6	6.5	1.1 [10]	....
....	10.41	329	306	23	7.2	6.7	0.5	....
....	8.62	261	250	11	5.6	5.4	0.2	....
....	11.36	355	323	32	7.7	7.0	0.7	....
....	10.52	224	198	26	4.5	3.9	0.6	....
....	9.63	258	246	12	5.6	5.3	0.3	....
....	10.81	299	299	...	6.5	6.5	...	....
....	8.74	200	200	...	4.3	4.3	...	....
....	9.73	257	257	...	5.8	5.8	...	....
....	8.16	264	256	8	...	...	...	....
....	9.05	307	299	8	...	...	...	....
....	7.98	360	291	69	7.8	6.3	1.5	....
1.013	....	348	331	17	7.6	7.2	0.4	....
1.012	....	239	239	trace	5.6	5.6	...	....
1.017	....	444	423	21	8.3	7.9	0.4	....
....	....	313	140	163	... [12]	...	...	1 : 1.16
1017	4.11	170	100	70	3.8	2.2	1.6	....
1018	6.82	240	160	80	5.5	3.7	1.8 [13]	....
1015	8.29	480	210	270	10.7	4.8	5.9 [14]	....
1010	....	209.5	146	63.5	6.9	4.8	2.1	1 : 0.43
....	....	399 + 84 % [15]	178	221	13.2	5.9	7.3	1 : 1.24

[13] Two preliminary days on the same diet.

[14] Three preliminary days on the same diet. Patient received daily 240 gm. beef (192 mg. creatin N.) and one day 240 c.c. soup.

[15] Of the creatin intake compared with the milk diet.

[16] Daily 250 gm. beef (225 mg. creatin N.).

TABLE I (*continued*).

Diagnosis.	Date. 1908	Wt. kg.	Diet.	Intake. Cal.	Intake. N. gm.	Urine. Amount.
Muscular dystrophy. No. 20. Miss T. Y. Age 21.	Av. of April 22 to 23	64.9 [17]	Milk and egg	1891	12.15	555
Muscular dystrophy. No. 21. S. Y. Age 18.*	Avs. of Sept. 4 to 10	33	Milk and egg	2316	10.61	682
	April 21 to 23	33 [18]	Milk and egg	2122	14.10	720
	May 1 to 3	32.2 [19]	Cereal	2431	5.19	833
	May 13 to 15	33.6 [20]	Cereal	2276	5.14	820
	May 6 to 8	32.5 [22]	Beef	2851	17.66	718
	May 25 to 27	34.2 [24]	Beef	2453	13.79	705
Muscular dystrophy. No. 22. I. Sch. Age 17.	Avs. of Sept. 5 to 11	...	Milk and egg	2736	13.01	....
	Feb. 24 to 26	41.0 [25]	Milk and egg	1937	9.43	790
	Mar. 5 to 7	41.0 [26]	Milk and egg	2074	12.80	1285
	Mar. 15 to 17	.... [27]	Milk and egg	2570	14.28	940
	April 14 to 16	...	Milk and egg	2451	17.58	1227
	April 30 to May 2	40.8 [28]	Cereal	2603	5.72	925
	Mar. 24 to 26	...	Beef	2208	21.23	1090
	Mar. 29 to 30	...	Beef	....	21.00	....
Dystrophy. No. 23. Mr. L. I. Age 9.	Avs. of June 7 to 8	16 [32]	Milk and egg	2046	10.35	720
	June 12 to 13	16 [33]	Cereal	1933	4.52	815
	June 21 to 22	16 [33]	Cereal	2074	4.53	500
	June 2 to 3	15.3 [33]	Beef	2022	10.62	755

[17] Two preliminary days on the same diet.
[18] The preceding day on the same diet. [19] Four preliminary days on the same diet.
[20] Two preliminary days on the same diet.
[21] Of the creatin intake compared with milk period Sept. 4 to 10.
* Only a few of the experiments performed on this patient are here recorded.
[22] Two preliminary days on same diet. Daily 14.8 gm. beef N. (396 mg. creatin N.)
[23] Of the creatin intake compared with milk period April 21 to 23.
[24] Three preliminary days on the same diet. Daily 11.4 gm. beef N. (310 mg. creatin N.) two days each 240 c.c. soup besides.

TABLE I (*continued*).

Urine.	Output.				Coefficient.			Ratio of creatinin to creatin.
Specific gravity.	Total N. gm.	Creatinin and creatin N. gm.	Creati-nin N. gm.	Creatin N. mg.	Creatinin and crea-tin.	Creati-nin.	Creatin.	
1.028	8.99	175	50	125	2.6	0.7	1.9	1 : 2.5
....	....	234	53	181	7.1	1.6	5.5	1 : 3.4
1.025	11.89	180	59	121	5.5	1.8	3.7	1 : 2.1
1.014	4.30	193	52	141	6.0	1.6	4.4	1 : 2.7
1.015	4.75	215	63	152	6.5	1.9	4.6	1 : 2.4
1.027	12.11	448	129	319	13.5	3.9	9.6	1 : 2.5
		54 %[21]						
		67 %[23]						
1.023	12.86	653	133	520	19.4	3.9	15.5	1 : 3.9
....	....	263	55	208	5.7	1.2	4.5	1 : 3.8
1.022	10.04	290	60	230	7.2	1.5	5.6	1 : 3.8
1.018	13.57	340	70	270	8.4	1.7	6.7	1 : 3.8
1.023	12.91	222	70	152	5.3	1.6	3.7	1 : 2.2
1.019	14.53	240	70	170	6.0	1.7	4.3	1 : 2.5
1.015	6.22	136	44	92	3.4	1.1	2.3	1 : 2.1
1.021	20.09	670	110	560	16.1	2.6	13.5	1 : 5.2[30]
		99 %[29]						
....	21.71	900	120	780	21.9	2.9	19.0	1 : 6.6[31]
1016	6.80	122	44	78	7.6	2.8	4.8	1 : 1.7
1012	3.53	115	41	74	7.1	2.6	4.5	1 : 1.8
1015	3.81	122	44	78	7.6	2.8	4.8	1 : 1.8
1013	6.10	290	56	234	18.9	3.6	15.3	1 : 4.2

[25] Two preliminary days on the same diet. [26] Seven preliminary days on same diet.
[27] Three preliminary days on the same diet.
[28] Two preliminary days under cereal diet with 9 gm. N. intake daily.
[29] Of creatin intake compared with milk period Sept. 5 to 11.
[30] Daily 14.6 gm. N. (414 mg. creatin N.).
[31] Two preliminary days on the same diet.
[32] Three preliminary days on the same diet. The patient was on a milk diet for ten consecutive days. Figures omitted here, since they were of the same character as those recorded. [33] Three preliminary days on the same diet.

TABLE I (*continued*).

Diagnosis.	Date. 1908.	Wt. kg.	Diet.	Intake. Cal.	Intake. N. gm.	Urine. Amount.
Muscular dystrophy. No. 24. Mr. E. S. Age 42.	Avs. of Sept. 14 to Oct. 3	62.0 [34]	Milk and egg	2543	13.10	....
	May 18 to 20	...	Milk and egg	2491	14.67	587
	April 30 to May 2	...	Milk and egg	2110	12.15	613
	June 21 to 23	...	Milk and egg	2875	15.64	860
	May 12 to 14	...	Cereal	2326	5.44	660
	June 7 to 9	...	Cereal	2946	7.32	988
	May 7 to 9	...	Beef	3211	21.25	667
	May 25 to 27	...	Beef	2620	15.04	560

[34] In these nineteen days no marked variation from day to day was observed.
[35] Two preliminary days on the same diet.
[36] Three preliminary days on the same diet.
[37] The preceding day on the same diet.

TABLE I (*continued*).

Urine.	Output.				Coefficient.			Ratio of creatinin to creatin.
Specific gravity.	Total N. gm.	Creatinin and creatin N. mg.	Creatinin N. mg.	Creatin N. mg.	Creatinin and creatin.	Creatinin.	Creatin.	
....	....	248	183	65	3.9	2.9	1.0	1:0.36
1.025	9.65	229	159	70	3.6	2.5	1.1	1:0.62[35]
1.026	9.73	223	156	67	3.6	2.5	1.1	1:0.62[36]
1.023	13.60	336	196	140	5.3	3.1	2.2	1:0.72[37]
1.023	4.90	248	207	41	4.0	3.3	0.7	1:0.21[37]
1.010	5.28	223	182	41	3.7	3.0	0.7	1:0.22[38]
1.028	14.07	723 100%[39]	263	460	11.7	4.3	7.4	1:1.7[40]
1.023	9.41	493 70%[39]	230	263	7.9	3.7	4.2	1:1.1[41]

[38] Three preliminary days on the same diet.
[39] Of creatin intake as compared with milk and egg period Sept. 14 to Oct. 3.
[40] Two preliminary days on the same diet. Daily 17 gm. beef N. (477 mg. creatin N.).
[41] Four preliminary days on the same diet. Daily 12.5 beef N. (350 mg. creatin N.).

TABLE II.

Influence of Protein Intake on the Elimination of Creatin and Creatinin in some of the Patients.

Name.	Diet.	N. Intake.	N. Output.	Coefficient.		
				Total.	Creatinin.	Creatin.
M. Sch.	Cereal	5.6	4.1	3.8	2.2	1.6
	Milk	9.4	6.8	5.5	3.7	1.8
I. Sch.	Cereal	5.7	6.2	3.4	1.1	2.3
	Milk	13.0	...	5.7	1.2	4.5
	"	9.4	10.0	7.2	1.5	5.6
	"	12.8	13.6	8.4	1.7	6.7
	"	14.3	12.9	5.3	1.6	3.7
	"	17.6	14.5	6.0	1.7	4.3
L. I.	Cereal	4.5	3.8	7.6	2.8	4.8
	"	4.5	3.5	7.1	2.6	4.5
	Milk	8.4	...	8.4	3.0	5.4

Bibliography.

[1] Folin: This journal, 1905, xiii, p. 85; Folin, Festschrift für Olaf Hammerstein, Reprint, Upsala, 1906.

[2] Klercker, Biochemisches Zeitschrift, 1907, iii, p. 45 (see also reference 12).

[3] Shaffer: This journal, 1908, xxiii, p. 11.

[4] Weber: Archiv für experimentelle Pathologie und Pharmakologie, 1908, lviii, p. 93.

[5] Mellanby: Journal of physiology, 1907–1908, xxxvi, p. 288.

[6] Von Hoagenhuyze and Verploegh: Zeitschrift für physiologische Chemie, 1908, xlvi, p. 415, and 1908, lvii, p. 161.

[7] Benedict and Meyers: This journal, 1907, xviii, p. 377.

[8] Leathes: Journal of physiology, 1906–1907, xxxv, p. 205.

[9] Froschbach: Archiv für experimentelle Pathologie und Pharmakologie, 1908, lviii, pp. 112–140.

[10] Closson: This journal, 1906, xvi, p. 252.

[11] Shaffer: *Ibid.*, 1908, xxii, p. 445.

[12] Hoagenhuyze and Verploegh: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 415.

[13] Benedict: Carnegie Institution of Washington, 1907, Publication No. 77.

[14] Benedict and Diefendorf: This journal, 1907, xviii, p. 362.

[15] Spriggs: Quarterly journal of medicine, 1907–1908, i, p. 63.

[16] Underhill and Kleiner: Journal of biological chemistry, 1908, iv, p. 165.

[17] Richards and Wallace: *Ibid.*, 1908, lvii, p. 179.

[18] Leffman: Zeitschrift für physiologische Chemie, 1908, lvii, p. 476.

[19] Gottlieb and Stangassinger: *Ibid.*, 1900, lii, p. 1; 1908, lv, p. 295 and p. 322.

[20] Hoagenhuyze and Verploegh: *Ibid.*, 1908, lvii, p. 265.

ACAPNIA AND SHOCK.[1] — III. SHOCK AFTER LAPAROTOMY: ITS PREVENTION, PRODUCTION, AND RELIEF.

By YANDELL HENDERSON

(With the Collaboration of JAMES RYLE COFFEY and FRANK ELMER JOHNSON).

[*From the Physiological Laboratory of the Yale Medical School.*]

CONTENTS.

I. Acapnia as an element in inhibition 66
II. Relations of the blood gases to peristalsis 69
III. Exhalation of CO_2 from exposed viscera 76
IV. The effects of aeration of the viscera 77
V. The production and relief of acapnial shock 80
VI. Conclusions 84

I. Acapnia as an Element in Inhibition.

WHEN the abdomen is opened, peristalsis ceases.[2] After reclosure the motility of the stomach and intestines does not return for a considerable period. Exposure and handling of the viscera induce also a loss of tonus, — as evidenced by the tympanites after surgical operations upon human beings. Ether anæsthesia even for operations other than laparotomy is frequently followed by flatulence.

The investigations of Bayliss and Starling,[3] Eliot and Barclay-

[1] For the two preceding papers of this series, see This journal, 1908, xxi, p. 126; and 1909, xxiii, p. 345; see also the latter volume, p. xxx, for an abstract of a future paper of this series.

[2] *Cf.* von Braam Houckgeest: Archiv für die gesammte Physiologie, 1872, vi, p. 266.

[3] Bayliss and Starling: Journal of physiology, 1901, xxvi, pp. 107 and 125.

Smith,[4] Cannon and Murphy,[5] Meltzer and Auer,[6] Magnus,[7] and many others have made it probable that the cessation of peristalsis and loss of tonus in exposed viscera are not due to a single simple process. According to Meltzer,[8] "Opening of the abdomen is instrumental in bringing out a two-fold inhibition — a reflex inhibition and an inhibition of a local peripheral mechanism." Perhaps each of these processes will in the ultimate analysis need to be resolved further into several distinct elements. It is not, however, the object of this paper to attempt a solution of this general problem, but to contribute a new point of view and some experimental evidence on certain phases of the topic to which relatively little attention has been paid. Briefly stated, the points to be considered are:

1. Stimulation of afferent nerves causes hyperpnœa. From the excessive pulmonary ventilation reflexly induced by laying open the abdomen, — or indeed by any surgical operation, — there results a diminution in the CO_2 content of the arterial blood. Furthermore, ether is a respiratory stimulant. Even in operations under morphin and ether in dogs, and probably also in men,[9] respiration is constantly excessive, and the arterial CO_2 at nearly all times subnormal. This acapnia is a factor in the central, or reflex, inhibition of which the splanchnics are the efferent path.

2. When the viscera are exposed to the air, exhalation of CO_2 occurs, and the local acapnia which results is a factor in the peripheral inhibition.

3. General acapnia from hyperpnœa and local acapnia from exposure are the initial causes of surgical shock after laparotomy.

[4] Eliot and Barclay-Smith: Journal of physiology, 1904, xxxi, p. 297.

[5] Cannon and Murphy: Journal of the American Medical Association, 1907, xlix, p. 840.

[6] Meltzer and Auer: Zentralblatt für Physiologie, 1907, xxi, no. 3, and This journal, 1907–1908, xx, p. 259. Also Auer, This journal, 1907, xviii, p. 347; and 1909, xxiii, p. xvii.

[7] Magnus: Archiv für die gesammte Physiologie, 1908, cxxii, p. 210 (refs. to previous papers).

[8] Meltzer: Archives of internal medicine, July, 1908, (review and discussion of literature).

[9] In man under ether the heart rate is nearly always more rapid and often far more rapid than in normal life. It was shown in the first paper of this series (*Loc. cit.*) that under such conditions the heart rate is an index which varies inversely as the arterial CO_2.

The starting-point of the reasoning and experimentation which have led to these conclusions was the well-known fact that the exposed and quiescent viscera of a rabbit are thrown into violent peristalsis by occlusion of the trachea. It was formerly supposed that this phenomenon and the other functional stimulations of asphyxia were caused by lack of oxygen. The course of recent investigations has tended to show, however, that deficiency of this element exerts a purely paralyzing influence upon respiration,[10] — at least in respect to the direct effect in mammals. The excitant influences of asphyxia upon respiration and arterial pressure are really due to excess of CO_2. It is probable that in like manner the excessive peristalsis of asphyxia is caused by hypercapnia, not by anoxhæmia.

Conversely, it is a matter of common knowledge that respiration is increased by mental excitement, by pain, and by ether anæsthesia in some stages. Doubtless hyperpnœa from any of these causes involves excessive pulmonary ventilation. It is not necessary that the breathing should be violent in order to be "excessive," in the sense in which the word is here used. A slight increase in rate or depth brings about a diminution of the CO_2 content in the arterial blood, and ultimately of that in the tissues of the body. It is certain from Haldane and Priestley's[11] investigations that even a slight decrease is something against which the body is normally protected by nature with the utmost care. Even a small degree of acapnia induces marked alterations in the functional activity of many nerve centres and of some peripheral mechanisms. Accordingly the failure of motility in the stomach and intestines during and subsequent to hyperpnœa from anger or sorrow, from ether excitement, and from irritation of afferent nerves, may be due in part to a greater or less degree of arterial or of general acapnia.

When a tissue is exposed to the air without the protection of the skin, marked alterations rapidly develop. As described by Crile,[12] the "exposure particularly affects the vaso-motor mech-

[10] *Cf.* HALDANE and POULTON: Journal of physiology, 1908, xxxvii, p. 390. For a review of the literature, see SCHENCK: Ergebnisse der Physiologie, 1908, vii, p. 65; also HENDERSON, Y.: This journal, 1908, xxi, p. 130.

[11] HALDANE and PRIESTLEY: Journal of physiology, 1905, xxxii, p. 225.

[12] CRILE: Surgical shock, 1899, pp. 130, 135, 136, 147. Similar observations were described by VON BRAAM HOUCKGEEST: *Loc. cit.*

anism. If a bloodless field of operation, the thigh for example, be exposed, it soon becomes suffused with blood, all the vessels become dilated, the translucency of the tissue is lost, and further dissection then becomes bloody." The abdominal viscera exhibit these reactions in an especially high degree; after exposure "even the clear transparent peritoneal spaces in the mesentery display vessels and sometimes become red." The intestine also undergoes a rapid loss of tonus, either as a result of the congestion or directly from the same causes as those which affect the blood vessels. Handling the intestine accelerates the development of these conditions. But even when mechanical irritation is avoided and the loop under observation is kept warm and moist, extreme loss of tonus and intense congestion may occur. The amount of CO_2 in solution in the tissues and fluids of the body is such that it must exhale readily from an exposed surface, and a local acapnia in the immediately underlying tissues must result. It is sometimes a practice of surgeons to "protect" exposed viscera with cloths moistened in warm saline and frequently changed. The conditions thus maintained are as unfortunate as if they had been specially designed for the production of local acapnia. Our experiments show that, if the atonicity and congestion in an exposed loop of intestine has not gone too far, restoration of the CO_2 content of the tissues induces recovery of both myenteric[13] and vascular tonus. They show also that a degree of paralysis can be induced by warm moist aeration so complete that introduction of CO_2 gas into the lumen of the gut or asphyxiation of the subject fails to elicit any reaction.

II. Relations of the Blood Gases to Peristalsis.

The significance of the following series of experiments arises from the fact that no one previously (so far as we can learn) has ever seen directly, in the opened abdomen of an animal with spinal cord intact, the stomach, the small and the large intestine all performing their *normal* movements. It is probable that the condition which inhibited peristalsis in the investigations of previous observers was acapnia. We have data which show that a diminution in the CO_2 content of the blood usually occurs in animals

[13] *Cf.* observations of Bokai, quoted on p. 80 of this paper.

under operative conditions. The three experiments given below demonstrate that when this acapnia is prevented the forms of motility of the gastro-intestinal canal are practically identical with those shown by radiographs of unoperated animals. The objection cannot be made that the results of these experiments were due to asphyxia (anoxhæmia or hypercapnia), for the blood-gas analyses show that the oxygen supply was ample (note the analyses of the venous blood), and that the arterial CO_2 in two of the experiments was not considerably above that of normal life.

The experiments were performed upon three dogs, — each of about 10 kilos body weight. Each received 0.05 gm. morphin sulphate, and was then anæsthetized with chloroform *with as little hyperpnœa as possible.* The abdomen was laid open by an incision through the entire length of the linea alba. The omentum was cut out, and the intestines moved sufficiently to bring into the visible field the greater curvature of the stomach, the lower part of the ileum, and the ascending and transverse colon. A sheet of thin, flexible, transparent celluloid (of about the width of the animal's body, 2 cm. longer than the incision in the linea alba and with rounded corners) was inserted inside the body wall. A practically air-tight window was thus formed through which the viscera were clearly exhibited. The air in the space below the celluloid was expelled by means of a stream of CO_2 gas. In one case pendular movements in the small intestine manifested themselves immediately thereafter. No special precautions were taken against cooling, except that the experiments were performed during July (1907). The trachea was opened and a tube 15 mm. in diameter and 2 metres long attached. With this increase in the dead space of the respiratory tract, the breathing became deep and full, but the mesenteric arteries retained their bright red color. Thereafter, owing to the narcotic effect of the morphin which the animals had received, no further administration of anæsthetic was needed. The stomach was then distended with air [14] by way of the œsophagus. Bread mush slightly acidified with HCl was introduced far up in the large intestine. A part of the mush passed on into the ileum.

Within ten minutes after the establishment of these conditions, in all three experiments, peristalsis was in full activity in the

[14] Air is not a chemical stimulus. *Cf.* Bokai: *Loc. cit.* (p. 80 of this paper).

stomach, lower ileum, and upper colon. The movements were quite different from those observable when an animal is asphyxiated; and the characters of the movements in the stomach, ileum, and colon were in marked contrast each to the other. In the stomach the greater curvature of the antrum pylori, the pylorus itself, and a part of the fundus were visible. At the preantral groove constrictions developed, — in one subject every fourteen seconds and in another every eighteen seconds. These constrictions, when viewed in profile as they moved toward the pylorus, were 15 mm. deep and 25 mm. long. The waves moved at a rate such that one could be seen developing in the preantral groove as the preceding reached the pylorus. The pylorus remained contracted [15] and the duodenum quiescent throughout the periods of observation in all three experiments. The picture presented was identical with the radiographs obtained by Cannon from cats in that the diameter of the antrum was reduced at the trough of each wave to about half that between the waves.[16] It differed merely in the fact that only two waves, instead of three, were simultaneously visible, and that their rate was slower than in the cat. Roux and Balthazard [17] have found that this is normally the case in dogs, in which animals they saw, as we have, about four waves to the minute.

The pictures presented by both ileum and colon were identical with those seen when the radiographs of Cannon [18] are revolved in a zoëtrope. In the ileum the movements were those of rhythmic segmentation. Strikingly quick and vigorous were the alternate constrictions and relaxations and the corresponding blanching and reddening of the segments, as if a column of large frog hearts were beating in such mutual co-ordination that numbers 1, 3, 5, and 7 were in systole as 2, 4, 6, and 8 were in diastole, and *vice versa*. The contractions certainly involved complete occlusion of the lumen of the gut.

In the distended colon the entire visible portion (about 10 or 12 centimetres) exhibited a series of alternate rings of contraction and relaxation. The former were 8 mm. apart from trough to trough, and constricted the gut to about half the diameter of the intervening portion. They moved steadily from left to right (*i. e.*,

[15] *Cf.* Magnus, R.: Archiv für die gesammte Physiologie, 1908, cxxii, p. 210.

[16] Cannon: This journal, 1898, i, p. 364.

[17] Roux and Balthazard: Archives de physiologie, 1898, xxx, p. 18.

[18] Cannon: This journal, 1901, vi, p. 265.

anti-peristalsis) at a velocity of 3 centimetres per minute. In one case the large intestine was distended merely with air for fifteen minutes before the bread mush was introduced. The anti-peristalsis which was maintained during this period was not perceptibly different from that seen after the introduction of the bread mush.

All of these movements were maintained for periods of half an hour. Then the long tube attached to the trachea was replaced by a short cannula. With this diminution in the dead space of the respiratory tract respiration became shallow, the heart rate quickened, and blood pressure fell slightly. Within three minutes the intestines, both small and large, became quiescent. The sheet of celluloid was then removed from the abdomen. The intestines were as fresh and pink in appearance as when the abdomen was first opened. Immediately after removal of the celluloid, however, the vascular congestion always seen when the viscera are exposed to the air began to develop. Within fifteen minutes gastric peristalsis had ceased and the small intestine showed a notable loss of tonus. Before the long tube and celluloid were removed two samples of blood were withdrawn, — one from the femoral artery, the other from a cannula inserted through the jugular into the right heart, — and the oxygen and CO_2 which they contained were determined by the Barcroft-Haldane method.[19] After the removal of the long tube and celluloid the abdominal viscera were exposed for one hour to a current of air warmed to 35° to 38° and saturated with moisture. A continual handling of the intestines was involved in this process, but this was done gently. Hyperpnœa and tachycardia accompanied the manipulation, although ether was administered in quantities sufficient to maintain complete anæsthesia. Whenever the manipulation was stopped for a few moments, respiration became shallow or even ceased altogether for a brief period. The heart rate, on the contrary, continued rapid. Arterial pressure was not diminished, although the pulse was very narrow. The stomach and intestines became greatly congested, widely relaxed and atonic, and wholly irresponsive to stimulation. At the end of the hour two more samples of blood for analysis were taken from each animal. The percentage contents of oxygen and

[19] Barcroft and Haldane: Journal of physiology, 1902, xxviii, p. 234. The flasks used by us were three times as large as those of Barcroft and Haldane, and the blood samples were 3.0 c.c. instead of only 1.0 c.c.

CO_2 found in these and the earlier samples (calculated to 0° and 760 mm. of mercury pressure) are given in the table.

The significance of the figures for the CO_2 content of the blood during peristalsis is emphasized by the fact that (as we have found in a series of investigations to be published in detail in a later

TABLE I.

Showing Percentage of O_2 and CO_2 in the Blood during Peristaltis (Sample 1) and after Exposure and Handling of Viscera (Sample 2).

Animal.	Oxygen.		Carbon-Dioxid.	
	Sample 1.	Sample 2.	Sample 1.	Sample 2.
Arterial Blood.				
Dog 1	18.8	20.9	50.4	24.5
Dog 2	22.6	21.0	43.3	29.9
Dog 3	14.5	23.7	43.3	26.3
Venous Blood.				
Dog 1	12.8	10.6	60.2	29.2
Dog 2	13.5	8.8	57.9	43.0
Dog 3	10.5	16.6	47.0	29.9

paper) dogs which are etherized without morphin almost always develop, during the process of anæsthetization, a hyperpnœa which reduces the CO_2 content of the arterial blood below 35.0 per cent. Those which have received a moderate dose of morphin and are then etherized are less liable to hyperpnœa; but even these seldom, in our experience, reach the operating table with a CO_2 content in the arterial blood as high as 38.0 per cent. In profound anæsthesia under morphin and chloroform we find that the CO_2 content is 45.0 to 50.0 per cent or even higher. But when ether alone is used, in addition to the acapnia induced during the stage of excitement, every incision through the skin, or other operation involving stimulation of afferent nerves even in complete anæsthesia, is

accompanied by an augmentation of respiration and a corresponding diminution in the CO_2 content of the arterial blood. Furthermore dogs under ether show very little tendency, even when left perfectly quiet, to recuperate the normal percentage. On the contrary, it is not uncommon to find dogs in which hyperpnœa once started continues for long periods in spite of a maximum administration of ether by a mask. In man in prolonged operations under ether the heart rate usually shows a progressive increase, indicating probably, as we have pointed out in a previous paper, a corresponding diminution in the CO_2 of the arterial blood. It appears to us to be highly probable that the occurrence of acapnia affords the reason why such expert experimenters as Bayliss and Starling,[20] in their well-known investigations upon the motility of the alimentary canal (under A. C. E. anæsthesia), did not see the rhythmic segmentation in the small intestine and the anti-peristalsis of the colon which are certainly their normal movements.[21] Likewise Eliot and Barclay-Smith [22] found it necessary to destroy the spinal cord in all animals except the cat in order to observe any marked motility in the colon. Furthermore, what they saw (under ether anæsthesia) was so abnormal that they were led to doubt whether true anti-peristalsis occurs in this portion of the gut. Doubtless their animals were all in conditions of more or less acapnia. It is because of some mysterious inhibiting element in the condition of animals under operative conditions that so many of the functions of the abdominal viscera, etc., were not made clear until methods of observation under *normal* conditions were employed, especially by Pawlow and by Cannon. But the real nature of the abnormal and inhibiting condition which prevents or distorts these functions under anæsthesia and immediately after operation is unknown. We believe that fundamentally it is acapnia.

From all the blood-gas analyses in the literature no one inferred that the CO_2 content of the arterial blood in health is a constant, yet the investigations of Haldane and Priestley have demonstrated that this must be the case. On the contrary, the percentages found in the blood varied widely, — more commonly below than above 40 per cent. Therefore these data prove that the majority of the

[20] Bayliss and Starling: Journal of physiology, 1901, xxvi, pp. 107 and 125.

[21] *Cf.* Cannon: This journal, 1902–1903, viii, p. xxi.

[22] Eliot and Barclay-Smith: Journal of physiology, 1904, xxxi, p. 297.

animals from which the blood samples were taken — and correspondingly a majority of all animals and men under anæsthesia and operative conditions — were in states of more or less acapnia.

Great significance attaches to the facts that the movements observed in our experiments were the normal forms of motility, and that the blood gases were nearly the same as in normal life, — not asphyxial. Therefore it is not reasonable to suppose that the inactivity of the gut usual under anæsthesia and operative conditions is due to some inhibition other than acapnia, and that in our experiments the increase of CO_2 by the tracheal tube neutralized this inhibition by a stimulation. And if there is here no neutralization, there is then no longer need to invoke "prolonged inhibition" (in the wide but vague sense of some writers) to explain the phenomena of shock in the abdominal viscera. Whether or not this is true of the suppressions of secretory activity must be left for discussion in later papers. The data here presented indicate that the failures of motility at least are the expression of lowered tonus in the tissues of the body and altered activity in the centres of the central nervous system because of diminished CO_2.

These observations and the blood-gas analyses indicate also that the effects of asphyxia upon the intestine — the blanching, constriction, and violent pendulum movements — are mainly due to hypercapnia. Lack of oxygen without excess of CO_2, in the experiments of Magnus [23] upon the excised intestine, caused an increased tonus in the circular muscle. But the effect was slow in comparison with that in ordinary asphyxia, and the possibility that there was an increase of CO_2 in the saline bath does not seem to have been wholly excluded. We observed repeatedly that, if the dead space of the respiratory tract was increased by so long a tube that the blood in the mesenteric arteries lost its brightness of color, the movements of the intestines were for a few seconds accentuated and then ceased altogether. If the tube was then shortened until the blood recovered its brightness, the intestines again exhibited a brief period of exaggerated activity. These observations support the idea that lack of oxygen is not a stimulus, but that it merely paralyzes.

[23] Magnus: Archiv für die gesammte Physiologie, 1904, cii, p. 137.

III. Exhalation of CO_2 from Exposed Viscera.

The following experiments afford a basis for estimating the rate at which CO_2 exhales from exposed viscera. They show that the CO_2 content of uncovered tissue is reduced, for in successive periods of a half hour each the exhalation diminished. Crile[24] states that "where the omentum is made to cover the viscera, there is much less shock." These experiments show that this protection can be in part explained by a less rapid exhalation of CO_2 from viscera covered by it than from organs directly exposed.

Cats were placed under chloroform anæsthesia. The abdomen was opened by an incision in the median line. The large end of a glass funnel 8 to 10 centimetres in diameter was inserted through the cut so that the body wall and skin fitted air tight around it, like a button-hole around a button. The neck of the funnel was connected with a Pettenkofer absorption tube (2 metres in length and containing 400 c.c. of dilute baryta water) to which in turn was connected a suction pump on a water faucet. Air was admitted to the space above the viscera and below the inverted funnel by a small glass tube passed through the body wall. To the outer end of this tube was attached a vessel containing moist soda-lime and a wet sponge. There was no negative pressure under the funnel. Thus every minute 200 c.c. of moist air, at 18° and free from CO_2, were drawn over a definite area of peritoneal surface, and the CO_2 which exhaled was absorbed by the baryta. This slow current of air was continued for a half-hour. Then the baryta water was transferred to a flask. After sedimenting over night, 25 c.c. of the clear supernatant fluid, and an equal quantity of the original baryta water were titrated with a solution of oxalic acid (2.808 gm. in a litre of water, 1 c.c. of the acid representing 0.5 c.c. of CO_2 at 0° and 760 mm. of mercury pressure).

During the first half-hour period in two of the experiments the omentum was left covering the intestines. The exhalation of CO_2 under these conditions amounted to 7.2 c.c. from one cat and 6.4 c.c. from the other. In both of these experiments the funnel was then removed, the omentum pushed off of the viscera, and the funnel replaced as nearly as possible in its previous position. During the ensuing period of a half-hour the exhalation of CO_2 amounted to 17.4 and 15.0 c.c. respectively. The area of viscera exposed was 80 sq. cm.

In two other experiments the omentum was removed at the beginning, but only 50 sq. cm. were exposed under the funnel. The exhalations from this area during three successive periods of a half-hour each were 10.8, 7.8, and 6.0 c.c. respectively, in the first experiment; and in the second 8.8, 6.3, and 6.3. Only a slight degree of congestion developed in the intestines.

[24] Crile: *Loc. cit.*

From these data the exhalation from the abdominal viscera into the air during the first half-hour under the conditions of the experiments may be estimated at 0.15 to 0.20 c.c. of CO_2 per square centimetre of visceral surface exposed, and less than half this quantity through the omentum. The total amounts exhaled are insignificant in comparison with the entire CO_2 production of the body. A slight diminution in the amplitude of respiration would counterbalance the exhalation from the viscera, and thus prevent the development of a general acapnia. But in the tissue exposed (as the last two experiments show by the lessening exhalations in successive periods) the exhalation reduces the CO_2 content considerably below the normal. Furthermore, these experiments determine only the minimum rate of loss, for the viscera were quiescent and somewhat cool, and the current of air was very slow. No comparable exhalation of CO_2 occurs through the intact skin. Schierbeck[25] found an output of 0.35 gm. (or 182.1 c.c. at 0° and 760 mm. pressure) per hour from the entire skin at temperatures at which there was no sensible perspiration, and three to four times as much with perspiration. Taking the surface of the skin (for a body weight of 65 kilos) at 2 square metres, the output per sq. cm. of skin per half hour would be only 0.0045 c.c. of CO_2. The minimum rate of exhalation from a square centimetre of exposed peritoneum is forty times more.

IV. The Effects of Aeration of the Viscera.

When the abdominal viscera, or indeed any tissues, are exposed to the air, they undergo a loss of tonus and become congested. These changes occur also, although in a less degree, when the intestines are placed in a bath of warm saline. If the exhalation of CO_2 is a factor in these phenomena, we should expect that the conditions which would induce or prevent them could be inferred from the principles governing the diffusion of this gas. From a mass of wet paper pulp impregnated with CO_2 the exhalation would be more rapid if the mass were kept warm than if it were cold, more rapid if the surface were kept moist than if it became dry, more rapid if it were continually kneaded than if stagnant, more

[25] Schierbeck: Archiv für Physiologie, 1893, p. 119.

rapid if it were placed in a draft of air or a stream of water than if the surrounding medium were quiescent, and nearly as rapid if the mass were under water (or physiologic saline) as it would be in air, for the coefficient of solubility of CO_2 is 1. Applying these considerations to the problem of the local inhibition of peristalsis, it is evident that the point last mentioned would afford an explanation of the fact (otherwise puzzling) that peristalsis is not much more persistent in a bath of warm saline than in air. Thus Meltzer finds proof of a local inhibition in the intestine — which we would explain by the local acapnia — in the following experiment: After destruction of the spinal cord in the rabbit the peristalsis in the cæcum is exaggerated by the absence of the normal inhibitory control. The mere act of opening the abdomen does not, as it does in a normal rabbit, cause immediate cessation. Nevertheless very soon after exposure the movements subside, and "even in a warm bath of a physiologic solution the cæcal peristalsis ceased after ten or fifteen minutes." The three experiments cited in section II of this paper show that when both local and arterial acapnia are prevented even the more difficult task of maintaining motility in the large intestine of a dog with the spinal cord intact is readily accomplished. That this task is difficult under ordinary experimental conditions is shown by the fact that in the careful experiments of Eliot and Barclay-Smith, even after destruction of the spinal cord, anti-peristalsis was never seen in the colon of dogs.[26]

Professor W. B. Cannon tells me that he has recently observed two cats in which gastric peristalsis did not occur spontaneously after destruction of the spinal cord and opening of the abdomen under saline. He found that the attachment of a piece of rubber tubing (25 cm. in length) to the trachea was effective as a means of initiating motility.

The effects of exposure of the viscera, and particularly the intense vascular congestion, have been regarded as the results of mechanical irritation, cooling, and drying. Doubtless many investigators have felt the inadequacy of these three causes to account for all the phenomena observable, but no other factor has been thought of. In order to determine whether exhalation of CO_2 is this unrecognized fourth cause, we devised a method of treatment for the viscera (by means of the apparatus shown in

[26] ELIOT and BARCLAY-SMITH: *Loc. cit.*

Fig. 1) which fulfils the conditions, mentioned in the last paragraph, under which the diffusion of this gas would be most rapid. It consists in passing over the viscera a gentle current of air warmed to 35° or 38° and saturated with moisture. It affords an almost crucial test of the hypothesis of acapnial shock. If diminution of the CO_2 content were of no importance in loss of tonus, the conditions under which the viscera are placed by this

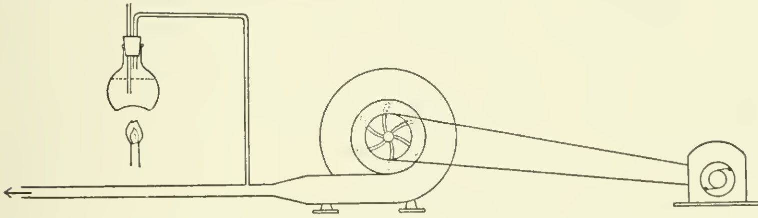


FIGURE 1. — This apparatus consists of a $\frac{1}{12}$ horse-power electric motor, a rotary air fan, and a flask in which water is boiled. Steam is discharged into the tube from which air, thus warmed and moistened, is directed upon the viscera. For cats one triple burner is placed under the flask, for dogs three large triple burners (*i.e.*, 9 Bunsen burners). For cats the tube is a piece of bicycle tire ("single tube"). For dogs it is a piece of steam fire-engine hose 8 cm. in diameter.

method would be nearly ideal. A surgeon who watched one of our experiments without knowing its purpose asked whether it would not be advantageous to use the method in the operating-room of a hospital, since cooling and drying are eliminated without mechanical irritation. The current of warm moist air is apparently so mild that the hands of the operator after exposure to it for two or three hours are not in the least reddened, nor is their skin softened or puckered. Yet in viscera exposed to it intense congestion rapidly develops. Furthermore *this treatment is a highly effective procedure for the production of shock.* The details of some of our observations on this topic are as follows:

These experiments were performed upon cats. Under chloroform anæsthesia the abdomen was opened by a median incision, and two loops of the small intestine were drawn out. One was held in the current of air. Almost immediately it blushed as markedly as does the ear of an albino rabbit when the cervical sympathetic is cut. Innumerable minute blood vessels previously invisible became apparent on the surface. At first these vessels were bright red, but at the end of fifteen minutes the tissue had acquired a peculiar dark, intensely congested, almost bruised appearance, although it had been handled very gently. The tonicity was so much reduced that the loop was of double its

original diameter. Mechanical irritation by pinching between the fingers now caused no contraction. The other loop of intestine had been wrapped in cotton wool moistened with normal saline at 18°, and had been placed so as to lie on the side of the animal out of the current of air. At the end of the fifteen minute period it was found to be only slightly congested, in good tonus, and normally irritable. Parts of the intestine which had remained wholly within the abdomen showed no perceptible alteration; the effects of the aeration were confined to the parts directly exposed. Doubtless mechanical irritation was a contributory factor in the alterations noted in the exposed loop. In another experiment one loop was laid on cotton wool and left untouched by the hand during twenty minutes of warm moist aeration, while a control loop was wrapped in thin sheet rubber to protect it from the air and was handled continuously for this period. Both became congested, but the former underwent by far the greater loss of tonus and motility. The animal was then asphyxiated; and the exposed loop recovered its tonus, and developed, in common with the rest of the intestine, pendular movements. Altogether, aeration of the intestine was tried upon four cats. In three of these experiments the exposed loop was later placed in warm saline saturated with CO_2. A few cubic centimetres of this gas were also introduced into the lumen of the gut by means of a hypodermic syringe. In two of the three cases a *marked and rapid recovery of tonus and motility* were observed.[27]

V. The Production and Relief of Acapnial Shock.

We have applied the treatment of warm and moist aeration to the entire abdominal viscera in experiments upon 15 dogs. Two examples of the production of shock in these experiments are described below. They are selected especially because in both a rapid recovery from the condition of shock was obtained by means of measures designed to relieve acapnia. In neither case was the condition of shock extreme — although both were near the point beyond which there is no return — before relief was begun. We have found that in other cases, in which the failure of the circulation had progressed further than in these, similar measures were not ultimately successful. The reason for the ineffectiveness of all methods of restoration in cases below an arterial pressure of 30 or 40 mm. will be shown in a later paper by data from blood-gas analyses. These two experiments are, however, especially signifi-

[27] This result was obtained also by Bokai: Archiv für experimentelle Pathologie und Pharmakologie, 1887, xxiii, p. 209.

cant because of the rapidity of the recovery which they show. Without measures for the replacement of the body's store of CO_2 the restoration of the animals to normal condition would have been a matter of many hours, if indeed it had occurred at all spontaneously. Even more important, however, is the demonstration which they afford that an animal is readily brought to the verge of shock by the mild procedure of aeration of the viscera. This fact is to be contrasted with the demonstration by Sollmann, Brown, and Williams [28] that pouring concentrated nitric acid or caustic soda into the abdominal cavity produces little effect upon respiration or arterial pressure within a period of an hour. Thus it appears that corrosion of the viscera is less effective than aeration as a means of inducing shock.

In Fig. 2 is shown the arterial pressure curve obtained in one of these experiments. The subject was an animal of the type which best withstands operations, a bull-dog in good condition. Yet aeration of the viscera for three hours lowered the arterial pressure by 50 per cent, and diminished the amplitude of the pulse curve to an even greater degree. The pulse in the femoral artery became barely perceptible to the finger. After the first twenty minutes of aeration the animal became completely comatose and no further administration of anæsthetic was needed. In the production of this condition the stimulation of afferent nerves seemed to play no part, for respiration was never accelerated. In this respect this experiment is rather exceptional. The amplitude of the respiratory movements was diminished to as great a degree as the pulse. The small intestine changed color from pink to a dark purple; it relaxed so completely that its diameter was doubled, and it ceased to respond to a pinch. The mesenteric veins and the venous radicles at the base of the intestine became engorged and nearly black. During the first half-hour after the aeration was discontinued and the abdomen closed, no spontaneous improvement in the condition of the animal occurred. Then 200 c.c. of Ringer's solution were slowly (during ten minutes) injected into the femoral vein. This fluid had previously, and while cold, been shaken thoroughly in a flask through which CO_2 was bubbled; it had then been warmed to 35°; and at the time of injection it was not only saturated with the gas, but contained also numerous small bubbles. The animal's

[28] Sollmann, Brown and Williams: This journal, 1907–1908, xx, p. 74.

respiration increased in amplitude almost immediately. A tube 15 mm. in diameter and 0.5 metre in length was attached to the trachea, and additional pieces of tubing gradually added until the total length was 1.5 metres. The respiration became deep and full, the heart rate slower, and arterial pressure began to rise. Ringer's solution saturated with CO_2 was introduced into the peritoneal cavity, and a stream of CO_2 gas from a Kipp generator was bubbled through this liquid from a tube inserted deep among the viscera. At the end of twenty minutes the animal had come out of coma, so that ether had again to be administered. Arterial pressure had risen considerably, and the pulse was of nearly normal amplitude. After an hour the Kipp generator was shut off, and the tube was removed from the trachea. Within five minutes after these changes respiration had again become shallow, the amplitude of the pulse had diminished, its rate had increased, and arterial pressure had begun to fall. The tube was then replaced on the trachea, and 150 c.c. of Ringer's solution saturated with CO_2 were injected into the femoral vein. A half hour later the tracheal tube was finally removed. Thereafter the condition of the nervous system, the circulation, and respiration were almost as nearly normal as in the period six hours earlier and before the abdomen had been opened. The viscera had recovered their normal appearance and tonus.

A demonstration of the production and relief of shock which differs in some respects from the experiment above discussed is afforded by Fig. 3. During the aeration of the abdominal viscera coma developed, respiration became shallow, the pulse narrow, the femoral artery constricted,[29] and the intestines congested. At the end of one hour the animal was by all these signs in a state of shock, yet arterial pressure had not fallen. Tracheotomy was then performed, and the sciatic nerve was pinched with a pair of artery forceps. Vigorous hyperpnœa resulted, and arterial pressure fell gradually. Ether was administered during this period in quantities sufficient to maintain anæsthesia, but not in such amounts as to account at all for the fall of arterial pressure. At the end of twenty minutes of hyperpnœa the pressure had dropped from 140 down to 55 mm. of mercury. The forceps were then removed from the

[29] For literature showing that the fall of arterial pressure in shock is not due to arterial relaxation (*i. e.*, vaso-motor failure), but on the contrary that the arteries are constricted, see Henderson, Y.: This journal, 1909, xxiii, p. 362.

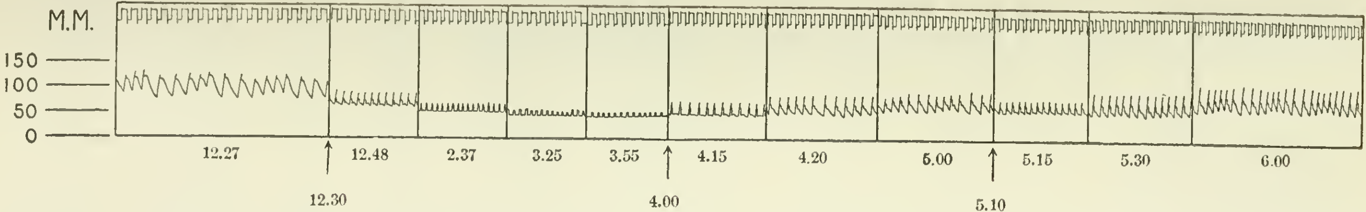


FIGURE 2. — (About half the original size). Experiment of Feb. 28, 1907. Vigorous bulldog of 10.5 kilos. Morphin sulphate 0.05 gm. chloroform, then ether. Time record in 0.5 seconds. Carotid pressure curve recorded by a Hürthle manometer. The abdomen was opened at 12.30, and the viscera were aerated until 3.25, when the viscera were replaced and the abdomen closed. At 4.00 measures for the re-carbonating of the blood and tissues were instituted (see text). At 5.10 they were discontinued for five minutes and a slight relapse occurred. Throughout the entire experiment the rate of respiration was never less than 11 nor more than 13 per minute. The amplitude of respiration, however, varied as greatly as, and synchronously with the amplitude of the pulse.

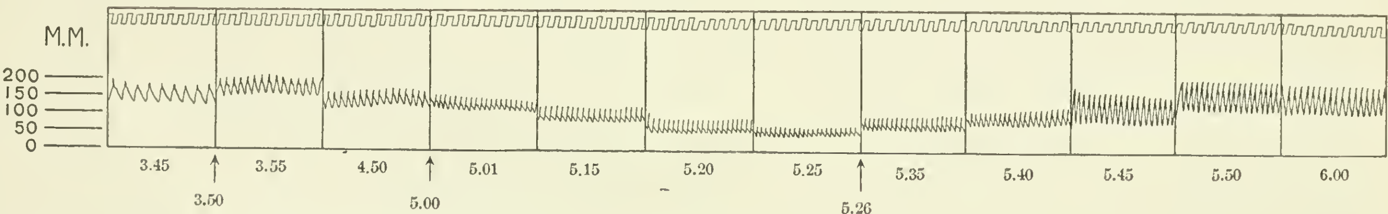


FIGURE 3. — Experiment of April 15, 1907. Dog of 5.0 kilos. Morphin sulphate, 0.03 gm. first chloroform, then ether. Time record in 0.5 second. Carotid pressure curve recorded by a Hürthle manometer. The abdomen was opened at 3.50, and the viscera were aerated until 5.20. At 5 tracheotomy was performed. From 5 until 5.20 the sciatic nerve was pinched at frequent intervals. Hyperpnœa was thus induced. At 5.26 120 c.c. of Ringer's solution saturated and bubbling with CO_2 were slowly injected into the femoral vein. At 5.30 a tube 15 mm. in. diameter and a metre in length was attached to the trachea. At 5.50 the animal had recovered from the state of coma and shock. The tube was removed and the trachea closed. At 6 no relapse had occurred, and except for a more rapid pulse rate the animal appeared to be in as good condition as at 3.45.

sciatic, the viscera replaced in the abdomen, and the cavity closed. During the next ten minutes the animal seemed on the verge of respiratory failure, but arterial pressure rose slightly (to 65 mm.). Then 120 c.c. of Ringer's solution, saturated and bubbling with CO_2, were injected into the femoral vein. As soon as the increased depth of respiration induced by the CO_2 had developed, a tube 15 mm. diameter and 1.0 meter in length was connected with the trachea. At the completion of an interval of fifteen minutes occupied by the processes of recovery (*i. e.*, "recarbonization" of the tissues) respiration was deep and full, the coma was relieved, and the administration of ether again became necessary, the pulse regained its normal amplitude, and arterial pressure rose to 150 mm. of mercury. The tube was then removed from the trachea, and the wound closed. No relapse occurred, and ten minutes later the animal was in every respect, except a more rapid pulse, as nearly in normal condition as prior to the opening of the abdomen two and a quarter hours previously.

V. Conclusions.

I. Acapnia, due to hyperpnœa, plays an important part in the central inhibition of peristalsis occurring under surgical operations. Local acapnia, due to direct exhalation of CO_2, is a factor in the loss of tonus in exposed viscera.

II. When loss of CO_2, both by way of the lungs and by direct exhalation, is prevented, and the blood gases are maintained nearly normal, peristalsis can be directly observed in the stomach and in the small and large intestines.

III. The minimum rate of exhalation of CO_2 from exposed peritoneal surfaces is 0.15 to 0.20 c.c. per sq. cm. in the first half hour, or 40 times the rate from the skin.

IV. Exposing the abdominal viscera to a current of air at body temperature saturated with moisture rapidly induces congestion and loss of tonus and motility.

V. Aeration of the viscera in this manner is an effective method for the production of shock. Restoration of the body's store of CO_2 is effective as a method of relief from all except the extreme stages of acapnial shock.

VI. These observations and others which are to be presented in later papers indicate that the CO_2 tension in the nerve centres

and in the tissues and fluids of the body is a factor in the maintenance of tonus (in the broad sense of the word) of the same order of importance as temperature, oxygen supply, osmotic pressure, and the equilibrium of anions and kations.[30]

I am indebted to Prof. Walter B. Cannon for valuable criticism upon the manuscript of this paper.

Preliminary note of further investigations upon the effects of acapnia. — I have obtained data which indicate that one of the conditions requisite to the contractility of the uterus is the normal CO_2 content of the blood. The tonus and motility of this organ also appear to be inhibited by acapnia, and accentuated by hypercapnia. In normal labor the pulmonary ventilation, in spite of the suffering, is not continually excessive. During each "pain" the partial pressure of CO_2 in the alveolar air of the lungs is even increased by the spontaneous holding of the breath. The obstetrician is able to some extent to control the duration and vigor of the contractions by commanding or discouraging this apnœa. It is probable that the ineffective "pains" of prolonged labor are in part due to inability of the subject to regulate respiration properly, *i. e.*, to inhibit the respiratory centre from responding to the intense stimulations of afferent nerves. Hyperpnœa occurs, acapnia develops, and diminution of uterine tonus and motility result. Upon this topic and upon the suppressions of gastric and pancreatic secretions by acapnia investigations are under way.

[30] *Cf.* HENDERSON, L. J.: This journal, 1908, xxi, p. 427.

THE RÔLE OF THE ASH CONSTITUENTS OF WHEAT BRAN IN THE METABOLISM OF HERBIVORA.[1]

By E. B. HART, E. V. McCOLLUM, and G. C. HUMPHREY.

[*Contribution from the Agricultural Chemistry and Animal Husbandry Departments of the University of Wisconsin.*]

IN a previous publication[2] it has been shown that wheat bran contains phytic acid in combination with potassium, magnesium, and calcium. In a later publication[3] it was demonstrated that this complex was in all probability responsible for the well-known laxative effect of wheat bran, — a phenomenon previously held to be due to the mechanical irritation occasioned by the coarse and fibrous construction of this material. In the course of the above investigation with milch cows data were procured indicating in certain cases a specific physiological function for this complex. Beside this laxative effect which manifested itself in all experiments, its withdrawal from the ration occasioned more or less disturbance of fat production, a regular reduction in the volume of urine produced daily, and an increased flow of milk. In addition to the above enumerated facts, withdrawal of phytin from a ration disturbed the œstrum periods of certain individuals, while in other individuals this disturbance was but occasional.

During this early investigation a limited amount of data was collected on the metabolism of the bases associated with phytin, and their channels of excretion. It is indeed entirely conceivable that the path of elimination of the ingested bases associated with phytin is responsible for such phenomena as the laxative effect and the

[1] Published with the permission of the Director of the Wisconsin Experiment Station of the University of Wisconsin.

[2] PATTEN and HART: Journal of the American Chemical Society, 1904, xxxi, p. 564.

[3] JORDAN, HART, and PATTEN: This journal, 1906, xvi, p. 268.

increased urine flow. The reversal of these two phenomena invariably took place on the withdrawal of phytin from the ration of milch cows. The other effects, such as disturbances of fat production, flow of milk, and cessation of the œstrum periods, — functions imbedded deeply in the maternal nature of the animal, — were not always highly pronounced, and when such disturbances did take place, are to be considered individualistic and at present unexplained. If it were possible to induce these last three disturbances with all individuals, then the specific relation of phytin to these physiological processes might be seriously considered, but such does not appear to be the case.

It was to study further the action of the components of the phytin complex when administered separately as salts, their channels of excretion and general relation to the phenomena of constipation and diuresis, with the consequent effect on milk secretion, that this investigation was undertaken.

The scope of the following experiment involved:

1. A study of the metabolism and channels of excretion of the base and acid constituents of phytin, as well as equivalent quantities of these bases in the form of chlorides and sulphates.

2. The effect of the supply and the channel of excretion of these elements on diuresis, the character of the feces and milk flow, and the complete composition of the milk.

3. The effect of withdrawal of phytin and the reduction of the crude fibre content of the ration on the character of the excreta.

4. Physiological action of prepared potassium phytate.

General Plan.

1. Feeding to the same animal during short and long periods of time, rations differing greatly in the amount of phosphorus, magnesium, or potassium, as phytin, or rations low in natural phytin, but supplemented with magnesium and potassium, as a chloride or sulphate, or potassium as a phytate.

2. A reduction of the amount of the crude fibre in the ration of low phytin content secured by lowering the proportion of washed bran and increasing the starch content of the ration.

3. Abrupt or gradual changes from rations of high phosphorus,

magnesium, and potassium content, to rations low in all or only one of these ingredients.

4. The nutritive plane of the rations to be maintained the same except in the variation of the ash constituents.

5. The ration fed to be carefully weighed and samples of the excreta quantitatively collected and preserved for analysis.

Experimental Part.

The cow selected was a vigorous grade Holstein, in fair flesh and with a ravenous appetite. Her keen appetite secured a complete consumption of the ration during the entire experiment. The animal was kept in an especially arranged and warmed room. She was fed from a tight box which would allow recovery of all uneaten food. The daily ration was given in two equal proportions, morning and night, and water offered at definite times. She was weighed daily; all excreta were quantitatively collected. The weights represent what was voided during the twenty-four hours from 6 A. M. The animal was milked at a definite time, twice a day, morning and night.

The rations employed were made up, as in previous experiments, of oat straw, wheat bran, rice, and wheat gluten. This ration affords a high phytin intake, consequently a high phosphorus, magnesium, and potassium consumption. A basal ration, low in phytin and consequently low in phosphorus, magnesium, calcium, and potassium, was secured by extracting whole wheat bran with water after a period of soaking. The calcium content of the basal ration is somewhat increased over that of the standard ration owing to the percentage increase of this element in the washed bran. Nevertheless the daily consumption of this element is below the daily output. With the basal ration as a starting-point additions of materials whose influences were to be studied could be secured.

Sufficient feeding materials were prepared or set aside for a presumed length of the experiment and carefully sampled, for analyses. The milk, urine, and feces were weighed and sampled after being carefully mixed. Reserve samples of milk and urine, preserved with formaline and toluol, were set aside. Five-pound samples of fresh feces were dried at 60° C. for reservation. Nitrogen determinations were made on the fresh feces. Standard

methods of analyses were used in most cases.[4] Fat was determined by the Babcock method, and casein by the centrifugal process checked at intervals by the chemical method. It is only necessary to state that this method gave close agreement with the chemical determinations.

Sequence of Rations.

An initial period of two weeks was consumed in adjusting the animal to the ration used. Actual records began November 26.

Ration 1. Fed from November 26 to December 5.

Transition Period. This was begun December 5 and was completed on December 9. Previous experience had shown that a sudden withdrawal of phytin from the ration resulted in constipation. To avoid this effect the withdrawal was made gradual by the daily substitution of two pounds of washed bran for two pounds of whole bran. The wheat gluten was slightly increased from day to day until on December 9 the animal was receiving two pounds. Her appetite remained keen.

Ration 2. December 9 to December 23.

Ration 1. December 23 to December 30. On the evening of December 23 the animal was suddenly changed to the ration of whole bran.

Ration 2. December 30 to January 11. December 30 a sudden change from the whole bran to the washed bran ration to which was added 135 gm. of potassium sulphate and 200 gm. of magnesium chloride. These quantities of salts supplied approximately the amounts of magnesium and potassium withdrawn by substituting washed bran for whole bran.

Ration 1. January 11 to January 21. This change was made suddenly.

Ration 2. January 21 to February 1. A sudden change from the whole bran ration to the ration in which the crude fibre intake was equivalent to that of ration 1 was made on January 21. This ration of lower crude fibre content was secured by reducing the intake of washed bran from 10 to 5.3 pounds. In addition 4.7 pounds of wheat starch were substituted for the withdrawn washed

[4] The thanks of the authors are due Mr. Harry Steenbock for valuable assistance in collecting a part of the analytical data.

bran and the amount of gluten was raised to 2.7 pounds. This period was for the purpose of thoroughly testing the laxative effect of phytin. It was thought not impossible that the increased intake of crude fibre with the washed bran ration might be responsible for the constipated condition. Constipation nevertheless resulted after a lapse of ten days.

Ration 1. February 1 to February 6.

Ration 2. February 6 to February 14. A sudden change from the whole bran ration to the washed bran ration, plus 135 gm. of potassium sulphate, equivalent in potassium to the quantity supplied by the whole bran ration. The appetite was keen and the entire ration was consumed.

Ration 1. February 14 to February 19.

Ration 2. February 19 to February 29. A sudden change from whole bran to the washed bran ration to which was added 115 gm. of potassium chloride, or the potassium equivalent to the quantity supplied by the whole bran ration.

Ration 1. February 29 to March 10.

Ration 2. March 10 to March 16. A sudden change from the whole bran ration to the washed bran ration, plus 150 gm. of crude potassium phytate. This material was prepared by extracting bran with 0.2 per cent of hydrochloric acid, allowing the strained extract to settle in barrels. The extract was made alkaline with sodium hydroxide and precipitated with barium chloride. The precipitate was thoroughly washed, carefully decomposed with sulphuric acid, filtered from the barium sulphate, and neutralized to phenolphthalein with potassium hydroxide. The solution was evaporated to dryness on the water bath and the residue ground and used directly. The material had a pleasant taste and odor.

Discussion of Data.

The tables displayed in the following pages were made up of either daily records or were averages of periods in which it was believed the animal was adjusted to the ration. Below are given the amounts of various feeds used daily, together with their composition.

TABLE I.

THE RATIONS FED.

Ingredient.	Ration 1.	Ingredient.	Ration 2.
	pounds.		pounds.
Oat straw	10	Oat straw	10
Bran	10	Washed bran . . .	10
Rice meal	7	Rice meal	7
Wheat gluten . . .	1½	Wheat gluten . . .	2

TABLE II.

COMPOSITION OF THE FEEDING STUFFS USED.

Feeds.	Water.	Protein.	Fat.	P_2O_5.	CaO.	MgO.	K_2O.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Oat straw . .	8.52	6.81	1.75	0.45	0.318	0.270	1.560
Bran	9.11	15.12	4.50	3.50	0.178	1.128	1.700
Washed bran .	7.55	10.80	4.25	0.31	0.228	0.181	0.098
Rice meal . .	12.43	8.84	0.40	0.25	0.010	0.052	0.130
Wheat gluten .	6.37	75.00	1.60	0.47	0.108	0.044	0.098

Comparative nutritive value of the two rations. — The following table presents the numerical data covering this point:

TABLE III.

QUANTITIES AND PERCENTAGES OF DIGESTIBLE NITROGEN AND DRY MATTER FED.

DATE.	P_2O_5 Daily.	CaO Daily.	MgO Daily.	K_2O Daily.	Dry matter daily.			Nitrogen daily.		
					Fed.	In Feces.	Digested.	Fed.	In Feces.	Digested.
	gm.	gm.	gm.	gm.	gm.	gm.	per cent.	gm.	gm.	per cent
Dec. 1–4	190.5	23.5	65.4	152.6	11698	3963	66.1	286	92.6	64.1
Dec. 19–22	46.7	26.0	22.5	80.4	11982	4370	63.5	283	87.8	68.9
Feb. 10–13	46.7	26.0	22.5	153.3	11982	4343	63.7	283	83.4	70.5

There is nothing to indicate but that the animal received an abundance of digestible organic nutrients on either ration. The dry matter digested daily from ration 1 was 17 pounds, and from ration 2 16.7 and 16.8 pounds; while the amounts of digested protein were 2.6, 2.6 and 2.7 pounds respectively. From this it is apparent that whatever physiological differences manifested themselves they must be attributed to other variants in the ration.

Influence of the supply of ash materials either as phytin or other salts on the organic components of the milk. — In the table that follows are recorded the results of the gradual transition period (December 3 to 29) from whole bran to washed bran, as well as data illustrating the effect where the change in the ration was

TABLE IV.

Effect of Supply of Bran and Ash Constituents on Composition of the Milk.

Date.	Total P_2O_5 fed.	Total CaO fed.	Total MgO fed.	Total K_2O fed.	In Milk.				
					Solids.	Protein.	Casein.	Fat.	Sugar.
	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.
Dec. 3	190.5	23.5	65.4	152.6	10.6	2.81	1.90	2.85	4.94
Dec. 4	190.5	23.5	65.4	152.6	10.8	2.80	1.95	3.20	4.80
Dec. 5	161.4	23.9	55.7	138.0	10.8	2.67	2.00	3.15	4.98
Dec. 7	103.5	24.7	39.3	108.9	10.9	2.70	2.00	3.18	5.02
Dec. 9	46.7	26.0	22.5	80.4	10.7	2.80	2.00	3.00	4.90
Dec. 15	46.7	26.0	22.5	80.4	10.3	2.68	1.95	2.85	4.77
Dec. 21	46.7	26.0	22.5	80.4	10.7	2.70	2.10	2.85	5.15
Dec. 22	46.7	26.0	22.5	80.4	10.7	2.70	1.90	2.85	5.15
Dec. 29	190.5	23.5	65.4	152.6	10.9	2.78	1.95	2.85	5.27
Dec. 30	46.7	26.0	61.9	153.3	11.0	2.75	2.00	3.00	5.25
Jan. 6	46.7	26.0	61.9	153.3	10.9	2.78	2.05	3.10	5.02
Feb. 5	190.5	23.5	65.4	152.6	11.2	2.81	1.90	2.90	5.49
Feb. 6	46.7	26.0	22.5	153.3	11.2	2.78	1.95	2.85	5.57
Feb. 14	46.7	26.0	22.5	153.3	10.9	2.81	1.95	3.00	5.09
Jan. 19	190.5	23.5	65.4	152.6	11.2	2.87	1.95	3.10	5.23
Jan. 20	41.5	21.5	18.7	78.1	11.1	2.93	1.90	3.20	4.97

made in a single day, January 19 to 20. In addition, the table includes records of the influence of a sudden change from a whole bran ration of large phytin intake to the washed bran ration plus salts added as magnesium sulphate and potassium chloride (December 29 to January 6); an equivalent to the magnesium and potassium removed in the washed bran as potassium chloride alone (February 5 to 14); the effect of the continuation of the respective ration initiated on December 30 and February 6 is shown in the records for January 6 and February 14. These data clearly indicate, we think, that with this animal there was no significant fluctuation in the composition of the milk as the result of a wide divergence in the quantities and forms of the ingested ash constituents. It, of course, must be remembered that at no time was the animal starving for magnesium or potassium, as indicated by the fairly close balance between the income and outgo of these elements during all the periods (see Table VIII).

Influence of the supply of ash elements on their percentage content in the milk. — In Table V are displayed results showing the

TABLE V.

PERCENTAGES OF PHOSPHORUS, CALCIUM, MAGNESIUM, AND POTASSIUM IN THE MILK.

Date.	Total P_2O_5 fed.	Total CaO fed.	Total MgO fed.	Total K_2O fed.	Amounts in the milk.			
					P_2O_5.	CaO.	MgO.	K_2O.
	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.
Dec. 3	190.5	23.5	65.4	152.6	0.178	0.135	0.019	0.193
Dec. 4	190.5	23.5	65.4	152.6	0.172	0.136	0.018	0.191
Dec. 5	161.4	23.9	55.7	138.0	0.172	0.130	0.020	0.181
Dec. 7	103.5	24.7	39.3	108.9	0.171	0.132	0.018	0.190
Dec. 9	46.7	26.0	22.5	80.4	0.175	0.131	0.015	0.165
Dec. 15	46.7	26.0	22.5	80.4	0.168	0.128	0.017	0.170
Dec. 21	46.7	26.0	22.5	80.4	0.168	0.125	0.013	0.148
Dec. 22	46.7	26.0	22.5	80.4	0.178	0.130	0.017	0.154
Dec. 29	190.5	23.5	65.4	152.6	0.164	0.125	0.016	0.157
Dec. 30	46.7	26.0	61.9	153.3	0.172	0.128	0.020	0.146
Feb. 5	190.5	23.5	65.4	152.6	0.178	0.118	0.019	0.175
Feb. 6	46.7	26.0	22.5	152.3	0.180	0.118	0.018	0.185

effect of a wide variation in the intake of ash elements on the percentage of these constituents in the milk.

The table emphasizes the constancy of composition of the milk in respect to the ash elements, even when a wide variation in intake of these elements prevailed. The form of intake of phosphorus, magnesium, and potassium, whether as phytates, sulphates, or chlorides, appeared to have no influence on the amounts secreted by the mammary cells.

Influence of the supply of ash elements on the yield of milk solids and the excretion of urine. — In order to show the effect of the supply of phosphorus, magnesium, and potassium, as phytates, sulphates, or chlorides on the flow of milk and the volume of urine excreted, periods of short duration, but representing adjusted conditions of the animal to the ration, were selected.

There is nothing in the data presented that indicates with this animal a constant relation between the amounts or form of the ash elements ingested in the ration and the amount of milk, or quantity of solids produced. Even when we consider a transition period involving the gradual withdrawal of phytin, there was no effect on the volume of milk produced. On December 4, with a high phytin ration, the amount of milk secreted was 16,889 gm., while on December 9, or at the termination of the transition period, and corresponding to the lowest intake of phytin, the flow was 17,025 gm.

The consistent and pronounced effect of the supply of phytin (December 1 to 4), of potassium and magnesium as chlorides and sulphates (January 6 to 9), of potassium alone as a chloride (February 25 to 28), or of potassium as a phytate (March 13 to 16) on the volume of urine produced daily, when compared with the low intake of the ash elements recorded in the other periods, is striking. When this effect is considered in relation to the daily water consumption, there is seen to be no close parallelism. However, the proportion of water excreted through the urinary tract is normally in herbivora such a small proportion of the total intake that fluctuations amounting to 10 pounds in the daily consumption affected the volume of urine produced but slightly (see period February 25 to 28 and March 13 to 16).

It appears from this experiment that the volume of urine produced was not so closely related to the amount of water consumed as it was to the quantity and form of the ash elements in the food.

TABLE VI.

EFFECT OF SUPPLY OF BRAN ASH CONSTITUENTS AND ADDED SALTS ON YIELD OF MILK AND MILK SOLIDS AND EXCRETION AND COMPOSITION OF URINE.

Date.	P_2O_5 daily fed.	CaO daily fed.	MgO daily fed.	K_2O daily fed.	Yield of milk daily.	Casein daily.	Fat daily.	Solids daily.	Yield of urine daily.	Water drunk daily.	Per cent in urine.			
											P_2O_5.	CaO.	MgO.	K_2O.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	pounds.				
Dec. 1–4	190	23	65.0	152	17,467	340	515	1,860	7,342	116.6	0.008	0.009	0.10	0.96
Dec. 19–20 . . .	46	26	22.0	80	16,162	328	464	1,737	4,196	121.2	0.011	0.18	0.06	0.42
Jan. 6–9	46	26	62.0	153	13,961	282	418	1,506	8,729	133.6	0.008	0.10	0.05	1.14
Jan. 28–31 . . .	42	22	18.7	78	14,051	295	431	1,573	4,502	102.5	0.006	0.21	0.07	0.18
Feb. 25–28 . . .	46	26	22.0	153	13,223	...	...	...	8,431	116.9	...	...	...	...
Mar. 13–16 . . .	81	26	22.0	126	12,723	...	...	...	7,034	126.4	...	...	...	...

A glance at the table also shows the pronounced variation in the percentage composition of the urine, with respect at least to calcium and potassium. This will be discussed later.

TABLE VII.

Relation between the Nitrogen and Ash Excretion.

Date.	N.		P_2O_5		CaO		MgO		K_2O	
	Fed.	Outgo.	Fed.	Outgo.	Fed.	Outgo.	Fed.	Outgo.	Fed.	Outgo.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Dec. 1–4	286	300	190	181	23.5	50.0	65.4	68	153	168
Dec. 19–22	283	263	47	67	26.0	56.0	22.0	18	80	77
Feb. 10–13	283	275	47	58	26.0	61.9	22.0	13	153	126

The data embodied in the foregoing table emphasize and extend what has previously been shown to be true with other animals, namely, that the nitrogen and phosphorus excretion have no close direct relation to each other. This is equally true in respect to potassium and magnesium, which rose and fell in the amounts excreted with great regularity and dependent on the amounts ingested. Of course at no time was the animal starving for potassium or magnesium, and this interpretation applies only when the supply was still considerably above the animal's requirements. In the case of lime the supply was a constant one, consequently affording no data on this point. That there is a definite and more or less constant metabolism of the ash elements of the cell which bears a definite relation to the amounts of nitrogen suffering degradation in cellular metabolism is entirely possible.

Physiological effect of the various rations on the character of the feces and on the œstrum periods. — Again, abundant proof of the laxative effect of phytin was accumulated. Even when the amount of crude fibre incident to a large consumption of washed bran was reduced in quantity and a sudden change made from a whole bran ration to one with an equivalent quantity of crude fibre but without phytin, constipation resulted. When the washed bran ration was supplemented with a quantity of magnesium and potassium, as chlorides and sulphates, equivalent to these bases, removed in the washing process, a sudden change from the whole bran to the

washed bran ration could be made without in any way modifying the character of the feces. They remained normal. When the substitution for the removed bases was confined to potassium alone, either as a sulphate or chloride, and the change from the whole bran ration to one of washed bran made, the character of the feces was invariably changed. While constipation did not result, the feces became drier and firmer. When potassium was supplied as a phytate to a washed bran ration, the character of the feces was normal and soft.

It is interesting in this connection to record the fact that the difference in the percentage of moisture in feces appearing normally dry and formed, and those of a softer character, was not so large as we supposed. A so-called dry and formed excreta contained 83.5 to 84.5 per cent of water, while one produced during a laxative condition contained 87 to 89 per cent of water.

No disturbance of the œstrum periods was observed.

Balance of income and outgo of phosphorus and the ash elements and their channels of excretion. — The data embodied in the following table cover those parts of the periods in which it was believed that the animal had reached an adjusted condition. In most cases averages for 4 days are given.

The constancy in the amounts of the ash elements secreted in the milk independent of the quantity and form ingested in the food, has already been mentioned.

The principal channel for the excretion of phosphorus and magnesium was the gut. Increased intake of these two elements raised their output in the urine, but generally the proportion was relatively small. The form of intake of magnesium did not affect the path of output materially.

There was also a constant excretion of calcium through the gut, this being the main channel for this element. However, in periods of low phosphorus intake, there was a considerably increased output of this element in the urine. At no time in the experiment was the intake of calcium equivalent to the output.

Potassium was eliminated by both the urinary and intestinal tracts. The amounts of potassium eliminated through the gut appear to bear a close relation to the amounts of ingested phosphorus. A high intake of potassium accompanied by a high amount of phosphorus increased the elimination by the gut.

During the later periods of the experiment there appeared to

be a storage of magnesium and phosphorus, especially the latter at periods of high intake of this element.

TABLE

INCOME AND OUTGO

Date.	Food.	Daily intake, gm.				Daily output milk, gm.			
		P_2O_5	CaO	MgO	K_2O	P_2O_5	CaO	MgO	K_2O
Dec. 1–4	Whole bran	190.5	23.5	65.4	152.6	30.4	23.2	3.4	33.6
Dec. 19–22	Wash bran	46.7	26.01	22.5	80.4	27.6	19.8	2.6	26.6
Dec. 27–28	Whole bran	190.5	23.5	65.5	152.6	27.6	19.5	2.7	24.0
Jan. 6–9	Wash bran $MgCl_2$ K_2SO_4	47.7	26.0	61.9	153.6	23.4	18.2	2.7	22.3
Jan. 18–19	Whole bran	190.5	23.5	65.4	152.6	23.5	17.5	2.8	22.3
Jan. 28–31	Wash bran Starch	41.5	21.5	18.7	78.1	24.1	18.5	2.6	23.4
Feb. 5	Whole bran	190.5	23.5	65.4	153.3	24.7	16.4	2.6	23.0
Feb. 10–13	Wash bran K_2SO_4	46.7	26.0	22.5	152.6	26.4	19.4	2.7	25.6
Feb. 17	Whole bran	190.5	23.5	65.4	152.6	23.4	16.7	2.4	22.1
Feb. 25–28	Wash bran KCl	46.7	26.0	22.5	153.0	23.1	16.5	2.4	22.0
Mar. 13–16	Wash bran K Phytate	80.6	26.0	22.5	126.3	22.2	15.9	2.3	20.9

General Discussion.

The data obtained from this experiment covered daily observations of three and one-half months. At no time was the appetite of the animal impaired. She remained strong and vigorous throughout the entire period. She also maintained her body weight. One of the most striking facts brought out was the constancy in ash content of the milk, in spite of the considerable fluctuation in the amounts ingested in the food in the various periods. This is not so surprising, however, when we remember the constantly accumulating evidence that the animal secretions are elaborated quite independent of a considerable variation in the character of the food.

The effect of withdrawal of phytin on fat elaboration in the milk was not apparent.

There was also no quantitative disturbance in the production of the other organic constituents of the milk incident to the with-

VIII.

OF ASH ELEMENTS.

Daily output feces, gm.				Daily output urine, gm.				Daily total output, gm.			
P_2O_5	CaO	MgO	K_2O	P_2O_5	CaO	MgO	K_2O	P_2O_5	CaO	MgO	K_2O
150.5	24.5	57.2	64.4	0.63	.64	7.3	70.8	181.5	48.3	68.0	168.8
39.5	28.3	12.7	31.9	0.47	7.8	2.8	18.4	67.6	55.9	18.1	76.9
145.3	30.5	59.9	57.5	2.3	.29	3.0	86.6	175.2	50.3	65.6	168.1
36.2	25.0	41.6	29.2	0.67	8.4	4.5	98.9	60.3	51.6	48.8	150.4
126.3	21.8	46.9	57.9	5.2	1.0	4.3	56.7	155.0	40.3	54.0	137.4
30.4	21.2	8.1	27.6	.27	9.7	3.1	8.4	55.4	49.4	13.8	59.4
120.9	18.3	42.3	11.6	20.0	0.45	11.2	24.6	65.6	35.1	56.1	159.2
31.0	19.4	7.7	28.7	.64	5.8	2.9	71.9	58.0	44.6	13.3	126.2
77.5	20.1	38.9	22.8	....	....	....	....	....	....	....	....
26.5	19.2	16.5	34.4	....	....	....	....	....	....	....	....
32.4	22.8	23.2	39.8	....	....	....	....	....	....	....	....

drawal of phytin. From this it appears that when such disturbances have been produced, as in previous experiments, they must be considered as individualistic, but that we do not have in the body phytin a specific chemical entity directly regulating and imperatively concerned in the process of fat production. It is of course not here questioned but that the components supplied by phytin are important sources of the ash constituents in the animal's metabolism.

With this animal there was also no disturbance in the flow of milk consequent upon the withdrawal of phytin or any other supplied salts.

The flow of urine was directly related to the supply of phytin, as well as to certain of its components, when these were supplied as salts in the ration. This was equally manifested where the displaced phytin was substituted by potassium and magnesium as

sulphates and chlorides, or by potassium alone as a chloride or phytate. The increased output of potassium in the urine which always accompanied an increased intake strongly suggests a close relation between the diuresis produced with the whole bran and its high potassium content. The percentage of potassium in the urine, however, does not remain constant, even with increased flow. When the volume increased, the percentage of potassium often rose to 1 per cent, while in the period of low output it was but one tenth that amount.

The constipating effect incident to withdrawal of phytin was always manifest. When, however, the phytin was replaced with magnesium sulphate, a laxative effect was produced, but when this substitution was made with potassium sulphate or chloride, an unmistakable dryness of the feces resulted. Again, when the displaced natural phytin was substituted by a prepared potassium phytate, a laxative condition resulted. A glance at Table VIII will reveal the fact that the principal channel of excretion of magnesium in the cow is the gut. The inference from a consideration of the facts lends strong color to the theory that the phosphorus and magnesium carried by whole wheat bran are largely responsible for this action. It is to be observed that when the washed bran was supplemented with crude phytin, the excretion of magnesium in the gut was increased.

The lime supply in the ration of the entire period was manifestly deficient. The output was approximately 50 gm. daily, while the intake was but 25 gm. The popular notion that wheat bran is particularly useful as a building material for growing animals, due to high ash content, needs qualification. It is high in total ash, but its content of lime is relatively low. Ten pounds of wheat bran supplied but 8 gm. of calcium oxide. This period of feeding covered one hundred and ten days, and consequently entailed an approximate loss of 2500 gm., or 5½ pounds of lime. The data from the Rothamsted Experiment Station on the ash constituents of various animals affords an approximate estimate of the total lime in our animal. At the beginning of the experiment it was about 24.2 pounds. This means that during the period of our experiment there had been a loss of about 25 per cent of the entire lime content of the animal. A certain and definite percentage content had been maintained in the milk, and an apparent waste, possibly indicating general cell metabo-

lism had been excreted in the feces and urine. This large loss above that used for milk production could have had no other source than the skeleton. This supports what our experiments with pigs have shown, namely, that the skeletal tissue can vary its ash content within quite wide limits, thereby acting as a supply house over considerable periods of time for certain ash constituents that may be deficient in quantity in the food. During the periods of high phosphorus feeding there appeared to be a storage of phosphorus and especially of magnesium. This condition prevailed more particularly during the later periods of the experiment. This would make clear how it was possible to withdraw the needed supply of lime from a calcium-phosphorus complex and still retain the phosphorus. However, it is also possible that the constant withdrawal of lime did not involve the calcium phosphate of the skeleton, but that the calcium carbonate, which is also supposed to be present, was involved in this interchange; but this is mere supposition.

During periods of low phosphorus feeding there was constantly an increased output of calcium in the urine, apparently again involving the metabolism of a calcium-phosphorus entity. The total output of calcium was greater during these periods than during periods of high phosphorus feeding. It is apparent from the data that the daily loss of calcium oxide due to milk production and cell metabolism was at least 50 gm. The animal weighed 1150 pounds and produced about 30 pounds of milk daily.

The periods of low phosphorus feeding also afford data on the output of this element at periods of phosphorus starvation. The average amount of phosphorus pentoxide lost daily through the milk and cell metabolism was approximately 60 gm. It is an interesting fact that in spite of deficient supplies of these two elements during considerable periods there was nevertheless an apparent waste. A part was constantly being metabolized and passed out of the reach of the reconstructive processes. Whether this means that the form in which the ash elements are presented to the cell before metabolism is different from that existing after such processes have occurred, is, of course, mere conjecture.

The supply of magnesium and potassium compounds in the food at all periods was equal to or greater than that excreted.

Summary.

1. A high potassium intake accompanied by a high phosphorus intake gave a high potassium content in the feces, although a considerable portion of potassium was also excreted in the urine.

2. A high potassium intake accompanied by a low phosphorus intake gave a low potassium content in the feces, with a high output of this element in the urine.

3. A high potassium intake accompanied by a low phosphorus and a high magnesium intake gave a high potassium output in the urine.

4. Magnesium when supplied as a chloride or as a phytate was largely excreted in the gut.

5. Phosphorus and calcium were also principally eliminated by this channel.

6. A low phosphorus intake was accompanied by a high calcium output in the urine.

7. A deficient calcium intake was nevertheless accompanied by a considerable output of this element in the gut. This same statement is also true of phosphorus.

8. When calcium or phosphorus was deficient in quantity in the food, the skeletal tissues appeared to be ready sources of supply. The average quantities of calcium oxide and phosphorus pentoxide metabolized and excreted daily by this animal during periods of deficient supply were, respectively, 50 and 60 gm.

9. The supplies of potassium and magnesium were in all periods equal to or above the amounts eliminated.

10. Variations, within wide limits, in the form and quantity of supply of potassium, magnesium, or phosphorus, did not influence the percentage content of these elements in the milk.

11. With this animal there was no appreciable fluctuation in the percentage of organic constituents in the milk relative to the supply of phytin.

12. Marked diuresis was produced by the quantity of phytin supplied. A high potassium and magnesium intake, as sulphate and chloride, produced a similar effect, as did potassium alone when supplied as a chloride. This would indicate that the high potassium intake accompanying the whole bran ration was responsible for this phenomenon.

13. Sudden withdrawal of phytin produced constipation. This was even manifested when the intake of crude fibre was reduced to that of normal bran.

14. The laxative action is more easily understood when it is remembered that the channel of excretion of phosphorus, calcium, and magnesium especially, and a part of the potassium, when supplied in wheat bran, is by way of the gut.

15. The "margin of safety" provided in the skeletal tissues in the animal precludes against immediate disastrous results consequent on a sudden deficit in the intake of phosphorus or calcium.

THE EFFECT OF SMOKING UPON THE BLOOD PRESSURES AND UPON THE VOLUME OF THE HAND.[1]

By JAMES W. BRUCE, JAMES R. MILLER, and DONALD R. HOOKER.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

Historical.

THE effect of nicotine upon the cardio-vascular system is very well defined. Wertheimer and Colas [2] state that the acceleration of the heart rate which follows the injection of nicotine is present whether the extrinsic nerves to the heart are intact or severed, and that an increase of arterial tension occurs even after the destruction of the medulla and spinal cord. The action upon both the heart and blood vessels is, however, greater when the nervous system is intact, demonstrating a central as well as a peripheral effect.

Langley [3] concludes, in an investigation published in the same year (1901), that "nicotine stimulates sympathetic nerve cells; the evidence that it paralyzes them is incomplete." [4] He observed, further, that nicotine in dilute solutions (0.1 per cent in 0.7 per cent NaCl) when applied to sympathetic ganglia produces exciting effects of longer duration, although slower in onset than in stronger solutions.

Of recent years attention has been focused upon the action of tobacco, especially of tobacco smoke. Cushny [5] states that in whatever form tobacco is consumed, nicotine absorption occurs. That

[1] Reported before the American Physiological Society, December, 1908.

[2] Wertheimer et Colas: Archives de physiologie, 1891, p. 341.

[3] Langley: Journal of physiology, 1901, xxvii, p. 224.

[4] Lest this statement, as quoted, should be misleading in regard to the work of Langley and others in tracing nerve paths by the use of nicotine, we ought perhaps to call attention to the fact that the paralyzing action of nicotine is presumably, in large part at least, upon the endings of the preganglionic fibres.

[5] Cushny: Pharmacology and therapeutics, 1903, p. 276.

tobacco exerts a deleterious effect upon the animal organism when administered in sufficient quantity is evident from the recent literature on the subject. Thus Richon and Perrin [6] observed that the subcutaneous injection of the aqueous extract retarded growth in rabbits. After the injections were stopped the animals continued to develop normally. Fleig [7] studied the effect of inhalation of smoke, the subcutaneous injection of aqueous extracts of smoke and of nicotine, and the salts of nicotine on rabbits. The results of such procedures were qualitatively the same, causing the animal to abort or to produce weakly viable young. When the dosage was low, the effect was less pronounced, and with weak fumes the young were able to return to normal some time after the treatment was stopped. Lesiur [8] states that denicotinized tobacco is entirely without toxic effect upon animals. This statement is supported by the work of Lehmann,[9] who concludes that nicotine is the most important and practically the only toxic substance in tobacco smoke. Investigating the effect of tobacco on man, Boveri [10] has noted a distinct effect upon the muscular power of healthy individuals after smoking. He observed, using Mosso's ergograph, that the use of tobacco resulted in a short, preliminary period of slightly increased power, followed by a long period during which the power was distinctly less than in control experiments. Finally, mention should be made of the clinical picture known as Toxic angina.[11] This condition is apparently limited to smokers, and is attributed to disturbances in the coronary arteries brought on by the use of tobacco.

Upon the blood pressure and heart rate the inhalation of tobacco smoke acts similarly to the injection of nicotine. Thus Fleig and De Visme [12] give a tracing which shows the effect upon a curarized dog. After a transient fall in blood pressure, accompanied by a decrease in the heart rate and a decrease in the volume of the kidney, there is a considerable rise in carotid blood pressure, co-

[6] Richon et Perrin: Comptes rendus de la Société de Biologie, 1908, lxiv, p. 563.

[7] Fleig: Comptes rendus de la Société de Biologie, 1908, lxiv, p. 683.

[8] Lesiur: Comptes rendus de la Société de Biologie, 1908, lxiv, p. 9.

[9] Lehmann: Münchener medicinische Wochenschrift, 1908, lv, p. 723.

[10] Boveri: Deutsche medicinische Wochenschrift, 1906, xxxii, p. 1439.

[11] Osler: Practice of medicine, 1901, p. 764.

[12] Fleig et De Visme: Comptes rendus de la Société de Biologie, 1907, lxiii, p. 578.

incident with an increase in the rate of the heart. As the blood pressure begins to rise, the volume of the kidney momentarily increases. This slight increase in renal volume is followed by a distinct decrease in volume which occurs while the arterial pressure is still rising. Finally, dilatation of the kidney sets in before, and appears, on the tracing, to initiate the fall in arterial pressure. The preliminary effect in this experiment is undoubtedly, as Pachon [13] points out, due to vagus action and presumably is of central origin.

Attention may also be called to the clinical view that tobacco is one of the etiological factors in the production of arterio-sclerosis. Numerous attempts have been made to produce arterio-sclerosis experimentally in animals by repeated injections of nicotine and by feeding extracts of tobacco.[14] This work has resulted in demonstrating degenerative changes in the muscular coats of blood vessels unaccompanied, however, by involvement of the intima. The evidence therefore is negative so far as true arterio-sclerosis is concerned. Whether the injury to the muscular coats is the result of mechanical or toxic effects cannot be stated.

The only reference to the immediate effect of smoking upon the blood pressure in man with which we are acquainted was published by Hesse [15] in 1907.[16] This observer determined the maximum

[13] Pachon: Comptes rendus de la Société de Biologie, 1908, lxiv, p. 116.

[14] Saltykow: Zentralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, 1908, p. 654.

[15] Hesse: Deutsches Archiv für klinische Medicin, 1907, lxxxix, p. 565.

[16] Since this was written a paper by Lee (Quarterly journal of experimental physiology, 1908, i, p. 335) has become accessible to us. Lee observed a continuous rise in blood pressure (systolic) during the smoking period. Immediately after smoking was stopped the pressure began to fall and continued to fall until the normal was reached. Moderate and excessive smokers were less affected than novices. In the latter the rise in pressure was sharp, and in one case gave way to a fall of 50 mm. Hg with the symptoms of general collapse. In all of Lee's experiments (seven in number) the smoke was inhaled. The rise in pressure noted was, for novices, 10–20 mm. Hg, for moderate smokers about 10 mm. Hg, and for excessive smokers 2–4 mm. Hg. This observer also studied the effect of tobacco smoke upon animals, his results confirming the previous work cited above, except in the case of one rabbit which was submitted to 70 inhalations of fifteen to twenty minutes each, over a period of five months. This rabbit, which had gained steadily in weight, showed artheromatous changes postmortem. The conclusion is reached that "arterial disease may result from prolonged tobacco smoking" brought about by the mechanical injury to the vessels caused by sudden and repeated elevations of arterial tension.

and minimum blood pressures and the heart rate before and after smoking on about 25 individuals. The maximum pressure was determined by the palpation method, the minimum pressure by Strassburger's method. The Riva-Rocci instrument was used. Hesse's results varied somewhat, but he observed that, as a rule, both pressures rose and the heart rate was increased. The maximum pressure tended to rise more than the minimum, so that the pulse pressure was increased. In two cases this author compared the effect of natural with denicotinized tobacco. The effect was practically the same. Also in both of these cases, comparing natural with denicotinized tobacco (the only cases in which the blood pressure was observed subsequently to the use of tobacco), the blood pressure fell to or below normal within twenty minutes. The heart rate also showed a marked tendency to fall below normal, although the change was somewhat more gradual than in the case of the pressures. It is difficult to explain these results except upon the assumption that by far the major part of the effect observed is due to psychical influences, in which case it would not be justifiable to assume that tobacco smoke as such exerts any influence upon the cardio-vascular system. This negative inference is supported by the similarity of action between the natural and nicotine-free cigars, although it must be borne in mind that commercial nicotine-free tobacco doubtless contains some nicotine.

Experimental.

The following experiments were performed to ascertain the demonstrable effects upon the heart rate, arterial pressure, and the volume of the hand which follow moderate smoking.

Subjects. — Both were healthy men, accustomed to moderate smoking, twenty-one years old.

Methods. — Arterial blood pressure was determined upon the right arm with the Erlanger sphygmomanometer, the palpation method being used for systolic pressure. Volume changes in the left hand were recorded with Mosso's plethysmograph, a rise in the curve indicating vaso-constriction, and a fall in the curve vaso-dilatation. The work was conducted in a private room, and all extraneous factors were, as far as possible, excluded. The experiments lasted from one and a half to three hours, the subject

sitting quietly during that time. During the observations it was found convenient to permit the subject to aimlessly draw pictures on the arm of the chair. This procedure offered sufficient diversion to maintain a fairly even psychic condition. In some of the experiments an attempt was made to determine venous pressure. The method employed was not, however, suited to the experimental

BLOOD-PRESSURE

SUBJECT M.

Exp. No.	Max.			Min.			P. P.			P. R.		
	B.	D.	A.	B.	D.	A.	B.	D.	A.	B.	D.	A.
XI	113	..	..	75	..	..	38	..	..	60	..	..
	113	..	..	70	..	..	43	..	..	62	..	..
XII	119	..	..	75	..	..	44	..	..	84	..	..
	120	..	..	..	..	..	..	..	..	72	..	..
	119	..	..	75	..	..	44	..	..	72	..	..
	120	..	..	75	..	..	44	..	..	64	..	..
III	110	..	+ 9	70	..	+ 5	40	..	+ 4	68		+ 4
VI	108	..	+ 8	70	..	+ 5	38	..	+ 3	60	+ 12	+ 10
IX	112	..	+ 3	70	..	+ 3	42	..	0	66	..	+ 6
X	114	..	+ 5	75	..	+ 5	39	..	0	66	+ 18	+ 14

Max. = maximum pressure. Min. = minimum pressure.
P. P. = pulse pressure. P. R. = heart rate.
B = before smoking. D = during smoking.
A = after smoking.

conditions and was finally abandoned. Pipes, cigars, and cigarettes were used. There was no evident difference in the effects, consequently special notes in this regard are not always entered in the protocols. An electric signal was employed to write upon the plethysmographic record, so that the observer could readily indicate the time when events, such as blood-pressure determinations and psychical disturbances, took place.

Experiments. — After the subject was entirely accustomed to the experimental conditions, thus insuring in so far as possible an even psychic condition, the records of the experiments were preserved. The number of experiments culminating satisfactorily is not large, nevertheless the results are suggestive. In the accompanying table we have gathered together all the blood-pressure

DETERMINATIONS.

Subject B.												
Exp. No.	Max.			Min.			P. P.			P. R.		
	B.	D.	A.	B.	D.	A.	B.	D.	A.	B.	D.	A.
XIII	101	..	..	70	..	..	31	..	..	88	..	..
	100	..	..	65	..	..	35	..	..	84	..	..
	100	..	..	70	..	..	30	..	..	80	..	..
XIV	105	..	..	70	..	..	35	..	..	80	..	..
	102	..	..	70	..	..	32	..	..	84	..	..
	103	..	..	70	..	..	33	..	..	84	..	..
I	95	..	− 2	65	..	0	30	..	− 2	80	..	0
II	95	..	+ 5	70	..	0	25	..	+ 5	68	..	+ 10
IV	99	+ 3	..	70	0	..	29	+ 3	..	86	− 2	..
	..	..	..	..	..	..	..	..	..	..	+ 2	..
	..	..	..	..	..	..	..	..	..	..	+ 6	..
V	105	..	+ 3	65	..	+ 2	40	..	0	88		+ 12
VII	103	..	− 3	65	..	0	38	..	− 2	90	..	+ 13
VIII	93	..	+ 6	65	..	+ 5	28	..	+ 1	90	+ 6	0

determinations, together with the observations of the heart rate. It will be seen that, after smoking, there is, as a rule, an increase in the heart rate. The increase is not great and may be absent altogether, even when the other records (blood pressure and hand volume) show distinct changes. The maximum and minimum pressures usually rise slightly, the maximum the more, so that the pulse pressure tends to increase.

The following experiments are given in detail, since they correspond to the plethysmographic tracings reproduced in the accompanying plate. In all of the protocols the same nomenclature

FIGURE 1. — Plethysmographic tracings of the left hand. The Roman numerals refer to the experiments, the Arabic numerals to the experimental procedure. The vertical lines (indicated by +) on the tracings delimit the smoking periods. The curves read from left to right. A rise in the curve indicates decrease in the hand volume. The scale at the side gives volume changes in cubic centimetres.

is used. Under "Procedure" are given the time of observations and the various experimental steps, together with notes of extraneous factors. Under "Ref. pleth." are given the numbers corresponding to those on the plethysmographic tracings. "Max.," "Min." and "P.P." indicate maximum and minimum arterial blood pressure and pulse pressure. "P. R." indicates the heart rate. In the brief discussion of these experiments reference will be made to the plethysmographic tracings.

The curve shows (Exp. I) considerable variations, two of which (3 and 4) may be due to the hot water drunk. Its general trend is, however, quite even until the smoking period (5–6), after which it first tends to elevate itself and then to remain constant. The sudden rise just before 5 is presumably of psychic origin. The heart rate remained quite constant. Apparently the peripheral vaso-constriction following the smoking period was either compensated or insufficient to markedly affect the blood-pressure readings by the method employed. There is, however, a slight increase in maximum and minimum pressure and heart rate over the determinations made just before smoking was begun.

The curve shows (Exp. III) a sharp and considerable rise just

EXPERIMENT I.

MAY 7, '08. SUBJECT B.

Procedure.	Ref. pleth.	Max.	Min.	P. P.	P. R.
10.26 A. M.	..	..	..	..	..
10.32 A. M.	..	94	..	..	84
10.40 A. M.	..	..	65	29	80
10.46 A.M.	..	..	..	..	80
10.58 A. M.	..	95	..	..	..
Cup hot water . . .	2	..	..	..	..
Second cup water . .	3	..	..	..	..
11.10 A. M.	..	95	63	32	84
Third cup water . .	4	..	..	..	76
11.25 A. M.	..	90	70	25	..
11.30 A. M.	..	..	60	30	..
Smoked two and a	5	..	..	..	..
half Cigarettes . .	6	..	..	..	..
	..	93	65	28	80
11.55 A. M.	7	..	..	..	..

EXPERIMENT III.

MAY 11, '08 SUBJECT M.

Procedure.	Ref. pleth.	Max.	Min.	P. P.	P. R.
Start	1	..	..	..	..
11.05 A. M.	..	110	70	40	68
Smoking period . .	2, 3	..	..	..	..
11.30 A. M.	..	118	75	43	84
11.45 A. M.	..	121	75	46	60
11.53 A. M.	..	..	..	..	72

preceding and coincident with the beginning of the smoking period. These sudden changes are undoubtedly psychic in origin. It is to be noted, however, that the curve remains consistently elevated throughout the remainder of the experiment. There is a decided increase in both pressures, accompanied by an increase in the pulse pressure. The heart rate apparently played no part in the change. There is a transient increase in rate, amounting to 16 per minute, which disappeared within fifteen minutes and was even converted into a lessened rate without affecting the plethysmographic curve or the blood pressures.

EXPERIMENT IV.

May 11, '08. Subject B.

Procedure.	Ref. pleth.	Max.	Min.	P. P.	P. R.
Start	1	..	..	..	..
3.17 p. m.	..	..	..	..	84
	..	99	70	29	88
Moved arm	2	..	..	..	..
Smoking begun . . .	3	..	..	..	..
3.45 p. m.	..	..	..	..	84
4.02 p. m.	..	..	..	..	88
4.05 p. m.	..	103	70	33	..
4.15 p. m.	..	..	..	..	92
4.20 p. m.	..	..	70	..	..
4.25 p. m.	..	101	..	31	..
4.30 p. m.	4	..	..	..	..

Shows the effect of prolonged smoking. The first part of the curve is quite regular, except for two elevations, which were presumably coincident with the determination of maximum and minimum pressures on the opposite arm. Smoking was begun at 3, after which there is distinct evidence of a slow but progressive vaso-constriction. Upon the curve thus elevated, we again see the transient effects of extraneous factors. The blood pressures show little change. There is a slight increase in the maximum, the

minimum remaining stationary, so that the pulse pressure varies directly with the maximum pressure. The heart rate shows a consistent tendency to slowly increase.

EXPERIMENT VIII.

MAY 18, '08. SUBJECT B.

Procedure.	Ref. pleth.	Max.	Min.	P. P.	P. R.
10.40 A. M.	1	..	..	..	92
	2	90–94	..	..	..
	3	..	65	27	..
Ven. Press. Det. . .	4	..	..	..	..
	5	93+	..	..	..
	6	..	..	..	88
Ven. Press. Det. . .	7	..	..	..	..
Talked of smoking .	8	..	..	..	..
11.10 A. M. started pipe	9	..	..	..	..
Deep breath	10	..	..	..	..
Two deep breaths .	11	..	..	..	..
Inhaled smoke . . .	12	..	..	..	..
Said head dizzy . .	13	..	..	..	..
Smoking stopped . .	14	..	..	..	95
Deep breath	15	100	..	..	..
	16	..	70	30	..
Ven. Press. Det. . .	17	..	..	..	..
	18	..	..	..	..
Deep breath	19	..	..	..	90
	20	98	..	..	..
Two deep breaths .	21	..	70	28	..
11.50 A. M. Exp. ended	22	..	..	..	..

The curve exhibits many slight irregularities which find no explanation in the protocol. The most marked of the sudden changes,

occurring at 13, is accompanied by the note "Said head was dizzy." The effect of smoking was to cause a slight vaso-constriction. Both pressures were affected. There was a slight increase in the pulse pressure. The heart rate was greatest just at the end of the smoking period, but approached the control value as the experiment progressed.

EXPERIMENT IX.

May 18, '08. Subject M.

Procedure.	Ref. pleth.	Max.	Min.	P. P.	P. R.
H. left room	1	..	..	..	..
3.35 P. M.	2	..	..	..	68
3.42 P. M.	3	114	..	..	..
3.45 P. M.	4	..	70	44	..
Ven. Press. Det. . .	5, 6	..	..	..	..
4.00 P. M.	7	..	..	..	64
H. entered }	8	110	..	..	..
Talked }	9	..	..	..	..
4.06 P. M. cigar . . .	9	..	..	..	..
No apparent cause .	10	..	..	..	..
4.25 P. M door opened	11	..	..	..	..
4.30 P. M. smoking stopped	12, 13	..	..	..	72
Moved	14	..	..	..	..
4.37 P. M.	15	120	..	..	..
4.40 P. M.	16	..	70	50	..
Deep breath	17	..	..	..	..
Ven. Press. Det. . .	18	..	..	..	..
4.50 P. M.	19	..	75	..	..
Moved arm.	20	110	..	35	..
Exp. ended	21	..	..	..	..

The tendency of this curve to rise (from 5 to 9) before smoking was begun makes the interpretation difficult. The general effect is, however, that the smoking results in vaso-constriction. The

pressures are uncertain, but indicate a slight rise of both maximum and minimum. The heart rate is slightly increased.

EXPERIMENT XII.

MAY 19, '08. SUBJECT M.

Procedure.	Ref. pleth.	Max.	Min.	P. P.	P. R.
3.00 P. M.	1	. . . .	. .	. .	84
3.05 P. M.	2	118–120	. .	. .	. .
3.07 P. M.	3	. . . .	75	44	. .
Ven. Press. Det. . .	4	. . . .	. .	. .	. .
Yawn	5	. . . .	. .	. .	. .
3.18 P. M.	6	120	. .	. .	72
3.35 P. M.	7, 8	118–120	. .	. .	. .
3.45 P. M.	9	. . . .	75	44	. .
Ven. Press. Det. . .	10	. . . .	. .	. .	. .
	11	. . . .	. .	. .	64
4.15 P. M.	12	118–121	. .	. .	. .
4.17 P. M.	13	. . . .	75	44	. .
Ven. Press. Det. . .	14	. . . .	. .	. .	. .

This is one of the control experiments. The curve shows a slow and very slight vaso-dilatation throughout the experimental period. The blood pressures are strikingly constant. The heart rate shows a steady decrease.

Discussion. — Our results show clearly that a change in the cardio-vascular system follows smoking. Other factors, however, and especially psychic factors, of necessity complicate the results. How far, therefore, we are justified in stating that such change is a tobacco effect depends upon the possibility of excluding these factors. In describing our experimental procedure it was stated that special effort was made to maintain an even psychic condition. This effort consisted (1) in repetition until the subject was entirely accustomed to the procedure observed and (2) in permitting the subject to aimlessly draw pictures on the arm of the chair during

the experiments. The plethysmographic tracings show in consequence only transient irregularities as the result of psychic disturbances. As compared with the results which follow smoking these irregularities appear of insignificant moment. Their insignificance is further emphasized by the fact that they are not absent after smoking, but appear superimposed upon the elevated curve. The duration of the vaso-constriction which followed the use of tobacco may be conveniently summarized in the following figures:

	From end of smoking period.	From beginning of smoking period.
III.	20 min.	35 min.
I.	15 "	30 "
IV.	— "	60 "
VIII.	35 "	55 "
IX.	35 "	60 "

It is to be noted also that in none of these tracings is there evidence of a tendency to fall after the constriction has once set in. Unfortunately, we have no tracings to show the duration of such vaso-constriction, since it was not possible to continue our experiments over sufficiently long periods. In this connection we wish again to call attention to the results of Hesse cited above. He observed no difference in the effect upon the blood pressures of smoking natural and denicotinized cigars, and, what is of interest here, both the maximum and minimum pressures returned to or fell below normal (control) within twenty minutes. Our pressure determinations fail to show this quick reaction.

Even if we have shown that smoking as such does influence the cardio-vascular system, we do not believe that our evidence is sufficient to lend much support to the theory that tobacco is an etiological factor in arterio-sclerosis, at least in so far as this theory assumes a mechanical injury to the vessels. The effects of moderate tobacco smoking upon a man accustomed to its use would seem to be very little, if any, greater than the effects of those stimuli which are the necessary consequences of civilized life.

THE PRODUCTION BY HYDROGEN PEROXIDE OF RHYTHMICAL CONTRACTIONS IN THE MARGINLESS BELL OF GONIONEMUS.

By O. P. TERRY.

[*From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory of Purdue University.*]

THE normal movements of the body of a normal Gonionemus consist of a series of five to twenty-five or more rhythmical contractions, or pulsations, which squeeze water from the interior of the bell, and in so doing force the animal, apex of the bell forward, through the water. In general these contractions resemble closely the beat of the heart of the higher animals. As in the heart, these pulsations of Gonionemus are brought about by muscles under the control of a nervous system. In Gonionemus the nervous system consists of a ring of nervous tissue at the margin of the bell, in which are found many nerve cells, and from which nerve fibres ramify toward the apex of the bell. This nervous system has for one of its functions the initiation and co-ordination of the pulsations.

That the impulse to beat normally originates in this ring of nerve cells is easily proved, as was first done by Romanes, by cutting off this margin of the bell. At once all rhythmical movements of the bell cease when that bell is placed in normal sea water.

Such a marginless bell constitutes the material upon which the following experiments were performed. In describing the experiments the pulsations are called co-ordinated when the muscles of the marginless bell contract simultaneously, giving the appearance of the contractions of a normal non-mutilated specimen. This implies immediate relaxation of the muscles after a contraction. The pulsations are described as inco-ordinated, or clonic, when a wave of contraction starts at some point and travels rather slowly over the whole organism (sometimes back and forth several times).

Pulsations are described as tonic when, after a contraction, the organism remains contracted in a small hard ball.

Loeb[1] carried on experiments with a jelly fish, Polyorchis, found along the coast of California. His experiments dealt with the effects of various salt solutions on the pulsations of the marginless bell. It was also found that rhythmical pulsations can be initiated by the addition of a trace of carbon dioxide or any acid to the sea water. "About 1.5 to 2 c.c. of $n/10$ HCl to 100 c.c. of sea water is required for this purpose. Alkalies have the opposite effect."

I have confirmed these observations regarding the effect of acids and carbon dioxide upon Gonionemus. These results seem to be directly opposed to those upon the heart muscle, in which acids have an inhibitory action and alkalies the opposite.

Lillie[2] remarks: "Acids are known, even in low concentrations, to be very injurious to contractile processes: weak solutions of acid quickly suppress muscular activity. . . . Again, it [acid] must be present in relatively low concentration in the external medium."

Lillie says accumulation of acids, produced by metabolic processes, causes polarization of the surface of the contractile elements and inhibits further contraction. This is hard to reconcile with the fact that an excess of carbon dioxide and other acids in the surrounding medium stimulates the marginless bells of Polyorchis and Gonionemus to contract.

Experiments with hydrochloric acid.[3] —

$n/100$ HCl — No l. p.,[4] fairly co-ordinated pulsations in 2 sec., contractions ended by tonic condition.

$n/250$ HCl — No l. p., 10 co-ordinated pulsations in 15 sec.

$n/300$ HCl — (1) L. p. 30 sec., 90 co-ordinated pulsations.

(2) L. p. 10 sec., at the end of the 20th co-ordinated pulsation the solution was diluted with an equal amount of fresh sea water and 150 more fairly co-ordinated pulsations resulted.

$n/500$ HCl —No stimulation.

[1] Loeb, J.: The dynamics of living matter, 1906, p. 87.

[2] Lillie, R. S.: This journal, 1908, xxii, p. 75.

[3] In all of the following experiments the reagents were added to fresh sea water in such a way that a minimum of change occurred in the normal sea water. For example, in making up $n/500$ HCl, 2 c.c. of $n/10$ HCl in distilled water were added to 98 c.c. of sea water. The freshly trimmed bells were then gently placed in the solution.

[4] "L. p." stands for latent period, being the time elapsing between the placing of the bells in the various solutions and the response by contraction.

Experiments with nitric acid. —

n/250 HNO_3 — No l. p., 46 inco-ordinated pulsations.

n/300 HNO_3 — No l. p., 75 fairly co-ordinated pulsations.

n/400 HNO_3 — L. p. 5 sec., 13 co-ordinated pulsations, followed by inco-ordinated pulsations for 1½ min.

n/450 HNO_3 — L. p. 20 sec., 200 co-ordinated pulsations in 2 min., inco-ordinated pulsations for 2 min. more. Solution was then diluted to *n*/600, and 103 good pulsations followed in groups of 20, 45, and 38 with a few seconds intervening between the groups. Faint rhythmic pulsations followed for about 10 min.

n/500 HNO_3 — L. p. 1 min., 29 slow co-ordinated pulsations.

n/600 HNO_3 — No stimulation.

Experiments with acetic acid. —

n/250 $HC_2H_3O_2$ — L. p. 10 sec., 30 inco-ordinated pulsations.

n/300 $HC_2H_3O_2$ — L. p. 1 min., 40 co-ordinated pulsations.

n/400 $HC_2H_3O_2$ — L. p. 3½ min., 75 co-ordinated pulsations. After 25 more fair pulsations solution was diluted to n/500, and 250 slightly inco-ordinated pulsations resulted.

n/450 $HC_2H_3O_2$ — L. p. 2 min., 81 co-ordinated pulsations in 1 min. Solution then diluted to *n*/600, and 20 more good pulsations resulted. Three drops of *n*/10 acetic acid were then added, and pulsations began again. Immediately diluted solution to about *n*/600, and 90 more co-ordinated pulsations followed.

n/600 $HC_2H_3O_2$ — No stimulation.

Experiments with sulphuric acid. —

n/100 and *n*/200 H_2SO_4 — No l. p., 7 to 10 co-ordinated pulsations. Dilution did not prolong the contractions.

n/400 H_2SO_4 — L. p. 70 sec., 27 co-ordinated pulsations, followed by a few inco-ordinated pulsations; pause for 1¼ min., and 105 slightly inco-ordinated pulsations resulted.

n/500 H_2SO_4 — L. p. 2½ min., 50 co-ordinated pulsations in 35 sec.

n/700 H_2SO_4 — No stimulation.

Experiments with carbon dioxide.[5]—

1 part CO_2 charged sea water plus 1 part fresh sea water — L. p. 1½ min., 3 fair pulsations.

1 part CO_2 charged sea water plus 3 parts fresh sea water — (1) L. p. 1¼ min., 75 co-ordinated pulsations in 45 sec. followed by 75 slightly inco-ordinated pulsations.

[5] The carbon dioxide used was generated in a commercial portable siphon in sea water; an overcharge resulting.

(2) L. p. 1½ min., 8 co-ordinated pulsations in 6 sec., followed by inco-ordinated pulsations. Then specimen was placed in fresh sea water, and slightly inco-ordinated pulsations followed slowly for 4½ min., when 10 co-ordinated pulsations resulted, and then all movements stopped. Twenty-four hours later this same specimen was placed in 1 part CO_2 charged sea water plus 2 parts fresh sea water. L. p. 2 min., then 30 co-ordinated pulsations resulted in 20 sec. Solution was then diluted with abundant fresh sea water and more or less rhythmical, co-ordinated pulsations occurred for 7 min.

1 part CO_2 charged sea water plus 5 parts fresh sea water — L. p. 20 sec., 30 co-ordinated pulsations. Dilution with equal parts fresh sea water caused 70 co-ordinated pulsations to occur.

Lingle[6] has shown that strips of the ventricle of the turtle's heart beat rhythmically for a time in a pure sodium chloride solution; the contractions may be prolonged by the addition of calcium ions or of pure hydrogen peroxide, or by merely allowing pure oxygen to bubble through the solution. Hydrogen peroxide will not initiate beats in strips of turtle's ventricle in Ringer's solution.

With the idea of prolonging the pulsations initiated by an acid in the marginless bell of Gonionemus, I added to an $n/500$ HNO_3 solution (in sea water) a little neutral hydrogen peroxide ("Dioxygen") and found that the pulsations were practically uninfluenced, so far as I was able to determine, either in number or strength. It was possible, however, to initiate pulsations with a much weaker strength of acid if a trace of hydrogen peroxide was also added.

Experiments with nitric acid plus hydrogen peroxide. —

$n/500$ HNO_3 in 50 c.c. of sea water plus 1 drop of hydrogen peroxide — L. p. 2 min., 18 co-ordinated pulsations; a short pause, then 5 more co-ordinated contractions.

$n/2000$ HNO_3 in 50 c.c. of sea water plus 1 drop of hydrogen peroxide — L. p. 1¾ min., 27 co-ordinated pulsations; pause of 1½ min., 35 co-ordinated pulsations; pause of ½ min., 23 co-ordinated pulsations; pause of ¾ min., 3 co-ordinated pulsations.

10 c.c. CO_2 charged sea water plus 40 c.c. dilute hydrogen peroxide (1 part H_2O_2 and 500 parts fresh sea water) — L. p. 2 min., 11 fair pulsations only.

The effect of neutral hydrogen peroxide was then determined upon the marginless bells when in otherwise fresh sea water. It

[6] LINGLE: This journal, 1900, iv, p. 265; 1902, viii, p. 75.

was found that it alone caused rhythmical pulsations even in very dilute solution. The pulsations were, if anything, more in number and greater in individual strength than those caused by carbon dioxide or acids alone.

Experiments with neutral hydrogen peroxide alone. —

1 part 3 per cent H_2O_2 in 100 parts of sea water — L. p. 10 sec., 30 co-ordinated pulsations. Removed to fresh sea water and a few more pulsations followed.

1 part 3 per cent H_2O_2 in 200 parts of sea water — L. p. 1 min., 20 co-ordinated pulsations.

1 part 3 per cent H_2O_2 in 400 parts of sea water — L. p. 2 min., 103 co-ordinated pulsations.

1 part 3 per cent H_2O_2 in 500 parts of sea water — L. p. 1 min., 50 co-ordinated pulsations; pause of 30 sec., 40 co-ordinated pulsations; pause of 5 sec., 21 co-ordinated pulsations; pause of $1\frac{1}{2}$ min., 10 co-ordinated pulsations; pause of 15 sec., 5 co-ordinated pulsations; pause of 15 sec., 77 co-ordinated pulsations. (A very few tiny bubbles of oxygen gas were observed on some of the bells after about 10 min. immersion.)

1 part 3 per cent H_2O_2 in 1000 parts of sea water — No response for 30 min. at least. Specimen unobserved then for four hours, at the end of which time 35 slow, weak, rhythmic pulsations were observed.

Loeb has said that acids cause pulsations in the marginless bells of Polyorchis. "Alkalies have the opposite effect." This I have shown to be true with Gonionemus, but also that if a trace of hydrogen peroxide is added to alkaline sea water, pulsations (clonic, then tonic contractions) can occur and continue. The amount of alkali added may be as great as $n/40$ NaOH. The alkali, however, precipitates the magnesium ions, and in this strength the $Mg(OH)_2$ is evident as a gelatinous suspension which slowly settles. In this case a number of the hydroxyl ions are lost from solution.

The beat in such a solution may be due in part to the removal of the inhibitory action of the magnesium ions. This is indicated, according to Loeb,[7] by the clonic, then tonic condition of the contractions; and also by the fact that the marginless bells in sea water made alkaline with NaOH (no H_2O_2 being added) tend to contract slowly into a small hard ball.

[7] Loeb, J.: This journal, 1902, viii, pp. 81, 91.

Experiments with sodium hydrate and hydrogen peroxide. —

40 c.c. $n/400$ NaOH in fresh sea water plus 2 drops H_2O_2 — L. p. $1\frac{1}{4}$ min., 168 co-ordinated pulsations in alternating series of slow and fast contractions; pause of 10 sec., then 5 good pulsations; pause of 2 min., then 11 more slow pulsations at intervals of about 2 sec.; pause of 8 min., then 1 pulsation; pause of 10 min., then 1 more pulsation.

40 c.c. $n/200$ NaOH in fresh sea water plus 2 drops H_2O_2 — L. p. $1\frac{1}{4}$ min., 31 co-ordinated pulsations; pause of 5 sec., 41 co-ordinated pulsations; pause of 3 sec., 78 co-ordinated pulsations; pause of 2 sec., 1 co-ordinated pulsation; pause of 5 min., 6 co-ordinated pulsations.

40 c.c. $n/100$ NaOH in fresh sea water plus 2 drops H_2O_2 — L. p. $1\frac{1}{4}$ sec., 30 co-ordinated pulsations; pause of 5 sec., 27 co-ordinated pulsations; then followed by 40 co-ordinated pulsations at intervals varying from $\frac{1}{4}$ to 5 min.

40 c.c. $n/50$ NaOH in fresh sea water plus 2 drops H_2O_2 — L. p. $1\frac{3}{4}$ min., 1 co-ordinated pulsation; pause of 5 sec., 54 co-ordinated pulsations; pause of 3 sec., 70 co-ordinated pulsations; pause of 2 sec., 2 co-ordinated pulsations; pause of 5 sec., 6 co-ordinated pulsations; pause of 3 sec., 13 co-ordinated pulsations; pause of 2 sec., 11 co-ordinated pulsations; pause of 3 sec., 3 co-ordinated pulsations; pause of 10 sec., 2 co-ordinated pulsations; pause of 10 sec., 2 co-ordinated pulsations; pause of 1 min., 18 co-ordinated pulsations; pause of 2 sec., 10 co-ordinated pulsations; pause of $3\frac{1}{2}$ min., 3 co-ordinated pulsations; pause of $1\frac{1}{2}$ min., 2 co-ordinated pulsations.

Benedict [8] claims that NaCl exhaustion in strips of turtle's ventricle is due to loss of tonus (which may be possible in the bell of Gonionemus after cutting off the nervous system), and that calcium chloride, sodium carbonate, and oxygen increase this tonus and thus initiate the contraction. Alkalies also tend to increase tonus, at least aid recovery after sodium chloride exhaustion. "It is very interesting to note that not one of these substances (calcium chloride, dextrose, lithium chloride, cane sugar, oxygen, air, hydrogen peroxide, sodium carbonate, and Ringer's solution) is capable of producing beats in a fresh strip."

I have shown above that hydrogen peroxide initiates pulsations in the marginless bell of Gonionemus, even in alkaline solution. Experiments with pure oxygen bubbling through the fresh sea water gave negative results, no pulsations resulting during four hours' observation. However, lack of time prevented complete

[8] Benedict: This journal, 1908, xxii, p. 16.

experimentation; it is possible that pulsations may have begun six or eight hours later, as, in one case where hydrogen peroxide was used in minute traces (1 drop to 40 c.c. sea water), pulsations were not observed until five or six hours later.

When the bells were placed in sea water to which sufficient sodium hydrate was added to make $n/50$ solution (not considering the loss by precipitation of magnesium) and oxygen allowed to bubble through, eight or ten isolated co-ordinated pulsations occurred at intervals of one to ten minutes.

Experiment with sodium hydrate plus pure oxygen. —

$n/50$ NaOH in sea water through which pure oxygen bubbled for 25 min. — L. p. 16 min., 1 co-ordinated pulsation; pause of 1 min., 1 co-ordinated pulsation; pause of 1 min., 1 co-ordinated pulsation; pause of 2 min., 1 co-ordinated pulsation; pause of 10 min., 1 co-ordinated pulsation.

Summary.

These experiments indicate that hydrogen peroxide initiates the pulsations in the marginless bell of Gonionemus in normal sea water by increasing the oxidative processes. Oxidations occur much more easily in alkaline than in neutral or acid media, which may explain the pulsations caused by pure oxygen in alkaline solutions and not in fresh sea water. Also it is unreasonable to suppose that exactly the same effects are produced by oxygen when in solution in normal amount for sea water and when there is a supersaturation. The stimulating action of hydrogen peroxide and the non-stimulation by oxygen in normal sea water may be due to the fact that oxygen is liberated from the hydrogen peroxide in an atomic condition. The stimulating action of oxygen in alkaline solution is probably due to increased tendency to oxidation.

STUDIES IN THE PHYSIOLOGY OF THE CENTRAL NERVOUS SYSTEM. — I. THE GENERAL PHENOMENA OF SPINAL SHOCK.[1]

By F. H. PIKE.

[*From the Physiological Laboratories of the University of Chicago and of the Harvard Medical School.*]

THE generally accepted teaching for many years has been that the spinal cord is the great reflex centre of the central nervous system. In the lower vertebrates, such as the tortoise, there is little doubt that the spinal cord is a true reflex mechanism, since nothing short of the death of, or severe injury to, its constituent neurones will stop such reflexes. In the frog and higher vertebrates any sudden injury to the cord which completely blocks conduction to the brain or to the medulla oblongata, such as crushing it or cutting it across transversely, will cause a cessation, more or less temporary, of the reflexes of the skeletal muscle supplied by nerves arising below the level of the injury. "This phenomenon is shock."[2] Two explanations are open to us at this point: (1) that the reflex mechanism in these animals normally involves other structures in the central nervous system lying above the level of the injury and that the reflexes have stopped because their normal pathway is blocked, or (2) that the reflex mechanism is the same in all vertebrates, despite the profound morphological changes occurring in the central nervous system as we ascend in the phylum, and that the reflexes stop because of some other reason than the first given. The second explanation has been the one generally, but not unanimously,[3] accepted, and much time has been given to

[1] A preliminary note appeared in Science, 1908, N. S. xxviii, p. 808.

[2] MARSHALL HALL: Synopsis of the diastaltic nervous system, London, 1850. The earlier literature is given by ECKHARD, Beiträge zu Anatomie und Physiologie, Giessen, 1881, ix, pp. 29–192.

[3] BASTIAN: Medico-chirurgical transactions, London, 1890, xxiii, pp. 151–217; ROSENTHAL and MENDELSSOHN: Neurologisches Centralblatt, 1897, xvi, p. 978, and the papers there cited. Also, PÁNDI, Archiv für die gesammte Physiologie, 1895, lxi, p. 465, and Neurologisches Centralblatt, 1904, xxiii, p. 449.

the consideration of the nature and cause of spinal shock and to the development of a symmetrical hypothesis. The second explanation is widely regarded to-day as a definite settlement of the point. Edinger,[4] for example, states that all instincts and reflexes are functions of the palæëncephalon, and not of the neëncephalon.

That the cause of spinal shock lies in the central nervous system itself and not in the fall of blood pressure produced by spinal transection is shown by the experiments of Owsjannikow,[5] who found that section of the splanchnic nerves did not cause spinal shock, although the blood pressure was greatly lowered; and raising the blood pressure by stimulation of the peripheral end of the splanchnic nerves did not remedy the condition of shock when the spinal cord was transected. It has been pointed out by Sherrington[6] that the whole animal is affected alike in the matter of low blood pressure, but the reflexes of the brain and the part of the spinal cord anterior to the lesion are not affected. The immediate effects of the transection are, therefore, not due to the low blood pressure. But any one who has worked with decerebrated animals and has noticed the slowly failing, irregular, or even spasmodic respiration which comes on after removal of a large part of the spinal cord has no doubt been impressed with the remote effects of a very low blood pressure. That the respiratory disturbances are not due to the stimulation of afferent fibres in such cases may be shown by freezing the cord and transecting it while frozen, as will be described later in the paper. Subsequent removal of the cord is followed by the same conditions as before, although there has been no stimulation of afferent fibres. Such remote effects of continued low blood pressure are shown by Goltz's[7] dogs, which died when the entire lumbar cord was destroyed at one time, even as late as several days after the first transection. Such remote effects have, in my opinion, been too generally overlooked in discussing the nature and causes of surgical shock.

Further literature on spinal shock is given by Rosenthal, Loeb, Goltz, Sherrington, and Walton in the various articles to be cited

[4] Edinger: Journal of comparative neurology and psychology, 1908, xviii, p. 437.

[5] Owsjannikow: Arbeiten aus den physiologischen Anstalt zu Leipzig, 1874, p. 374.

[6] Sherrington: Schäfer's Text-book of physiology, 1900, ii, p. 847.

[7] Goltz: Archiv für die gesammte Physiologie, 1873, viii, p. 460.

in this paper. A more formal discussion of the literature will be given in later papers of the series.

In the series of studies on the central nervous system, I purpose to examine into the question of shock from the point of view of the phylogenetic development of function of this system. The rôle of the central nervous system in the evolution of the vertebrate phylum has been dealt with very little from the functional side, and most of the interpretations of the adaptation of animals to their environment have been made by morphologists. It is a truism that the one best fitted to interpret in terms of function the various adaptations existing in nature is the trained student of function, but the paucity of literature upon this phase of the subject is evidence of the minimal extent to which the physiologist has entered into his heritage.

It does not seem possible that the application of the principles of evolution to the functional study of the central nervous system can be much longer deferred. But it is clear that whoever traces the functional evolution of the central nervous system, endeavoring to bring some order into the chaos of literature on the central nervous system, perhaps by establishing functional types that shall render to physiology the same service that structural types have rendered to morphology, must reckon with the idea of shock. And, conversely, the defenders of the idea of shock must reckon, more fully than has been done hitherto, with the facts of organic evolution, morphological and functional. The idea of shock has not yet been examined in its proper biological perspective.

In the light of its phylogenetic development we may also inquire into the two theories of the organization of the central nervous system now current, (1) the segmental theory as developed by Goltz [8] and Loeb,[9] and (2) the theory of cortical localization of function. As Goltz has shown, the theory of cortical localization of function cannot be true if his idea of shock, *i. e.*, a long inhibition resulting from the stimulation of efferent fibres, is true, and, although the doctrine of cortical localization of function has not been without defenders, *e. g.*, Hitzig,[10] and is even now the dominant theory, the hypothesis of spinal shock has passed almost un-

[8] Goltz: Archiv für die gesammte Physiologie, 1892, li, pp. 570–614.

[9] Loeb: Comparative physiology of the brain, New York, 1900, *passim*.

[10] Hitzig: Physiologische und klinische Untersuchungen über das Gehirn; gesammelte Abhandlungen, Berlin, 1904.

challenged. The predominant tendency has been, not so much to demand a proof of the existence of spinal shock, but to assume its existence and debate about the mechanism and nature of a more or less purely hypothetical entity. I shall show in later papers (1) that any idea of shock is inconsistent with the theory of cerebral localization, and (2) that it is possible to develop a theory of the comparative physiology of the central nervous system without postulating shock.

In connection with Professors Stewart and Guthrie,[11] I have published some experiments in which the main phenomena of spinal shock were duplicated by methods which did not involve the anatomical rupture of any conduction pathways, and with doubtful stimulation of efferent inhibitory pathways. In subsequent experiments the phenomena of spinal shock have been duplicated in still greater detail by the method of cerebral anæmia, which does not involve the anatomical rupture of any conduction pathways, although it totally blocks them physiologically, and by freezing the cord, — a procedure which does not stimulate any efferent inhibitory fibres. These experiments are given in detail in the present paper.

It may be pointed out here that what is said applies to spinal or experimental shock, as distinguished from surgical shock or "collapse." In my opinion they are entirely different phenomena. Neither will the general phenomena of so-called protoplasmic shock or "block" be considered here.[12]

I wish to express here my obligation to Professor G. N. Stewart in developing the line of experimental work which has led up to my present conception of the central nervous system; to my colleagues in the University of Chicago for many criticisms and suggestions, adverse as well as favorable; to Professor W. B. Cannon for many suggestions and also for the privilege of working in the Harvard laboratory, and to many clinical friends for data on the condition of the reflexes in the human subject in cases of disease of, or injury to, the central nervous system. If progressive changes have occurred in the phylogenetic development of the central nervous system, we might reasonably expect to find certain phenomena of shock more marked in the human than in the monkey — the highest

[11] Pike, Guthrie, and Stewart: This journal, 1908, xxi, p. 359.

[12] Sherrington: Schäfer's Text-book, 1900, ii, p. 846.

animal type so far studied in the physiological laboratory. Clinical data acquire, therefore, a peculiar interest when considered from the point of view of the evolutionist.

The Doctrine of a Long Inhibition as the Cause of Spinal Shock.

A subject which has so many bearings on the fundamental conceptions of the central nervous system as spinal shock may afford the opportunity for a searching examination which, under other circumstances, might be hypercriticism. Accordingly, we may inquire into (1) the conditions which such a hypothesis must fulfil, and (2) the evidence in favor of such a hypothesis. That the idea of shock, surgical and spinal, has been the excuse for much loose thinking, and that in all probability it has been erroneously held responsible for certain respiratory phenomena, are well shown by Porter.[13]

As generally expressed, the hypothesis of inhibition postulates a depression of function of the neurones below the level of the transection. It is evident to any one who has worked with the exposed spinal cord that this depression cannot be general. If the brain stem of a dog be divided transversely at the level of the anterior corpora quadrigemina and the anæsthetic withdrawn, the exposed spinal cord soon becomes irritable, and a mere touch will cause extensive movements of the muscles supplied by motor nerves originating in that particular region, but it may be impossible to obtain any reflexes of the skeletal muscles. It has been observed [14] that stimulation of the peripheral end of the cut pyramidal tract in the monkey will give as great a response, or even a greater, than when these pathways are stimulated with the brain and cord intact. There can be, therefore, no "motor paralysis" nor any depression of the neurones in the pyramidal paths. It has been shown that the pyramidal fibres do not end [15] about motor cells in the anterior horn, but about cells in Clarke's column and probably about other

[13] Porter: Journal of physiology, 1895, xvii, p. 455; Boston medical and surgical journal, 1908, clviii, p. 73.

[14] Sherrington: Schäfer's Text-book of physiology, 1900, ii, p. 847.

[15] Schäfer: Journal of physiology, 1899, xxiv, p. xxxii; von Monakow: Archiv für Psychiatrie, 1895, xxvii, pp. 1, 386. See also Redlich: Neurologisches Centralblatt, 1897, xvi, pp. 818-832.

cells in the gray matter. Von Monakow's view that the fibres of the pyramidal tract do not enter into direct connection with the motor neurones, but act upon the latter through intercalated dendraxones, receives a certain degree of confirmation. Impulses passing down the pyramidal tracts must therefore pass over at least two synapses before reaching the motor neurones. The conductivity of these synapses has not been affected in the least, and there is here no discoverable depression sufficient to account for the failure of the reflexes. Any such effect must have been exerted in some other locality. It would appear to be more than questionable, as Beevor[16] suggests, whether the pathway between the endings of the pyramidal tracts and the motor neurones is exactly the same pathway as that involved in the reflexes, supposing for the moment that the reflex arc lies through the spinal cord alone. If the two pathways were identical, there is no apparent reason why the reflexes should fail after spinal transection, since the efferent motor path is so obviously open. The inhibitory effect must therefore be exerted either upon some part of the afferent pathway or upon some undiscovered part of the efferent pathway. There is no apparent decrease in conductivity or excitability of the afferent nerves, and the only remaining places where one might look for a break in the arc are (1) the synapse between the afferent fibre of the posterior root and the cell of Clarke's column, or (2) in an intermediate neurone interposed between the posterior root fibre and the cell of Clarke's column; (3) it is conceivable, perhaps even probable, that the reflex arc might consist of the posterior root fibre, an intermediate neurone other than the one in Clarke's column and the motor neurone in the anterior horn.[17] But, whatever constitutes the reflex arc, the inhibitory effect must be exerted upon the afferent rather than upon the efferent or motor part of the pathway.

All reflex arcs are not affected alike by such inhibition. The visceral reflexes are affected scarcely more in man and the monkey by transverse lesions of the cord than they are in the rabbit or the cat,[18] but the return of the reflexes of the skeletal muscles, as will be pointed out in greater detail in a later paper, is far less in the former than in the latter.[19] And in the monkey weak electrical or

[16] BEEVOR: Journal of the American Medical Association, 1908, li, p. 89.

[17] VON LENHOSSEK: Der feinere Bau des Nervensystems, 2 Auf., Leipzig, 1895, p. 405. Cited by SHERRINGTON, SCHÄFER'S Text-book, 1900, ii, p. 810.

[18] SHERRINGTON: SCHÄFER'S Text-book, 1900, ii, p. 847.

[19] MOORE and OERTEL: This journal, 1899, iii, p. 245.

mechanical stimulation of the central end of the posterior root of a spinal nerve will elicit reflex movements of the skeletal muscles at a time when far stronger stimuli applied to skin or to afferent nerve trunks causes no response.[20] I have seen reflex movements in cats after transection of the cord on stimulation of the central end of a posterior root (lumbar) at a time when there was no reflex response to stimuli applied to skin or nerve trunks. On high spinal transection or on decerebration the reflexes of the hind limbs return sooner than those of the fore limbs. The hind limbs suffer less than the front on removal of the cerebral motor cortex. The inhibitory effect must be exerted in increasing degree upon the reflex arcs for the skeletal musculature as we ascend in the vertebrate phylum. To bolster up the theory of inhibition, it must be shown that (1) there are more inhibitory fibres in man and monkey than in any other animal, or (2) that these fibres are more easily excitable and produce far greater effects in man than in the lower vertebrates. It is well known also that after the reflexes have returned following transection of the cord, a second transection has no further effect.[21] Why these hypothetical efferent fibres should be capable of but one effective stimulation is a matter of considerable interest.

Shock is exerted in the aboral direction only. There are many afferent fibres the stimulation of which produces well-known inhibitory effects. Any theory of inhibition must explain why or how these afferent fibres escape stimulation by a process which is supposed to be such a tremendous stimulus to efferent nerves. And, again, it is a well-known fact that the tetanizing current from an induction coil is a far more effective stimulus for most efferent nerves than simple cutting; yet no one has ever been able to produce spinal shock by any sort of stimulation which did not destroy the activity of the neurones. Why the less efficient stimulus should produce so much greater effect when applied to the spinal cord is a point requiring further explanation on any hypothesis of inhibition.

A theory of shock must also explain why shock is less severe in young animals. Babák [22] has noticed that in larval frogs transection of the spinal cord produces no shock. To interchange premise and conclusion, as Babák does, and explain the absence of shock by

[20] SHERRINGTON: SCHÄFER'S Text-book, 1900, ii, p. 847.

[21] SHERRINGTON: Integrative action of the nervous system, New York, 1906, p. 246.

[22] BABÁK: Zentralblatt für Physiologie, 1907, xxi, p. 9.

saying that the inhibitory fibres in the cord have not developed is hardly the most rigid kind of a demonstration. It is necessary to show that some of these fibres are as yet incapable of conductivity or excitability.

The whole necessity for any theory of shock lies, as has been pointed out, in the fact that another assumption has already been made, — the assumption that the spinal cord has exactly the same function throughout the vertebrate phylum. It is scarcely necessary to point out here that no independent proof has ever been adduced that the reflexes for the skeletal muscles in higher vertebrates occur through an arc involving the cord alone when the whole central nervous system is intact, and Rosenthal's results [23] cast doubt upon the validity of such a postulate even in the frog. It may be mentioned here, too, that the doctrine of spinal shock and spinal reflexes has proved unsatisfactory from the clinical point of view.[24] That certain reflexes — the maintenance of a peculiar state of tonus of all the skeletal muscles — present in normal and in decerebrated frogs permanently fail when the basis of the mid-brain is severed from the medulla oblongata has been observed by Verworn.[25]

The Experimental Evidence on Spinal Shock.

It has recently been pointed out [26] that it is possible to duplicate many of the phenomena of spinal shock by occlusion of the head arteries. The functions of the brain and medulla oblongata totally fail during the consequent anæmia. The reflexes of the skeletal muscles are abolished and reflex vaso-motor effects are no longer obtainable. In experiments where the occlusion period was long and the damage to the cerebral cells consequently great, the scratch reflex was often noticed in the period of recovery. The statements in the literature [27] are that anæmia of the brain does not produce

[23] Rosenthal and Mendelssohn: *Loc. cit.*

[24] Walton: Journal of nervous and mental disease, 1902, xxix, p. 337; Walton and Paul: *Ibid.*, 1906, xxxiii, p. 681.

[25] Verworn: Archiv für die gesammte Physiologie, 1896, lxv, p. 63.

[26] Pike, Guthrie, and Stewart: This journal, 1908, xxi, p. 359; Journal of experimental medicine, 1908, x, p. 490.

[27] Asher and Lüscher: Zeitschrift für Biologie, 1899, xxxviii, p. 499; Asher and Arnold: *Ibid.*, 1900, xi, p. 271.

shock in rabbits. Also, removal of the cortex has a smaller effect than section below the pons.[28]

Section of the brain stem by the knife in cats just above the tentorium, *i. e.*, through the anterior corpora quadrigemina, produces no increase of shock over that produced by anæmia of the brain and cervical cord, if practised so late in occlusion or so early in resuscitation that the afferent paths to the respiratory centre are non-conductive, even though the efferent paths from the respiratory centre still remain open.[29] This is perhaps an additional argument against the view that mechanical excitation of efferent inhibitory fibres is a factor in spinal shock.

When the anatomical section is made at an earlier period in occlusion, when the anæmia has not yet produced block of the conducting paths, it causes shock no deeper than that due to the anæmia. Our first statement, that anæmia of the brain and cervical cord did not itself produce shock in the remainder of the cord, was based upon a study of the reflexes after short periods of anæmia. This study did not begin sufficiently early in the resuscitation period, as we had not then appreciated the fact that after a short period of anæmia the shock phenomena disappear rapidly, not, as after shock produced by anatomical section, through the opening of spinal reflex paths, but by restoration of the normal long paths to the brain in the resuscitation period. The facts really show, however, that our first statement was incorrect. Further proof of this will appear below.

If, then, cerebral anæmia produces spinal shock in cats, it should be possible to reproduce, in greater detail than we had previously done, the classical phenomena of spinal shock. The failure of the reflexes should be as complete during cerebral anæmia as after transection of the spinal cord. It should be possible, for example, to get back as many of the reflexes of the skeletal muscles when the head arteries were permanently ligated as could be obtained in the same time after transection of the spinal cord. It would, of course, be hopeless to attempt to keep an animal alive by means of artificial respiration for a number of days, and any such comparisons are, of necessity, limited to a few hours. All the animals were deeply etherized before beginning the experiment. A tracheal cannula was then inserted to provide for artificial respiration when it should

[28] SHERRINGTON: Integrative action of the nervous system, New York, 1906, p. 246.

[29] STEWART and PIKE: This journal, 1907, xx, p. 61.

become necessary. After the first cessation of movement in animals whose cerebral arteries had been ligated, the anæsthetic was discontinued, as total anæmia of the brain is equivalent to decerebration. Similarly, the anæsthetic was discontinued after decerebration. As illustration of these facts, we submit the following protocol, in addition to those previously published: [30]

Experiment of February 23, 1908. — Adult female cat. Ether. Tracheotomy. Stimulation of the central end of left sciatic nerve before ligation of the head arteries gave a marked increase in blood pressure.

3.25 P. M. Head arteries permanently clamped.

3.38 P. M. Stimulation of the sciatic has no effect.

3.45 P. M. Stimulation of the sciatic has no effect. The right hind foot is drawn up when pinched.

3.51 P. M. Stimulation of the sciatic causes slight rise in pressure. The reflex contraction of the hind legs is also increased somewhat at this time.

From this time to

433 P. M. The effect of stimulation of the sciatic nerve increased gradually, the vaso-motor response being slightly greater at successive intervals. The first appearance of the scratch reflex occurred at this time.

4.44 P. M. Stimulation of the left sciatic nerve caused rise in blood pressure. The right hind leg was strongly contracted during this stimulation, and kicked vigorously several times when the stimulation was stopped.

4.55 P. M. Spinal cord exposed in mid-dorsal region. Touching it with the point of the knife caused a great rise in blood pressure.

4.59 P. M. Divided spinal cord transversely. Right hind leg drawn up when pinched 30 seconds later.

5.01 P. M. Stimulation of the sciatic causes rise in blood pressure.

5.03 P. M. Scratching ribs with point of forceps causes kicking of hind legs and rise of blood pressure.

5.07 P. M. Result of the stimulation of sciatic was doubtful. Irregularities began to appear in the heart rhythm. The beat became slower, causing a greater excursion of the mercury column of the manometer. In two minutes the heart had ceased to beat. Post mortem examination showed that section of the spinal cord was complete, the knife passing just below the roots of the sixteenth spinal nerve.

The fatal effect of section of the spinal cord during the period of anæmia of the brain or early in the resuscitation period has been

[30] PIKE, GUTHRIE, and STEWART: This journal, 1908, xxi, p. 359.

noticed previously,[31] but the discussion of the significance of these results cannot be given here.

It will be noticed (1) that all the reflexes fail at a certain time, more or less variable according to the individual peculiarities of the animals:[32] (2) that there is a gradual return of reflex contraction of the hind legs on pinching the foot, the contraction occurring (*a*) on the same side and (*b*) involving the other side later. The return of the homolateral and crossed reflexes occurs here in the same order as we have observed in the fore limbs on reëstablishment of the cerebral circulation;[33] (3) irregularities resembling Traube Hering curves appear in the blood-pressure tracing, and soon a reflex rise of pressure appears in response to stimulation of the central end of the sciatic. Whether this is a true vaso-motor reflex in all cases is questionable. In the curarized cat, mentioned later in the paper, true vaso-motor reflexes were not obtained as early after occlusion of the head arteries as such blood-pressure changes usually appear in non-curarized animals. (4) The movements of the hind legs become increasingly facile and the scratch reflex may be evoked by stroking the ribs. (5) The whole posterior part of the animal becomes increasingly irritable, so that a light touch may cause kicking of the hind legs, movements of the tail, and contraction of the abdominal and lower intercostal muscles. The return of the reflexes is often somewhat slower when the cerebral circulation is stopped than it is after transection of the cord.

Occasionally apparently contradictory results are obtained. The reflexes of the skeletal muscles may fail for a time, although slow, convulsive respiratory movements occur and the blood pressure remains well up, possibly higher than normal. Stimulation of the central end of the sciatic nerve causes an unusually great change in the blood pressure, although it may evoke no reflex response of the hind limbs. Such a condition is shown in the —

Experiment of May 14, 1908.—Dog, nearly or quite grown. Ether. Tracheotomy. Blood pressure from cannula in left carotid. Central end of left sciatic and right vagus nerves prepared for stimulation.

2.33 P. M. Stimulated sciatic nerve; rise of blood pressure.

2.37 P. M. Stimulated right vagus nerve. Rise of blood pressure.

[31] Stewart *et al.*: Journal of experimental medicine, 1906, viii, p. 311; This journal, 1907, xx, p. 71.

[32] Stewart *et al.*: *Ibid.*, viii, pp. 289-321.

[33] Stewart *et al.*: *Loc. cit.*

2.39 P. M. Tied off right carotid and right subclavian arteries.

2.43 P. M. Tied off left subclavian artery.

2.45 P. M. Started artificial respiration.

2.54 P. M. Stimulated the central end of vagus. Marked rise of blood pressure.

2.55 P. M. Stimulated sciatic nerve. Marked rise of blood pressure. Very faint reflex in right hind leg.

2.57 P. M. Front legs perfectly limp. No reflexes.

3.00 P. M. Stimulated sciatic. Marked rise of blood pressure. The front leg perfectly limp, while the left hind leg is rigid, *i. e.*, extensor muscles are tonically contracted.

3.03 P. M. Stimulated vagus nerve. Rise of blood pressure. Corneal reflex still present.

3.05 P. M. The front limbs are becoming rigid, *i. e.*, tonically contracted.

3.06 P. M. Both fore limbs rigid; the right drawn up and the left extended.

3.10 P. M. Draws up left front foot when pinched, and pushes it out again when stimulation ceases.

3.46 P. M. Stimulated sciatic nerve. Some rise in blood pressure.

3.49 P. M. Cut spinal cord across.

3.50 P. M. Reflex in left hind leg as strong as ever.

3.51 P. M. Stimulated sciatic. Possibly a slight rise of blood pressure.

4.30 P. M. Stimulated sciatic. Slight rise of pressure, then a fall, with return to normal before stimulation ceased. Hind limb reflexes very active by pinching. Wags tail after stimulating sciatic.

4.41 P. M. Stimulated sciatic. Slight rise of pressure. Dog raised tail up and held it rigid for a moment. No corneal reflex at this time.

4.45 P. M. Stimulated vagus. Slight rise of pressure with return to normal. Hind limbs very active.

4.55 P. M. Cut spinal cord across the second time. Blood pressure rose to twice its usual height and then fell.

4.56 P. M. Shook tail violently when foot was pinched.

5.00 P. M. Stimulated sciatic. Slight fall of blood pressure with return to normal.

The first section of the spinal cord was at the level of the third or fourth dorsal segment. The second section was about two segments lower.

The attempt was made to eliminate all the bulbar and cephalic centres by anæmia, as we had done in cats. Instead of this a differential effect probably resulted. The limpness of the fore limbs after tying the head arteries could not have been due to any failure

of function in the upper part of the spinal cord, since the respiratory and vaso-motor centres, as shown by stimulation of the vagus, were active. Nor could it have been due to the effect of ether, since the hind legs were rigid. It must have been due, therefore, to the paralysis by anæmia of the cerebral cortex and possibly also the subcortical and basal ganglia as low as the posterior corpora quadrigemina, or to the anæmia of the fore limbs themselves. That the posterior corpora quadrigemina were not affected was shown by the persistence of the corneal reflex. Some shock might have been expected on section of the cord, since the reflex arcs might have been established above this level. The effect on the reflexes of the skeletal musculature was, however, very slight. The effect of the second transection was inconsequential. The respiration became slow and labored immediately after the first transection and soon ceased.

Every part of the encephalon anterior to the pons, or, at most, to the posterior corpora quadrigemina, as indicated by the wide pupils (due to failure of third nerve centre) and final absence of the corneal reflex, had been rendered inactive by the anæmia, and the inactivity of the anterior part of the encephalon was sufficient to bring about the failure of the reflexes of the skeletal muscles. Such cases are not very rare. The results are very similar to those following anatomical transection of the brain through the posterior corpora quadrigemina.

Later, the respiration and vaso-motor tone may also fail. These cases, as stated before,[34] are probably due to individual differences in the anastomotic channels to the brain and medulla oblongata.

More rarely, the blood pressure, nearly normal or occasionally higher than normal, and respiration may persist throughout the occlusion period. If, at the end of thirty or forty minutes, when the muscular reflexes have returned in some degree, the spinal cord be transected in the upper or mid-dorsal region, the skeletal reflexes are unaffected, while vaso-motor reflexes are abolished. As an illustration of this point, we may cite the —

Experiment of May 12, 1908. — Young cat, nearly grown. Ether. Tracheotomy. Blood pressure from left carotid. Central end of right sciatic nerve prepared for stimulation.

Central stimulation of the sciatic before ligation of the cerebral arteries caused rise in the blood pressure.

[34] STEWART *et al.*: *Loc. cit.*

11.06 A. M. Ligated head arteries in the usual way.

11.27 A. M. Blood pressure low, but respiration still continues. Artificial respiration kept up all the time.

11.28 A. M. Touched right hind leg. Blood pressure rose very high, and animal struggled. Pupils are widely dilated, with furrowed and sunken cornea. No corneal reflex on ordinary stimulation, but eyelids tremble when strong pressure is made on eyeball near lids.

12–12.30 P. M. The spinal cord, which had been exposed, was now divided transversely at level of third or fourth dorsal segment.

12.15 P. M. Stimulation of the central end of the left sciatic, which had been freshly prepared, caused a very slight rise in blood pressure. Stimulation with a slightly weaker current just before section of the cord caused a marked rise in pressure.

The reflexes of the hind legs were unaffected by transection of the cord.

12.23.30 P. M. Artificial respiration stopped.

12.25.30 P. M. Asphyxial convulsions. Blood pressure, which rose at first, has now fallen again. Micturition.

12.30.30 P. M. Hind legs straightened out spasmodically. Experiment stopped.

This condition corresponds, as nearly as can be expected from the diversity of the methods employed and the inability to control the degree of anæmia in various parts of the brain, to successive transections of the neural axis at (1) a high and (2) afterward at a low level. Very rarely, in fact only once in the entire series of experiments on cerebral anæmia begun nearly four years ago, have we found an animal in which the vaso-motor response to stimulation of the sciatic was not completely abolished for a time either by the anæmia or by subsequent anatomical transection of the spinal cord. In this animal stimulation of the central end of the sciatic during the period of anæmia produced some change, although at times a relatively small one, in blood pressure each time it was stimulated. And furthermore, transection of the spinal cord in the dorsal region did not affect these vaso-motor reflexes. It is worthy of remark that this was in a young animal. Although the phenomena were perfectly definite in this case, attempts to verify the facts on other young cats have so far failed.

It is possible, therefore, to reproduce with great exactness the phenomena of spinal shock observed after transection of the neural axis at various levels, by methods which do not involve the anatomical rupture of any conduction pathway.

The long-continued stimulation of efferent inhibitory pathways by anæmia is an improbable occurrence. It may be shown that anæmia does not stimulate efferent motor fibres. As has been pointed out in a previous paper,[35] the upper part of the phrenic nerve becomes anæmic during occlusion of the head arteries, and, while its excitability may increase, I have never seen a case of spasm of the diaphragm or a twitching of its muscular fibres due to the anæmia of the phrenic nerve. Again, after the cessation of the first convulsions immediately following the occlusion of the head arteries, the hind limbs relax for a considerable time, usually twenty to thirty minutes or even longer. But during this time there is a transition area of the cord which is receiving but little blood intervening between that portion which is receiving a full blood supply and that part which receives no blood at all. Somewhere in this transition area there would be excitation if anæmia, either partial or complete, excites nerves. But, as we have seen, there is no excitation of motor fibres. If the anæmia excites efferent inhibitory fibres alone, we have a method of rare exactness in physiology. Such stimulation of efferent inhibitory fibres is, however, extremely improbable, since anæmia of the upper part of the vagus does not cause any noticeable inhibition of the heart after the first rapid fluctuations are over.[36] Asphyxia alone does not cause prolonged spinal shock. An animal may be etherized and afterward asphyxiated until the heart ceases to beat. When the heart is again started and the blood oxygenated by artificial respiration, the animal rapidly recovers, and the reflexes quickly return in all their former vigor. I shall show, in another paper, that asphyxia temporarily abolishes vaso-motor as well as muscular reflexes, and that its general effects are closely similar to those of anæmia.

If the hypothesis of a long inhibition is true, and if anæmia really does cause spinal shock by stimulation of the efferent inhibitory pathways, such a stimulation should produce shock once for all, and the restoration of the cerebral pathways should have no influence on the supposed spinal reflexes. But such is not the case. We[37] have already quoted, in considerable detail, experiments in which there was a relatively rapid and extremely complete return of all the reflexes after restoration of the cerebral circulation following

[35] Stewart and Pike: This journal, 1907, xix, p. 339.

[36] Stewart and Pike: *Ibid.*, xix, p. 349.

[37] Stewart *et al.*: Journal of experimental medicine, *loc. cit.*

rather short periods of anæmia. There could have been, therefore, no permanent effects of the stimulation of such efferent inhibitory fibres. It might be supposed that any stimulation of efferent inhibitory pathways due to anæmia would cease upon the reëstablishment of the cerebral circulation and the spinal reflex arcs again become passable. There seems little reason for such a supposition, however, as the convulsions, described in previous papers, occurring during the resuscitation period are, in all probability, due to central stimulation of motor neurones. That anæmia should stimulate efferent inhibitory fibres only, and that the blood, upon its return to the temporarily anæmic cells, should stimulate motor neurones only, is too fanciful a conception to be entertained. Any stimulation of efferent inhibitory fibres during the resuscitation period must certainly be overbalanced by the stimulation of motor fibres. It is noteworthy, also, in this connection that the strychnine-like effect, previously described, obtaining apparently throughout the spinal cord, so long as it is anatomically intact, is seen when only the brain and a few upper segments of the spinal cord have been subjected to anæmia. These results are incompatible with the hypothesis of a long inhibition or depression of function by stimulation of efferent fibres, but they may in some degree be harmonized, as will be shown later, with the idea of tonus changes in spinal shock.

But granting for the moment that anæmia does stimulate efferent inhibitory pathways, can we produce spinal shock by any other method which does not necessarily involve the anatomical rupture of the conduction pathways or the stimulation of inhibitory fibres? To answer this question, I have frozen the spinal cords of rabbits, cats, and dogs by means of an ethyl-chloride spray or by pouring liquid air directly on to the cord. The first data known to me on this subject are from an unpublished experiment by Professor D. J. Lingle. The brain of a frog was frozen by ethyl chloride. The reflexes ceased just as they did in a decerebrated frog. In my own experiments the animals were etherized and the spinal cord exposed in the upper dorsal region. If ethyl-chloride was used for freezing, the cord was, as a rule, gently lifted up and a sheet of rubber tissue passed under it, the nerve roots on each side of one segment being cut to facilitate the passage of the rubber. If the cord lay down in the spinal canal surrounded by the warm blood which oozed out from the vessels of the vertebral column, some difficulty was experienced in freezing it through with the ethyl-chloride spray. Im-

mediately before the spray was applied to the cord, the vaso-motor response was tested by electrical stimulation of the central end of one sciatic nerve. This often sufficed for the reflexes of the skeletal muscles also, but both crossed and homolateral reflexes were usually tested by pinching the foot or the tail or some area of the skin. It was shown, by this means, that the division of the dorsal nerve roots had no appreciable effect on the reflexes tested. When liquid air was used for freezing, the spinal cord was left undisturbed, the dura mater being opened. No difference in the effects could be observed because of the different manner of handling the cord.

Section of the nerve roots in the dorsal region, as already stated, had no noticeable effect upon either the crossed or the homolateral reflexes of the hind legs. That section of the posterior root may affect the tone of muscles connected with that segment [38] is well known. It was in order to meet the objection which might be raised against the experiments that a number of the cords were left undisturbed while freezing. When thoroughly frozen, the cords were usually divided. Their consistency at this time resembled wood or soft metal.

The phenomena attendant upon freezing the cord were in every case entirely comparable to those following cerebral anæmia or anatomical transection without freezing. The blood pressure, as measured by a manometer connected with a cannula in the carotid, fell more or less rapidly, depending upon the rapidity with which the cord was frozen. When liquid air was employed, the pressure fell almost perpendicularly (Fig. 1). With ethyl chloride a gradual fall might occur during most of the ten or fifteen minutes occupied in freezing the cord. The reflexes of the skeletal muscles failed as completely as after anatomical transection or after cerebral anæmia.

In illustration of these facts we may cite the protocols of two experiments on the rabbit and on the cat, respectively:

Experiment of February 25. — Adult rabbit. Ether. Tracheotomy.

10.45 A. M. The spinal cord was exposed in mid-dorsal region. The central end of the left sciatic nerve was stimulated to test reflexes of the hind legs. Crossed reflex present.

10.55 A. M. Began freezing cord with ethyl-chloride spray.

[38] VON CYON: Berichte der königlichen sachsischen Gesellschaft der Wissenschaft, Leipzig, 1865, reprinted in "Gesammelte physiologische Arbeiten," Berlin, 1888, pp. 197–202; WARRINGTON: Journal of physiology, 1898, xxxiii, p. 112.

11.05 A. M. Cord now frozen through, and divided transversely while frozen. There were no movements of any part of the animal while the cord was being frozen, nor when it was divided. No crossed reflex after freezing, but there was a slight contraction of the leg on the same side when right hind foot was pinched.

11.15 A. M. Fairly good reflex on same side when right hind foot is pinched or on stimulating the central end of the sciatic nerve, with the suggestion of a crossed reflex.

11.20 A. M. Good reflex on same side, and fairly strong crossed reflex.

11.35 A. M. Good reflex contraction of both hind limbs on stimulating central end of left sciatic nerve.

11.42 A. M. Strong reflexes of both hind limbs.

Experiment stopped.

Section of spinal cord complete below fifteenth spinal nerve.

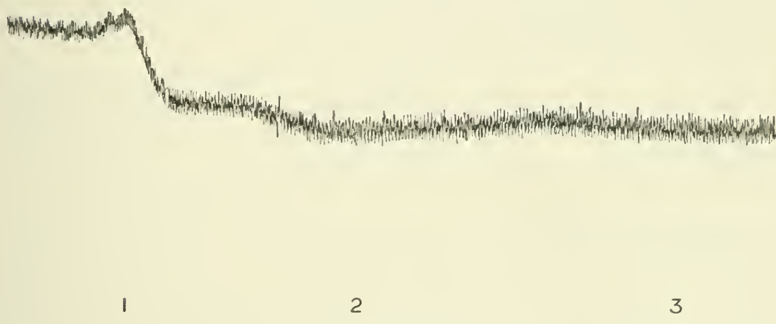


FIGURE 1. — Dog. April 9. Showing fall of blood pressure and absence of vaso-motor reflexes. Spinal cord frozen with liquid air at 1. Subsequent stimulation of the central end of the sciatic nerve at 2 and 3 one and one-half and three minutes respectively after freezing the cord was without effect. Four fifths the original size.

Experiment of February 25, 1908. — Adult cat. Ether. Tracheotomy. Spinal cord exposed in mid-dorsal region; two spinal roots cut on each side, so that a piece of sheet rubber could be drawn under it, separating it from the blood in the spinal canal. Blood pressure from cannula in left carotid. Central end of left sciatic nerve prepared for stimulation. Effect of stimulation on blood pressure was slight. The reflex contraction of the hind legs was good immediately before starting to freeze the cord.

3.48 P. M. Ethyl-chloride spray turned on cord.

4.07.30 P. M. The cord had been cut about half through. Spray maintained continuously. No movements of hind limb or tail during the time the spray was playing. The cord was now completely divided.

4.08 P. M. Ethyl-chloride spray stopped.

4.16 P. M. Stimulated sciatic nerve. No effect on blood pressure and no reflexes of the hind limbs. The right hind foot was drawn up slightly on pinching.

4.27 P. M. Stimulated sciatic. Slight rise in blood pressure. Good reflex contraction of right hind leg on pinching foot. No crossed reflex.

4.55 P. M. Stimulation of sciatic. Some fall of blood pressure.

5.07 P. M. Reflex contraction of right hind leg now much stronger.

5.20 P. M. Respiration stopped, possibly from ether, although little was given. Artificial respiration was begun. Natural respiration soon returned.

5.30 P. M. Scratching ribs on left side now causes movements of right hind foot.

5.35 P. M. Stimulated sciatic. Got crossed reflex of right hind leg.

5.37 P. M. Cut cord below first section. Rise of blood pressure. Struggles of hind limbs. Reflex contraction of right hind foot quite as strong as before.

5.43 P. M. Crossed reflex of right hind leg on stimulating left sciatic.

5.48 P. M. Reflex in right hind foot now shows excellent clonus. Ether, which had been given for a few minutes, was now stopped, and reflexes soon improved.

5.51 P. M. Clonus of right hind foot quite as strong as ever.

Experiment stopped.

We may cite in further illustration the —

Experiment of April 9, 1908. — Dog of 15 kilos. Ether. Tracheotomy. Cannula in left carotid artery. Spinal cord exposed in upper dorsal region. Central end of left sciatic nerve prepared for stimulation.

5.21 P. M. Stimulated sciatic. Rise of blood pressure.

5.25 P. M. Froze cord (about level of first or second dorsal) with liquid air. No movements of any kind occurred. Blood pressure fell rapidly (Fig. 1).

5.26.30 P. M. Stimulated sciatic. No effect.

5.28 P. M. Stimulated sciatic. No effect.

The anæsthetic is an objectionable and confusing feature in all experiments in which the cord is transected below the medulla oblongata. Attempts have been made to eliminate these factors from the problem, but without good results. For example, if the cord is frozen in the upper dorsal region and divided while frozen, variations in the degree of anæsthesia cause corresponding variations in blood pressure and in the reflex excitability of the part of the spinal

cord below the level of transection. It is evident that decerebration of the animal at this time can have no direct effect on the part of the spinal cord below the level of the first transection. Decerebration, when the spinal cord is intact, has a very slight immediate influence on blood pressure, vaso-motor reflexes, or respiration. Freezing the spinal cord does not stimulate any efferent inhibitory fibres, but when decerebration is done after previously freezing the cord and dividing it while frozen, the respiration soon becomes irregular and often ceases. Artificial respiration greatly prolongs life, but the hemorrhage incident to decerebration added to the fall of blood pressure following division of the spinal cord is incompatible with high reflex excitability for long periods of time. In another series of experiments undertaken for an entirely different purpose, ligation of both carotid and both vertebral arteries in the dog sometimes caused total insensibility for several hours without greatly affecting blood pressure or respiration. More often it produced mere drowsiness without insensibility. Somewhat similar results are shown in the protocol of the experiment of May 14, as quoted above. The method is therefore very inconstant in its results, but would seem to promise well for the few cases in which insensibility is produced. I have not so far had a really successful combination of the two methods (1) of producing insensibility by ligation of the four cerebral arteries and (2) of freezing the spinal cord. Some method of anæsthesia, however, is necessary when the blood-pressure changes caused by the stimulation of the sciatic nerve and of the vagus are to be compared. In some of the following experiments on the effect of the second transection of the cord, anæsthesia was maintained by ether, and in others the animals were decerebrated soon after the introduction of the tracheal cannula.

When the animal is decerebrated, the knife passing through the anterior corpora quadrigemina, the vaso-motor reflexes, as already stated, are not depressed, but may even be increased. The respiration continues unaffected unless the division is made so far back as to involve the part of the mechanism situated in the anterior or posterior corpora quadrigemina. The "shock" of the skeletal muscular reflexes is, however, severe. Early in the recovery, what is probably decerebrate rigidity makes its appearance. The fore limbs are rigidly extended and the jaws are tightly set. In cats the rigidity is less noticeable in the hind legs, and reflex drawing up of the feet on pinching makes its appearance here earlier than in the

fore limbs. A second transection of the neural axis, *e. g.*, in the lower cervical or upper dorsal region of the spinal cord, may affect the skeletal reflexes in some small degree, but never, in my experience, has it completely abolished them. The vaso-motor reflexes, on the contrary, are completely abolished below the level of the second transection and all respiratory movements, except those of muscles whose nerves arise above the level of the second section, cease permanently. After a time the vaso-motor reflexes return. Further transections, provided they are not too far removed from the level of first transection of the cord, are without effect. But the combination of the two operations — decerebration and transection of the spinal cord — seems, as already stated, to have a greater effect than either one alone. These facts are illustrated in the protocol of the experiment of March 14, 1908.

Experiment of March 14, 1908. — Young adult cat. Ether. Tracheotomy. Blood pressure from left carotid artery. Respiratory tracing from tambour connected with tracheal cannula. Skull trephined and calvarium removed. The cerebral hemispheres were removed rapidly, but an attempt was made to spare the basal ganglia. The object was to remove the cerebrum which, it might be supposed, would not produce shock but would destroy sensibility, allow the animal to recover from the effects of the anæsthetic, and then see whether the corneal reflex and swallowing would be affected by destruction of the basal ganglia.

10.05 A. M. Trephined skull.

10.15 A. M. Removed cerebrum. No further anæsthetic.

10.21 A. M. No reflexes in hind limbs, but strong corneal reflex present.

10.26 A. M. On pinching tail get slight movement at root.

10.32 A. M. Corneal reflex strong.

10.35 A. M. Put cotton in mouth; cat apparently swallowed.

10.50 A. M. Crossed reflex present in hind limbs. Slight movement of tail.

10.54 A. M. Movement of fore limb by pinching.

11.05 A. M. Twisting the hind legs is more effective in producing reflex movements than pinching the foot. Same also for the fore legs, but the hind-limb reflexes are more marked than the fore-limb reflexes. Strongly pinching the tail causes the head to be raised, drawn back, and rotated to the right.

11.10 A. M. The left ear is moved without obvious stimulation. There are a few small blood clots on the tip. The eyes and vibrissæ are moved when the ear is pinched. Pinching the nose also causes movements of the

vibrissæ, but they do not move much when they are pulled. The eye and the ear reflexes are much more active than the hind-limb reflexes.

11.28 A. M. Blowing in ear causes movement of it. On striking the board, the animal moves.

11.35 A. M. Pinched tail. Cat raised head. General movements of body, followed by deep respiratory gasp.

11.40 A. M. Pinched fore foot; cat raised head and body. Movements were such as a cat would make in attempting to rise.

11.43 A. M. Same observation as above on pinching hind legs.

11.49 A. M. Scratching side behind fore leg causes much stronger respiratory movements.

11.55 A. M. On gently tapping hind foot the cat made considerable effort to raise itself on to its feet by drawing hind feet off the board, raising its head, and pulling its fore feet forward.

12.12 P. M. Eyelids closed. Nictitating membrane well drawn over. Eyes looking well forward.

12.15 P. M. Cut spinal cord. Reflexes of skeletal muscles unaffected.

12.29 P. M. No reflexes could be elicited in fore or hind limbs, nor was there any corneal reflex.

Post mortem. Cord cut below fourteenth spinal nerve. Section complete. Head put into 4 per cent formalin and hardened *in situ.* The piece of cotton could not be found at *post mortem.* The stomach was so full of meat that the small piece of cotton might easily have been overlooked. The œsophagus was opened from cardia to pharynx, but no trace of cotton was seen.

April 8. The examination of hardened brain showed that the posterior and ventral portions of both occipital lobes were present, but more was present on the left side than on the right. Both anterior corpora quadrigemina intact, but the knife passed close to anterior portion of right corpus. A small portion of the optic thalamus was left, particularly on the left side, where a part of it projected out 4 mm. beyond the corpora mamillaria. From the profound shock effects produced by decerebration it might be thought that the basal ganglia had been injured. There must have been more of the brain left, however, than in the ordinary experiments, since raising the head had never been seen before. Although the second transection produced no immediate effects except the fall of blood pressure, the reflexes soon stopped, both anterior and posterior to the section, and the respiration became slow and labored. It is a question whether the failure of the reflexes and the respiration were an immediate or an indirect effect of the second transection.

Although the basal ganglia were not subsequently destroyed in the above experiment, transection through the anterior corpora quadrigemina was done in a similar experiment as soon as the corneal reflex returned after removal of the cortex and the withdrawal of the anæsthetic. The second transection (through the anterior corpora quadrigemina) had no effect on the corneal reflex.

Strychnine spasms, as has been pointed out previously,[39] may occur at any time during the occlusion period. It has been shown, also, that strychnine spasms will occur immediately after transection of the spinal cord in dogs. What may be mistaken for vaso-motor reflexes are also easily elicited at this time, since stimulation of the central end of the sciatic nerve will often cause a great rise in blood pressure. That this rise is due to the contraction of striated muscle is easily shown. No rise of blood pressure occurs in a strychninized and curarized animal at this time. Strychnine does not, therefore, have the same effect upon the spinal vaso-motor reactions that it has upon the reflex mechanism for the skeletal muscles. Although detailed consideration of this point cannot well be entered upon without taking into account certain other experimental data on the vaso-motor mechanism, best presented in a separate paper, the following condensed protocol will be given here for the sake of completeness:

Experiment of August 7, 1908. — Three-fourths grown cat. Ether. Tracheotomy. Blood pressure from cannula in left carotid. Central end of left sciatic nerve prepared for stimulation.

3.13 P. M. Head arteries ligated in the usual way. Ether then discontinued as soon as reflexes ceased, as cerebral anæmia is equivalent to decerebration.

3.16 P. M. Injection of strychnine (about 1/10 grain) subcutaneously.

3.24 P. M. More strychnine injected.

3.28 P. M. First strychnine convulsion appears. Stimulation of sciatic causes good rise of blood pressure, with extremely violent spasms.

3.30.30 P. M. About 10 c.c. of a 1 per cent solution of curare injected subcutaneously.

Stimulation of sciatic at intervals from

3.40 to 4.10 after the spasms of the skeletal muscles had ceased, caused no rise or only an extremely slight rise of blood pressure.

[39] STEWART *et al.*: Journal of experimental medicine, 1906, viii, p. 289.

Discussion of Results.

A detailed discussion of spinal shock would, at this stage, be quite premature, but a brief *résumé* does not seem out of place here.

As is well known, anæmia of the lower dorsal or lumbar cord alone does not produce permanent "shock." Spronck [40] and others have found that no permanent loss of reflexes and no permanent lesions of the spinal cord follow temporary ligation of the abdominal aorta if the period of anæmia has not been so prolonged as to cause death of the cells. Temporary anæmia of the upper part of the spinal cord and the brain will cause temporary "shock" everywhere below the region of anæmia. Freezing the spinal cord or brain causes shock below the frozen region. All the phenomena of spinal shock can be reproduced by interruption of the long conduction pathways of the spinal cord, regardless of any stimulation, or lack of stimulation, of efferent inhibitory pathways, if the time the animals are kept under observation is approximately the same after operation. Inhibition, therefore, has no important share in the production of spinal shock. The only essential factor in the production of spinal shock is the rupture of the long conduction pathways. Two possible interpretations are open to us: (1) It is conceivable that impulses from above are necessary to maintain the conductivity of the synapse between the afferent and efferent pathways concerned in the reflex arc. When the efferent fibres from the cerebrum, or whatever other portions of the brain which may be considered to give rise to them, are ruptured, or when their conductivity is blocked, the reflex arc becomes non-conductive. Under changed conditions it may regain more or less completely the primitive properties which it possesses in such animals as the turtle, but which it has lost in the gradual evolution of the vertebrate phylum. This idea enables us to retain the primitive reflex mechanism in the spinal cord, and to account in a general way for the increasing severity of spinal shock as we ascend in the vertebrate phylum. Von Cyon [41] observed that a skeletal muscle lost part of its tonus when the posterior root of its spinal nerve was divided. Loeb [42] suggests that this may throw some light on the question of spinal

[40] Spronck: Archives de physiologie, 1888, p. 1.

[41] von Cyon: *Loc. cit.*

[42] Loeb: Comparative physiology of the brain, 1900, p. 274.

shock. This idea is more or less prevalent under various guises, such as maintenance of tonus. The form which I have given is that suggested by Professor W. B. Cannon.

(2) Another possible explanation is that, in the morphological development of the nervous system as outlined by Herrick,[43] there may have been a concomitant shifting of function, and that the functional reflex arcs in higher animals pass through some part of the central nervous system above the spinal cord. The primitive pathways through the spinal cord persist morphologically, and may, under changed conditions, regain a part of their primitive function. I have previously suggested such a contingency in connection with the possible spinal origin of accelerator impulses to the heart.[44]

The possible effect of phylogenetic changes in modifying the reflex activities of the spinal cord has also been recognized by Professor Stewart.[45] In discussing the course taken by impulses leading to reflexes it is suggested that the question of the exact pathway is not so important as the question "whether, as a matter of fact, the spinal motor cells are most easily discharged by the impulses that reach them directly, or by the impulses that come down by the roundabout way of the cortex and the efferent fibres that connect it with the cord. It is evident that the answer to this question need not be the same for all kinds of animals. It may well be that in the higher animals, in which the cortex has undergone a relatively great development, the spinal motor mechanisms are more easily discharged from above than from below, while in lower animals the opposite may be the case." As I shall show later, certain of these primitive arcs probably persist in all animals.

The higher the type of animal, or, in other words, the more the central nervous system has departed from the primitive segmental type, the less complete will be the recovery from injury to, or disease of, the long conduction pathways, on this second hypothesis. We may, on this basis, readily explain the "deficiency phenomena" which Sherrington[46] describes in the monkey, without postulating any mysterious "Hemmungserscheinungen." And, as we would ex-

[43] HERRICK: Journal of comparative neurology and psychology, 1908, xviii, p. 393.

[44] PIKE, GUTHRIE, and STEWART: Journal of experimental medicine, 1908, x, p. 494.

[45] STEWART: Manual of physiology, 1900, 4th ed., p. 706: 5th ed., 1906, p. 706.

[46] SHERRINGTON: Philosophical transactions of the Royal Society, 1897, cxc, pp. 139-141.

pect on the hypothesis of a progressive change in the reflex pathways, the "multiformity and complexity of reflexes" in the monkey, "feeble and poverty-stricken as it is," when compared with the reflexes found in the cat or the dog on recovery from spinal transection, is still affluence when compared with the reflexes obtainable in the human after total transverse lesions. It makes little difference, from the theoretical point of view, whether we say, with Bastian [47] and others, that no reflexes return in the human subject after total transverse lesions of the spinal cord, or accept Senator's [48] verdict that, while it is proved beyond question that the tendon reflexes of the lower extremities may fail completely after lesions of the upper part of the spinal cord, even when the reflex arc is not demonstrably affected, such a total failure is probably not the absolute rule. On *a priori* grounds, one would expect a certain amount of variation here, as in other biological phenomena. Such questions are of interest to the clinician, but, whatever be his ultimate decision, the fact is plain that the effects of spinal transection are more severe in the human than in the monkey, and vastly more severe than in such mammalian types as the dog or cat. It is difficult for me to believe that a reflex arc, with no demonstrable lesions, should remain non-conductive for eleven years,[49] lacking only the tonus impulses from above to facilitate the passage of the afferent impulse, if it had been fully conductive up to the time of the accident to the spinal cord. The loss of certain afferent impulses, such as result from the division of the posterior spinal roots, for example, leads to chromatolysis and other degenerative changes in the motor cells of the cord,[50] but the loss of impulses from above does not, as Senator concedes, necessarily produce any such permanent effect in any neurones which might be regarded as forming a part of the spinal reflex arc, although Sherrington [51] had, the year previous to the publication of Senator's paper, put forward his hypothesis of "isolation dystrophy" to account for the permanent failure of certain spinal reflexes in the monkey after spinal transection.

It may be objected that a sufficient amount of time does not elapse between the transection of the cord and the reappearance of the

[47] Bastian: *Loc. cit.*

[48] Senator: Zeitschrift für klinische Medicin, 1898, xxxv, p. 18.

[49] Bowlby: British medical journal, 1890, i, p. 1132.

[50] Warrington: *Loc. cit.*

[51] Sherrington: Philosophical transactions of the Royal Society, 1897, *loc. cit.*

reflexes to permit of any change in the nature of the neurones constituting the spinal reflex arc. In reply, we may cite a single instance drawn from embryology. The accessory optic vesicles of the chick arise, reach their maximum development, and disappear within a space of three hours.[52] There is little reason for thinking that whatever functional change is necessary in the intermediate neurone in the spinal reflex arc should require a vastly greater time for its consummation, particularly in animals somewhere near the chick in the taxonomic scale, unless the neurone in question has so far departed from the primitive type that a resumption of primitive function is impossible. In older animals and in animals whose period of embryonic development is longer than that of the chick, cell processes might well be slower. In the monkey, however, the period of recovery from spinal transection does not apparently last more than five or six weeks.[53]

Porter[54] has shown that the commissural path at the level of the phrenic nuclei, which is probably closed to the passage of respiratory impulses ordinarily, almost immediately opens up when the spinal cord is hemisected at the level of the first or second cervical nerve, *e. g.*, on the right side, and the opposite, *e. g.*, the left, phrenic nerve is cut.

The evidence is perfectly clear, therefore, that the severity of spinal shock is greater in the monkey and in the human than in any other mammalian form, and more severe in mammals than in the lower orders of vertebrates. Spinal shock begins in those vertebrate forms in which the neopallium, as distinguished from the archipallium, first appears as part of the cerebral cortex, and the transverse segmentation of the spinal cord has added to it the longitudinal segmentation represented by the long conduction pathways of the cord.[55] It increases in severity and duration as the cerebral cortex and the long conduction paths of the cord increase in complexity. The effects of removal of the cerebellum, which appears much earlier, phylogenetically, than the part of the cerebrum known as the neopallium, are more severe and more permanent than those following removal of the motor areas of the cerebral cortex (Luciani). These facts, thus briefly stated, and many

[52] Locy: Anatomischer Anzeiger, 1897, xiv, p. 113.

[53] Sherrington: Philosophical transactions, *loc. cit.*

[54] Porter: Journal of physiology, 1895, xvii, p. 455.

[55] Herrick: *Loc. cit.*

others, the enumeration of which would require too much space at this time, have led me to believe that the mechanism of spinal shock consists, not in stimulation of efferent inhibitory pathways, nor in the loss of tone in levels below the transection, but in cutting off the normal conduction pathways for the reflexes. Recovery from shock, whether it be from spinal transection, removal of the motor areas or of other areas of the cerebral cortex, or removal of the cerebellum, consists, therefore, not alone in the failure or disappearance of a hypothetical inhibition nor in the regaining of tonus, but far more in the more or less complete assumption by more primitive structures in the line of phylogenetic development, of the function of the parts lost. These primitive parts have lain more or less dormant under ordinary conditions, but may again become active in some degree when the more recent structures have suffered irreparable injury. And the more complex the development of the structures appearing last in the phylogenetic chain, or, in other words, the more the central nervous system has departed from the primitive palæëncephalon, the less completely can the more primitive portions regain their primitive function. As regards the motor areas, we may consider this hypothesis to be such a modification or extension of the views of Luciani and Tamburini [56] as the phylogeny of the central nervous system may demand.

The decidedly less marked severity of spinal shock in young animals is sufficiently explained, not by assuming, as Babák [57] has done for the larval frog, that the efferent inhibitory fibres have not yet become excitable, but by supposing that Von Baer's law of recapitulation, known also as the law of biogenesis,[58] applies to function as well as to structure, and that those parts of the central nervous system which are the last to appear phylogenetically are also the last to reach their full functional development in ontogeny. The idea that the law of biogenesis applies to cerebral function is rendered far more than an assumption by the work of von Bechterew,[59] who showed that the myelination of the fibres of the pyramidal tract begins about the tenth day after birth.

[56] Luciani and Tamburini: Ricerche sperimentali sui centri psico-motori corticali; Reggio-Emilia, 1878; Brain, 1878–1879, i, p. 529; 1879–1880, ii, p. 234.

[57] Babák: *Loc. cit.*

[58] Sedgwick: Quarterly journal of microscopical science, 1894, xxxvi, p 35; Eigenmann: Mark anniversary volume, 1903, pp. 197–200.

[59] von Bechterew: Neurologisches Centralblatt, 1888, vii, p. 14; *ibid.*, 1889, viii, p. 513.

Considered in their relations to the spinal cord alone, it is difficult, perhaps impossible, to decide between the two hypotheses. Considered in their wider relationships, the evidence, in my opinion, favors the latter hypothesis. The detailed consideration of these points must, however, be postponed until I have presented further evidence drawn from the study of special reflex mechanisms, more particularly the vaso-motor mechanism, and the phenomena following destruction of the cerebral motor cortex by operation or disease, after which we may consider both hypotheses in their relation to the central nervous system as a whole along the lines suggested above.

HYDROLYSIS OF VITELLIN FROM THE HEN'S EGG.[1]

By THOMAS B. OSBORNE and D. BREESE JONES.

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

THE yolks of a large number of eggs were strained through cheesecloth, an equal volume of saturated sodium chloride solution added, and the mixture shaken with ether, containing a very little alcohol, until nearly all of the ether soluble substance was removed. The aqueous solution was then filtered through a felt of paper pulp and the perfectly clear solution dialyzed until all the globulin had precipitated. This precipitate was filtered out, redissolved in 10 per cent sodium chloride solution, and the clear solution again dialyzed. When the globulin had been completely reprecipitated, it was filtered out, washed with water, then with 50 per cent alcohol, and digested with a mixture of equal parts of absolute alcohol and ether. After digesting several days the vitellin was collected on a filter and washed thoroughly with ether. When dried over sulphuric acid, a colorless powder was obtained which was used for the hydrolysis.

Hydrolysis of Vitellin.

Four hundred grams of vitellin, equivalent to 345.6 gm. ash and moisture free, were dissolved in 1000 c.c. hydrochloric acid, specific gravity 1.1, by heating on a water bath for two hours. The hydrolysis was then completed by boiling the solution in an oil bath for fifteen and a half hours.

The hydrolysis solution was then concentrated, under diminished pressure, to a thick syrup and esterified according to the directions of Emil Fischer. The free esters were liberated, shaken out with ether and dried in the usual manner. After the salts were removed from the aqueous layer, the remaining amino-acids were subjected to a second esterification. After distilling off the ether from the

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

combined esters, at 760 mm. pressure, there were obtained 278 gm. of esters which were fractionally distilled as follows:

Fraction.	Temperature of bath up to	Pressure.	Weight.
I	80°	15.00 mm.	5.32 gm.
II	100°	12.00 "	24.98 "
III (*a*)	96°	0.20 "	48.52 "
III (*b*)	106°	0.15 "	23.86 "
IV	145°	0.19 "	48.87 "
V	200°	0.20 "	32.18 "
	Total		183.73 gm.

The undistilled residue weighed 58.00 gm.

Fraction I. — Repeated attempts were made to separate glycocoll ester hydrochloride from this fraction, but none was obtained. The amino-acids were then regenerated by boiling with water, the chlorine removed, and the free acids added to the amino-acids from Fraction II.

Fraction II. — This fraction was treated with concentrated hydrochloric acid, and an attempt was made to separate the hydrocholoride of glycocoll ester, but none was found. After saponifying by boiling with water, the solution was evaporated under diminished pressure and the hydrochloric acid removed with silver sulphate and the sulphuric acid with baryta. The filtrate from the barium sulphate was evaporated to dryness under diminished pressure, and the amino-acids thus regenerated together with those from Fraction I were extracted with boiling alcohol to remove proline. By fractional crystallization of the part insoluble in alcohol, 5.34 gm. of leucine, 6.46 gm. of valine, and 2.59 gm. of alanine were obtained.

The leucine was analyzed as follows:

Carbon and hydrogen, 0.1539 gm. subst., gave 0.3090 gm. CO_2 and 0.1410 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . = C 54.75; H 10.11 " "

The valine crystallized in the characteristic plates.

Carbon and hydrogen, 0.1448 gm. subst., gave 0.2728 gm. CO_2 and 0.1264 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.28; H 9.40 per cent.
Found . . . . = C 51.38; H 9.70 " "

The alanine crystallized in hard, dense prisms, which decomposed sharply at 290°.

Carbon and hydrogen, 0.1117 gm. subst., gave 0.1654 gm. CO_2 and 0.0792 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.45; H 7.86 per cent.
Found . . . . . = C 40.38; H 7.85 " "

The last fraction of amino-acids which separated from the final filtrate should contain glycocoll, if this were present. It was accordingly analyzed without recrystallizing, for if it contained glycocoll this should be indicated by a low percentage of carbon. As the analysis showed 42.36 per cent of carbon, little if any glycocoll could have been present, as the whole fraction weighed only about 1 gm.

Fraction III. — This fraction was saponified by boiling with water until the alkaline reaction had disappeared. The solution was then evaporated to dryness under diminished pressure and the proline extracted from the residue by boiling with absolute alcohol. The part insoluble consisted chiefly of leucine, which weighed 28.77 gm.

Carbon and hydrogen, 0.1500 gm. subst., gave 0.3026 gm. CO_2 and 0.1307 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . . = C 55.02; H 9.68 " "

From the filtrate of the leucine there were further obtained 3.77 gm. of copper aspartate which crystallized in the characteristic sheaves.

Copper, 0.1020 gm. subst., gave 0.0292 gm. CuO.

Calculated for $C_4H_5O_4N$ Cu $4\frac{1}{2}$ H_2O = Cu 23.07 per cent.
Found . . . . = Cu 22.87 " "

From the alcoholic extracts of Fractions II and III the proline was separated and weighed in the form of its copper salts, which contained 13.38 gm. of *l*-proline and 1.09 gm. of *r*-proline.

The *l*-proline copper was converted into the free acid and identified in the form of the phenylhydantoine which crystallized in beautiful prisms, melting sharply at 142°.

Nitrogen, 0.2154 gm. subst., required 20 c.c. N/10 HCl.
Calculated for $C_{12}H_{14}O_2N_2$ = N 12.96 per cent.
Found . . . . = N 13.00 " "

The air-dried racemic copper-salt was analyzed as follows:

Water, 0.1403 gm., lost 0.0155 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 11.05 " "

Copper, 0.1446 gm. subst., gave 0.0348 gm. CuO.
Calculated for $C_{10}H_{16}O_4N_2Cu \cdot 2\,H_2O$ = Cu 19.40 per cent.
Found . . . . = Cu 19.23 " "

Fraction IV. — Phenylalanine was removed from this fraction by shaking with ether in the usual way, and 6.38 gm. of the hydrochloride were obtained. The free phenylalanine decomposed at about 270° and gave the following analysis:

Carbon and hydrogen, 0.1709 gm. subst., gave 0.4111 gm. CO_2 and 0.1039 gm. H_2O.
Calculated for $C_9H_{11}O_2N$ = C 65.45; H 6.66 per cent.
Found . . . . = C 65.60; H 6.75 " "

From the aqueous layer, after saponification with baryta, there were obtained 5.57 gm. of aspartic acid, in the form of the barium salt. The free aspartic acid which resulted on decomposing the barium salt reddened at about 300° and gave the following analysis:

Carbon and hydrogen, 0.1765 gm. subst., gave 0.2350 gm. CO_2 and 0.0853 gm. H_2O.
Calculated for $C_4H_7O_4N$ = C 36.09; H 5.26 per cent.
Found. . . . . . . = C 36.31; H 5.37 " "

The filtrate from the barium aspartate, when freed from barium, concentrated to small volume and saturated with hydrochloric acid gas, yielded 6.99 gm. of glutaminic acid hydrochloride.

Fraction V. — From the ether extract of this fraction 4.59 gm. of phenylalanine hydrochloride were separated. The aqueous layer, after shaking out with ether, yielded 7.35 gm. of glutaminic acid in the form of the barium salt and 3.15 gm. as the hydrochloride.

The free glutaminic acid decomposed with effervescence at about 205°.

Carbon and hydrogen, 0.2001 gm. subst., gave 0.2984 gm. CO_2 and 0.1109 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81; H 6.12 per cent.
Found . . . . = C 40.67; H 6.16 " "

No copper aspartate could be obtained from this solution. Owing to an accident, a considerable part was lost at the outset of the attempt to separate glutaminic and aspartic acids, but since it contained no aspartic acid the loss fell only on glutaminic acid. As the quantitative determination of this acid was made by direct isolation of the glutaminic acid hydrochloride from a separate portion of the vitellin, the final result of the analysis was not affected by the loss.

Direct Determination of Glutaminic Acid.

There were taken for hydrolysis 86.26 gm. of ash and moisture free vitellin, which was dissolved by heating on a water bath for two hours in 300 c.c. of HCl, specific gravity 1.1. The hydrolysis was completed by further heating in an oil bath at about 130° for eighteen and one-half hours. The solution was then concentrated and saturated with HCl gas. After standing in an ice box for several weeks there separated 13.51 gm. of glutaminic acid hydrochloride, which, after deducting the ammonium chloride, is equivalent to 25.25 gm. of glutaminic acid, or 11.95 per cent.

Ether Distilled from the Esters at 760 mm.

The ether was treated with alcoholic hydrochloric acid, and the substance which separated after standing for several days was boiled with baryta until free from ammonia and worked up for glycocoll ester hydrochloride in the usual manner, but without success.

The Residue after Distillation.

The residue remaining after distillation of the esters, which weighed 58 gm., was dissolved in boiling alcohol, and after cooling 1 gm. of crystalline substance separated from the solution. The filtrate from this was saponified by boiling for five hours with 75 gm. of baryta, and, after removing the barium with an equiva-

lent quantity of sulphuric acid, 7.49 gm. of glutaminic acid hydrochloride were separated by the usual process.

Tyrosine.

A quantity of the vitellin equal to 45.04 gm. of ash and moisture free substance was hydrolyzed by boiling for eighteen hours with 150 gm. of sulphuric acid and 300 c.c. of water. After removing the sulphuric acid with an equivalent quantity of baryta, the solution was concentrated to crystallization, and after twenty-four hours the substance which separated was filtered out and recrystallized. The tyrosine after a second recrystallization weighed 1.50 gm., equal to 3.37 per cent.

Nitrogen, 0.1544 gm. subst., required 8.65 c.c. N/10–HCl.
Carbon and hydrogen, 0.1509 gm. subst., gave 0.3289 gm. CO_2 and 0.0866 gm. H_2O.

Calculated for $C_9H_{11}O_3N$ = C 59.67; H 6.08; N 7.73.
Found . . . . . . = C 59.43; H 6.37; N 7.84.

The filtrate from the tyrosine was used for determinations of the bases according to the method of Kossel and Patten.

Histidine.

The solution of the histidine = 500 c.c.

Nitrogen, 50 c.c. sol., required 2.32 c.c. 5/7 N–HCl = 0.2320 gm. N in 500 c.c. = 0.8561 gm. histidine = 1.90 per cent.

The histidine when converted into the dichloride melted at 233°.

Chlorine, 0.1076 gm. subst., gave 0.1335 gm. AgCl.

Calculated for $C_6H_{11}O_2N_3Cl_2$ = Cl 31.14 per cent.
Found . . . . = Cl 30.68 " "

Arginine.

The solution of the arginine = 1000 c.c.

Nitrogen, 50 c.c. sol., required 5.13 c.c. 5/7 N–HCl = 1.0260 gm. N. in 1000 c.c. = 3.1878 gm. arginine + 0.1700 gm. = 3.3578 gm. = 7.46 per cent.

The arginine was converted into the copper-nitrate double-salt for identification.

Copper, 0.1053 gm. subst., gave 0.141 gm. CuO.
Calculated for $C_{12}H_{28}O_4N_8Cu\ (NO_3)_2\ 3\ H_2O$ = Cu 10.79 per cent.
Found . . . . = Cu 10.69 " "

Lysine.

The lysine picrate weighed 5.5650 gm. = 2.1665 gm. lysine = 4.81 per cent.

Nitrogen, 0.1590 gm. subst., required 20.8 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 18.31 " "

Hydrolyses of vitellin from hen's eggs have been made by Hugounenq,[2] Levene and Alsberg,[3] and by Abderhalden and Hunter.[4] Since very wide differences exist between the results obtained by these investigators, we determined to make the hydrolysis just described in order, if possible, to obtain more definite information as to the proportion in which this important protein yields the several amino-acids. Hugounenq's results for most of the amino-acids are so far below those found by all the others who have analyzed this protein that they need no further consideration. Those reported by Levene and Alsberg for the mono-amino-acids are unquestionably too low, for they made their distillation at 15 mm. and the quantity of esters which they obtained corresponded to only about one half the yield which we secured. Levene and Alsberg call attention to their small yield of esters and state that much time elapsed between the first and second esterification. In view of these facts it is evident that a comparison of their results cannot be made with those of Abderhalden and Hunter or with ours.

In respect to the total amount of esters recovered after distillation, the quantity obtained by Abderhalden and Hunter, 158 gm., agrees fairly well with the 184 gm. which we obtained, and the

[2] Hugounenq: Comptes rendus des Séances hebdominaires de l'Académie des Sciences, 1906, cxlii, p. 173.

[3] Levene and Alsberg: The journal of biological chemistry, 1906, ii, p. 127.

[4] Abderhalden and Hunter: Zeitschrift für physiologische Chemie, 1906, xlviii, p. 505.

quantity obtained below 105°, namely, 97 gm., also agrees very closely with that which we got below 106°, namely, 103 gm. The results given by Abderhalden and Hunter for most of the amino-acids agree satisfactorily with those which we obtained, as shown by the following figures:

Hydrolysis of Vitellin from the Hen's Egg.

Substance.	Abderhalden and Hunter.	Osborne and Jones.	Substance.	Abderhalden and Hunter.	Osborne and Jones.
Glycocoll . . .	1.10	0.00	Tyrosine . . .	1.60	3.37
Alanine	+	0.75	Cystine . . .	....	not deter.
Valine	2.40	1.87	Histidine . . .	....	1.90
Leucine	11.00	9.87	Arginine . . .	....	7.46
Proline	3.30	4.18	Lysine . . .	....	4.81
Phenylalanine .	2.80	2.54	Ammonia . .	....	1.25
Aspartic acid . .	0.50	2.13	Tryptophane .	....	present
Glutaminic acid	12.20	12.95	Phosphorus .	....	0.94
Serine	?	?	Total		54.02

The most striking difference, and one which we cannot explain, is shown by glycocoll, which we were wholly unable to find, although persistent efforts were made to do so. The difference between the percentage of aspartic acid in these two analyses is relatively large, but from such data as are now available it would appear that determinations of aspartic acid are among the most uncertain of all of the protein decomposition products. We made no attempt to determine cystine or oxyproline.

HYDROLYSIS OF THE MUSCLE OF SCALLOP (PECTENS IRRADIANS).[1]

By THOMAS B. OSBORNE and D. BREESE JONES.

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

CHITTENDEN[2] found in the large adductor muscle of the American scallop (Pectens irradians) a relatively large amount of free glycocoll. It is therefore of interest to know whether the muscle substance of this mollusk is relatively rich in glycocoll or presents any other peculiar feature in the proportion of the amino-acids which it yields by hydrolysis.

A quantity of the muscles of the scallop were carefully removed from the still living mollusk and freed from all the other tissues. The muscles were then suspended in water, and each separately removed and dropped into a large volume of water containing toluol. After standing over night the muscles had swelled greatly and absorbed about two-thirds of the water. They were next dropped, one by one, into about 3 litres of water which was kept constantly boiling. The water in which they had stood over night was then added, and the whole boiled for fifteen minutes. The coagulated muscle substance was strained out on cheesecloth and disintegrated by crushing with the hand. It was then washed twice with distilled water and strained and pressed on cheesecloth after each washing. It was next suspended in water saturated with toluol, allowed to stand over night and then thoroughly pressed in a hydraulic press. After breaking up the press cake, it was digested for forty-eight hours with 95 per cent alcohol, squeezed out on cheesecloth, and again digested with 95 per cent alcohol. After digesting a third time with absolute alcohol, the residue was extracted with ether and dried in the air.

Thus prepared, it contained 13.13 per cent of moisture and 0.66

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

[2] CHITTENDEN: American journal of science and arts, 1875 (3), x, pp. 26-32.

per cent of ash. The ash and moisture free material contained 17.05 per cent of nitrogen.

Hydrolysis of Scallop Muscle.

Three hundred grams of the scallop muscle, equivalent to 258.6 gm. of ash and moisture free substance, were hydrolyzed in three separate portions by heating on a water bath for three hours with 600 c.c. of hydrochloric acid, specific gravity 1.1. The hydrolysis was then completed by boiling for twenty-four hours in an oil bath.

The glutaminic acid was separated from each of the hydrolysis solutions as the hydrochloride in the usual way, and after deducting the ammonium chloride which it contained, 14.08 gm., 13.83 gm., and 13.47 gm., respectively, were obtained from the three portions. With the 6.66 gm. subsequently obtained from the esters, the total glutaminic acid hydrochloride weighed 48.04 gm., equivalent to 38.5 gm. of glutaminic acid or 14.88 per cent of the muscle.

The free acid decomposed with effervescence at 202°–203°.

Carbon and hydrogen, 0.1701 gm. subst., gave 0.2546 gm. CO_2 and 0.0977 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81; H 6.12 per cent.
Found . . . . = C 40.82; H. 6.43 " "

The filtrate from the glutaminic acid hydrochloride was concentrated to a thick syrup, freed from water by evaporation with alcohol under reduced pressure, and esterified in the usual way. After separating the free esters with ether, the inorganic salts were removed from the aqueous solution and the esterification repeated. The esters thus obtained, which were freed from ether by distillation from a water bath at atmospheric pressure, were then distilled *in vacuo* with the following results:

Fraction.	Temperature of bath up to	Pressure.	Weight.
I	100°	9.0 mm.	28.49 gm.
II	100°	0.5 "	22.22 "
III	200°	0.4 "	58.32 "
		Total	109.03 gm.

The undistilled residue weighed 13.00 gm.

Fraction I. — The esters of this fraction were saponified by boiling with water until the alkaline reaction had ceased, when the

solution was evaporated to dryness under strongly diminished pressure and the proline extracted from the dried residue by boiling with absolute alcohol. From the amino-acids insoluble in alcohol, there were isolated 8.77 gm. of leucine. The filtrate from the leucine was evaporated to dryness and the residue esterified with alcohol and dry hydrochloric acid gas. After long standing at 0°, no glycocoll ester hydrochloride separated, even after repeating the process of esterification several times.

The amino-acids, when regenerated and freed from chlorine, yielded 2.20 gm. of leucine and 4.0 gm. of a mixture from which nothing definite could be separated.

The leucine had the following composition:

Carbon and hydrogen, 0.1381 gm. subst., gave 0.2772 gm. CO_2 and 0.1238 gm. H_2O.

Calculated for $C_6H_{13}NO_2$ = C 54.96; H 9.92 per cent.
Found . . . . . . = C 54.74; H 10.03 " "

Fraction II. — This fraction consisted almost entirely of the esters of leucine and proline. After saponifying by boiling in the usual way with water, the solution was evaporated to dryness under diminished pressure and the proline extracted with boiling alcohol. The part which was insoluble in alcohol yielded on fractional crystallization 10.93 gm. of not quite pure leucine.

On account of a very slight admixture of some substance which could not be readily separated, this leucine was converted into the copper salt, which gave the following results on analysis:

Copper, 0.1264 gm. subst., gave 0.0308 gm. CuO.

Calculated for $(C_6H_{12}NO_2)_2Cu$ = Cu 19.64 per cent.
Found . . . . = Cu 19.47 " "

Copper, carbon, and hydrogen, 0.1645 gm. subst., gave 0.0400 gm. CuO, and 0.2677 gm. CO_2, and 0.1079 gm. H_2O.

Calculated for $(C_6H_{12}NO_2)_2Cu$ = C 44.46; H 7.47; Cu 19.64 per cent.
Found . . . . = C 44.38; H 7.34; Cu 19.43 " "

The alcoholic extracts of Fractions I and II were joined and worked up for proline. After evaporating this solution under diminished pressure to dryness, the residue was extracted with absolute alcohol and the evaporation and extraction with alcohol repeated until the residue was completely soluble in absolute alcohol

and yielded no deposit on standing. The proline was then converted into the copper salt, and the lævo separated from the racemic by boiling with absolute alcohol. The racemic salt was freed from copper with hydrogen sulphide and the solution evaporated to dryness under diminished pressure. A small residue remained, insoluble in absolute alcohol, which was filtered out and the proline again converted into the copper salt.

Water, 0.2050 gm. subst., air dried, lost 0.0223 gm. H_2O at 110°.
Calculated for $C_{10}H_{16}O_4N_2Cu\ 2H_2O$ = H_2O 10.99 per cent.
Found . . . . = H_2O 10.88 " "
Copper, 0.1819 gm. subst., dried at 110°, gave 0.0496 gm. CuO.
Calculated for $C_{10}H_{16}O_4N_2Cu$ = Cu 21.81 per cent.
Found . . . . = Cu 21.78 " "

The copper salt of the lævo-proline, which was soluble in alcohol when dried at 100°, weighed 7.49 gm., equivalent to 5.91 gm. of lævo-proline.

The free acid was then regenerated and converted into the phenylhydantoine derivative. This latter crystallized from water in characteristic long prisms which melted at 143°.

Carbon and hydrogen, 0.1899 gm. subst., gave 0.4635 gm. CO_2 and 0.0966 gm. H_2O.
Calculated for $C_{12}H_{12}N_2O_2$ = C 66.67; H 5.57 per cent.
Found . . . . = C 66.57; H 5.69 " "

Fraction III. — The phenylalanine ester was removed by shaking this fraction with ether in the usual manner. The residue left by evaporating off the ether was saponified by heating with strong hydrochloric acid, and 10.92 gm. of phenylalanine hydrochloride, equivalent to 8.94 gm. of phenylalanine, were obtained.

The free phenylalanine, which resulted by treating the hydrochloride with an excess of ammonia, had the following composition:

Carbon and hydrogen, 0.1685 gm. subst., gave 0.4037 gm. CO_2, and 0.1038 gm. H_2O.
Calculated for $C_9H_{11}O_2N$ = C 65.45; H 6.66 per cent.
Found . . . . = C 65.34; H 6.89 " "

The final filtrate from the phenylalanine hydrochloride yielded a thick syrup, from which nothing could be directly separated.

This syrup was dissolved with boiling water, an excess of ammonia added, the solution decolorized with bone black, and concentrated. There was thus further obtained 3.74 gm. of free phenylalanine, making a total of 12.68 gm., or 4.90 per cent.

The aqueous solution, from which the ester of phenylalanine had been removed by shaking with ether, after saponifying in the usual manner with baryta, yielded 3.90 gm. of aspartic acid, as the barium salt, which was converted into the copper salt for analysis.

Copper, carbon, and hydrogen, 0.2013 gm. subst., gave 0.0583 gm. CuO, 0.1313 gm. CO_2, and 0.0920 gm. H_2O.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07; C 17.41; H 5.12 per cent.
Found . . . . = Cu 23.14; C 17.79; H 5.08 " "

The filtrate from the barium aspartate was freed from barium, the solution concentrated, and saturated with hydrochloric acid gas. There separated 6.66 gm. of glutaminic acid hydrochloride.

Chlorine, 0.1696 gm. subst., gave 0.1329 gm. AgCl.

Calculated for $C_5H_{10}O_4NCl$ = Cl 19.35 per cent.
Found . . . . = Cl 19.37 " "

The free glutaminic acid obtained from this hydrochloride decomposed with effervescence at 202°–203°.

Carbon and hydrogen, 0.2016 gm. subst., gave 0.3018 gm. CO_2 and 0.1127 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81; H 6.12 per cent.
Found . . . . . . = C 40.83; H 6.26 " "

The filtrates from the glutaminic acid hydrochloride, after being freed from chlorine, yielded 10.50 gm. of copper aspartate, which separated from a large volume of water in the characteristic sheaf-like groups of crystals.

Copper, 0.1609 gm. subst., air dried, gave 0.0461 gm. CuO.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07 per cent.
Found . . . . = Cu 22.89 " "

The filtrate from the copper aspartate, after removing the copper with hydrogen sulphide, yielded further 0.89 gm. of nearly pure leucine:

Carbon and hydrogen, 0.1115 gm. subst., gave 0.2261 gm. CO_2 and 0.0917 gm. H_2O.

Calculated for $C_6H_{13}NO_2$ = C 54.96; H 9.92 per cent.
Found . . . . = C 55.30; H 9.20 " "

Nothing could be separated from the undistilled residue, which weighed only 13 gm. The ether, which was distilled from the esters at 760 mm., was carefully examined for glycocoll, but none was found.

Tyrosine.

A quantity of ash and moisture free scallop muscle, weighing 25.87 gm., was hydrolyzed by boiling for twenty-four hours with a mixture of 49 c.c. of concentrated sulphuric acid and 181 c.c. of water. After removing the sulphuric acid quantitatively with baryta and washing the barium sulphate until free from tyrosine, the solution was concentrated on a water bath to crystallization. The substance which separated after twenty-four hours was dissolved in water, the solution decolorized by boiling with animal charcoal, and the tyrosine separated by concentration and cooling. By recrystallization 0.5058 gm. of pure tyrosine was obtained = 1.95 per cent.

Nitrogen, 0.1008 gm. subst., required 0.77 c.c. 5/7 N HCl.

Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.
Found . . . . = N 7.64 " "

The mother liquor and washings from the tyrosine were examined for bases according to the method of Kossel and Patten.

Histidine.

The solution of the histidine = 500 c.c.

Nitrogen, 50 c.c. sol., required 1.43 c.c. 5/7 N HCl = 0.1430 gm. N in 500 c.c. = 0.5277 gm. histidine = 2.04 per cent.

The histidine was converted into the dichloride for identification.

Chlorine, 0.1004 gm. subst., gave 0.1266 gm. AgCl = 0.0313 gm. Cl = 31.19 per cent.

Calculated for $C_6H_{11}O_2N_3Cl_2$ = Cl 31.14 per cent.
Found . . . . = Cl 31.19 " "

Arginine.

The solution of the arginine = 1000 c.c.

Nitrogen, 50 c.c. = sol., required 2.91 c.c. 5/7 N HCl = 0.5838 gm. N in 1000 c.c. = 1.8083 gm. arginine, + 0.1026 gm. = 1.9108 gm. = 7.38 per cent.

The arginine was converted into the copper-nitrate double-salt for identification.

Copper, 0.2195 gm. subst., gave 0.0300 gm. CuO.
Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2\ 3\ H_2O$ = Cu 10.79 per cent.
Found . . . . = Cu 10.93 " "

Lysine.

The lysine picrate weighed 3.8323 gm. = 1.4918 gm. lysine = 5.77 per cent.

Nitrogen, 0.2565 gm. subst., required 4.74 c.c. 5/7 N HCl.
Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$ = N 18.67 per cent.
Found . . . . = N 18.48 " "

Partition of Nitrogen.

The different forms of nitrogen yielded by hydrolyzing the scallop muscle by Hausmann's method, as modified by Osborne and Harris, were as follows:

Nitrogen as ammonia	1.08 per cent.
Basic nitrogen	4.52 " "
Non-basic nitrogen	11.05 " "
Nitrogen in magnesium oxide precipitate .	0.40 " "
Total nitrogen	17.05 per cent.

The nitrogen contained in the histidine, arginine, and lysine is 4.02 per cent, or 0.50 per cent less than the nitrogen precipitated by phosphotungstic acid, — a difference similar to that found for fish and chicken muscle, and probably due to basic substances of non-protein origin.

The results of this hydrolysis of scallop muscle, together with

those previously obtained with chicken and halibut muscle, are given in the following table:

	Scallop per cent.	Halibut per cent.	Chicken per cent.
Glycocoll	0.00	0.00	0.68
Alanine	...	...	2.28
Valine	...	0.79	...
Leucine	8.78	10.33	11.19
Proline	2.28	3.17	4.74
Phenylalanine	4.90	3.04	3.53
Aspartic acid	3.47	2.73	3.21
Glutaminic acid	14.88	10.13	16.48
Serine	...	...	...
Tyrosine	1.95	2.39	2.16
Arginine	7.38	6.34	6.50
Histidine	2.02	2.55	2.47
Lysine	5.77	7.45	7.24
Ammonia	1.08	1.33	1.67
Tryptophane	present	present	present
Total	52.51	50.25	62.15

Although the scallop contains relatively much free glycocoll, none was obtained by hydrolyzing the thoroughly washed muscle, notwithstanding the fact that every precaution was taken to isolate this amino-acid. The apparent lack of glycocoll from scallop and fish muscle and the relatively small quantity yielded by chicken muscle are in marked contrast to the amount of glycocoll yielded by ox muscle, as will be shown in a later paper. The fact that gelatine yields approximately 20 per cent of glycocoll would lead one to expect that the connective tissue of the muscle substance would yield enough glycocoll to make its presence noticeable in these analyses. As this has been found to be the case with ox muscle, it indicates that the connective tissues of these lower forms of life have a different structure from those of the ox.

It is probable that both alanine and valine were present among the products of hydrolysis of the scallop muscle, but it was impossible to separate either of them sufficiently pure for identification. The combined amount of these substances, however, was not large, for the mixture of amino-acids containing the alanine and valine weighed only 7.0 gm., equal to 2.4 per cent of the material hydrolyzed. The scallop muscle thus resembles that of halibut and chicken

in respect to the small proportion in which the lower amino-acids are yielded by hydrolysis.

The amount of glutaminic acid is nearly the same as that obtained from chicken muscle, but decidedly greater than that from the fish muscle.

The scallop muscle yields distinctly more arginine and less lysine than the other two muscles, and in this respect shows a distinct difference which is greater than any probable error involved in the analysis.

EXPERIMENTAL STUDIES ON THE PHYSIOLOGY OF THE MOLLUSCS — FOURTH PAPER.[1]

By LAFAYETTE B. MENDEL and H. GIDEON WELLS.

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

The Purines and Purine Metabolism of Sycotypus.

THE study of the nucleoproteins and their transformation under the influence of specific enzymes has been prosecuted with marked success during the past few years. Our ideas regarding the chemical constitution of the nucleic acids have become more definite, and experimental researches have contributed to a clearer understanding of the functions of the so-called "purine enzymes." The data gradually accumulated have, however, been derived almost without exception from the investigations upon the higher vertebrates.[2] The purine metabolism of lower forms has not yet received attention further than the occasional isolation of a purine compound as a constituent of invertebrate tissues.[3] Aside from the desirability of having some definite information regarding the purines in the lower animals, the recent controversy relative to the nature of the nucleic acids of the mammalian pancreas[4] emphasizes the importance of a broader study of these compounds. Again, the notable variations in the distribution of the purine-transforming enzymes in different organs and the same organs of different species lend additional interest to an extension of our knowledge. This is well illustrated by the experience gained in studies on mammalian em-

[1] The earlier papers will be found in This journal, 1905, xiii, p. 17; 1905, xiv, p. 313; 1906, xvii, p. 167.

[2] The literature is well reviewed by Bloch: Biochemisches Centralblatt, 1906, v, pp. 521, 561. *Cf.* also Vernon: Intracellular enzymes, London, 1908, Chapter II.

[3] *Cf.*, for example, Mendel and Bradley: This journal, 1906, xvii, p. 171; and unpublished studies from this laboratory.

[4] *Cf.* v. Fürth and Jerusalem: Hofmeister's Beiträge zur chemischen Physiologie, 1907, x, p. 174; 1908, xi, p. 146; Bang: *Ibid.*, xi, p. 76; Steudel: Zeitschrift für physiologische Chemie, 1907, liii, p. 539; Jones: Journal of biological chemistry, 1908, v, p. 1.

bryos in which the equipment in purine-transforming enzymes is appreciably different from what is found in the adult.[5]

The present experiments have been conducted with tissues of the gasteropod mollusc, Sycotypus canaliculatus, which has been employed in previous investigations in the laboratory.[6] Attention was first directed to the nature of the purines obtainable from the conspicuous glandular liver by hydrolysis, since this promised to throw light upon the character of the nucleic acids present.

The purines of the liver. — The liver was removed from about one-half bushel of the molluscs and separated as well as could readily be done from the other tissues of the animal, the chief admixture being the alimentary canal, which is intimately connected with the liver. In all, 750 gm. of fresh material were ground in a mortar and hydrolyzed by boiling fifteen hours with 5 per cent sulphuric acid. The undissolved residue left after filtering was again hydrolyzed five hours with fresh acid; the filtrates and washings were united, made slightly alkaline with sodium hydroxide, then slightly acid with acetic acid. A small precipitate that formed on neutralization was removed, and after concentrating the filtrate to 3 litres the purines were precipitated by the copper sulphate-sodium bisulphite method, and purified by reprecipitation.

After removing the copper, uric acid and the purines were sought and isolated in the usual way.[7] A very small amount of *uric acid* was obtained, 0.042 gm., giving the murexide test and crystallizing characteristically.

Guanine was obtained to the amount of 0.255 gm. (crude). Analyzed in the form of the hydrochloride, it yielded 15.4 per cent of H_2O (calculated for $C_5H_5N_5O \cdot HCl \cdot 2\ H_2O = 16.1$ per cent); nitrogen = 37.8 per cent (calculated for $C_5H_5N_5O_4 \cdot HCl = 37.3$ per cent).

Xanthine was isolated in small quantity, amounting to 0.128 gm. This product showed the solubilities of xanthine, and gave the Weidel reaction typically, but the total amount remaining was too small for analysis.

[5] *Cf.* Jones and Austrian: Journal of biological chemistry, 1907, iii, p. 227; Mendel and Mitchell: This journal, 1907, xx, p. 97.

[6] *Cf.* Mendel and Bradley: This journal, 1905, xiii, p. 17; 1905, xiv, p. 313; 1906, xvii, p. 167. The first of these papers contains an illustrated description of the anatomy of the animal.

[7] By the Krüger-Salomon method.

Adenine was isolated as the picrate (M. p. 276°–278° C., uncorr.). The total amount of adenine picrate obtained was 0.682 gm., which corresponds to 0.256 gm. of free adenine.

Hypoxanthine was isolated as the silver nitrate compound which weighed 0.378 gm., corresponding to 0.141 gm. free base. This salt was decomposed with hydrogen sulphide, and hypoxanthine nitrate obtained, crystallizing typically and containing 7.9 per cent of water of crystallization (calculated for $C_5H_4N_4O \cdot HNO_3 \cdot H_2O$ = 8.2 per cent). Nitrogen determination of the water-free nitrate gave 34.6 per cent (calculated for $C_5H_4N_4O \cdot HNO_3$ = 35.2 per cent).

The purines of the nidamental glands. — For purposes of comparison, the large whitish organ which lies immediately beneath the liver, and the function of which is the formation of the egg-case was also examined for purines in the same way. Five hundred and fifty grams of this tissue were obtained from the same lot of Sycotypus, but the yield of purines was very small. No uric acid was found, and the amount of guanine and xanthine obtained was too small to permit of analysis, although a few milligrams of each were separated and identified by their solubilities and the behavior with the Weidel and the nitric acid tests. A larger yield of *adenine* was obtained as the picrate, weighing 0.212 gm. (M. p. 274° uncorr.), corresponding to 0.079 gm. of *adenine*. There was also a very small amount of *hypoxanthine:* 0.044 gm. of the hypoxanthine silver nitrate, corresponding to 0.016 gm. of hypoxanthine.

It is evident from these experiments that the purines obtainable from glandular invertebrate tissue like the liver correspond with those derived from related tissues of higher forms. Although all four of the familiar purine bases were isolated and identified, the preponderance of guanine and adenine makes it probable that the molluscan nucleic acid itself corresponds with that of the thymus, liver, etc., of mammals, in containing these aminopurines alone. Since it is known that oxypurines readily arise by enzymatic reactions from the aminopurines of the nucleoprotein, it is generally assumed that the smaller quantities of hypoxanthine and xanthine frequently found on hydrolysis of glandular tissues have arisen through such changes.[8] Furthermore, the relative quantities of the purines obtained from the liver are not widely different from those

[8] *Cf.* Oppenheimer's Handbuch der Biochemie, 1908, i, p. 610.

recorded in previous tissue investigations. The significance of the uric acid found will be pointed out later.

The purine enzymes. — When mammalian glandular tissues are subjected to autolysis, the combined aminopurines are, as is well known, liberated through the agency of *nucleases* and are further changed into oxypurines by deamidizing enzymes, *adenase* and *guanase* (or a single amidase, according to Schittenhelm). In the presence of sufficient oxygen *oxidases* may further successively convert hypoxanthine into xanthine and the latter into uric acid. Finally, by the action of the *uricolytic enzyme,* uric acid is destroyed, with the probable formation of allantoïn. The specificity of the various enzymes mentioned is generally admitted at present. The following experiments with Sycotypus liver indicate the presence of nuclease and amidases in the molluscan tissue; no evidence of the occurrence of xantho-oxidase or uricolytic enzyme was obtained.

Autolysis of liver — nucleases and amidases. — Twenty-two hundred and sixty grams of fresh liver, with the contiguous portion of the alimentary canal, were ground fine, and subjected to autolysis in a closed vessel during twenty-six days at a temperature varying most of the time from 25° to 35° C., in 8 litres of water to which had been added considerable toluene and a little chloroform and thymol. During this period a large proportion of the insoluble sedimented material went into solution, and the fluid yielded a strong biuret reaction. It remained persistently amphoteric to litmus throughout the period of autolysis. After removing the sediment by coagulation and filtration, the purines of the fluid were precipitated with copper-sulphate-sodium bisulphite in the usual manner. The insoluble residue was hydrolyzed with 5 per cent sulphuric acid as in the first experiment, and it was noticeable that thorough hydrolysis did not greatly reduce the amount of insoluble residue beyond what was left after autolysis.

From the soluble products of autolysis there was isolated a relatively large amount of *xanthine,* which weighed 1.524 gm. after being once recrystallized and re-extracted with water at 35°. This was identified by its solubilities, by the Weidel test, by the typical crystals of the nitrate. Analysis of the xanthine dried at 125° gave 36.42 per cent of N (calculated 36.8 per cent).

Only a minute yield was obtained in the guanine fraction, amounting at most to a few milligrams. Presumably this material was *guanine,* on account of its insolubility in ammonia, and its failure

to give the Weidel test although reacting when dried with nitric acid followed by an alkali. The amount was, however, too small to be further identified.

Adenine seemed to be entirely absent, no precipitate being formed with picric acid.

Hypoxanthine, however, was abundant, there being obtained 0.709 gm. of typical hypoxanthine nitrate crystals. This corresponds to 0.444 gm. hypoxanthine. It was further identified by its typical silver salt and by analysis; the water of crystallization was found to be 8.27 per cent (calculated for $C_5H_4N_4O \cdot HNO_3 \cdot H_2O$, 8.29 per cent).

The total amount of purines obtained by hydrolysis of the *residue left after autolysis* was very small, and consisted almost entirely of *xanthine* and *hypoxanthine*. The xanthine fraction, after extraction with water and recrystallization, weighed 0.162 gm., while 0.219 gm. of hypoxanthine nitrate was obtained, corresponding to 0.132 gm. of free hypoxanthine. Only a very minute guanine fraction was obtained, — not enough to identify further than by its insolubility in ammonia, and a negative Weidel test with positive reaction to nitric acid followed by alkali. A very small precipitate of adenine picrate was obtained, weighing 0.016 gm. — too small for further purification and identification.

This experiment indicates the presence in Sycotypus tissues of an active *nuclease,* since in twenty-six days' autolysis the guanine and adenine were split out of the tissues almost quantitatively. Presumably the relatively considerable amount of xanthine and hypoxanthine found in the residue after autolysis represents merely that which was not washed out of the residue before the second hydrolysis; for the extremely bulky and fat-rich residue left after autodigestion of Sycotypus liver was very difficult to wash thoroughly.

The appearance of large quantities of xanthine and hypoxanthine coincident with the disappearance of the guanine and adenine from the tissues affords evidence that Sycotypus, like the higher organisms, possesses an *amidase,* or both *adenase* and *guanase.*

Xantho-oxidase and uricolytic enzyme. — In an earlier paper [9] the absence or tardy appearance of these oxidative and katabolic enzymes in embryonic mammalian tissues was associated with the character-

[9] Mendel and Mitchell: This journal, 1907, xx, p. 112.

istic synthetic functions of growing organisms. Most mammalian tissues contain a uric-acid-destroying enzyme, but the uricolytic and uricogenic enzymes are apparently independent of one another and do not necessarily run parallel in various tissues. The readiness with which a deamidization and an oxidation of purine precursors to uric acid occur by the enzymatic agency of some tissue extracts has lately been demonstrated by Schittenhelm.[10]

The following preliminary experiment was performed to determine the presence or absence of uricolytic enzymes in the liver: I. One hundred and thirty grams of fresh Sycotypus liver (adherent to which was also part of the alimentary canal) were ground fine, and extracted over night at room temperature with 400 c.c. water, toluene being added. The emulsion was strained through cheesecloth, and to it was added 0.3085 gm. of uric acid which had been dissolved in 100 c.c. boiling water with the aid of a weak sodium hydroxide solution added drop by drop. This mixture was then kept for sixty-four hours in a bath at 35°, in the presence of toluene, with a stream of air running through it. The purines were precipitated from this solution in the usual way, and the uric acid isolated and purified by reprecipitation from solution in concentrated sulphuric acid. The uric acid recovered weighed 0.296 gm., or 96 per cent of that which had been added, without allowance for any present in the extract itself.[11]

This experiment having indicated the probable absence of uricolytic enzymes, it was corroborated and controlled by the following set of experiments: II. Four hundred and fifty grams Sycotypus liver were ground fine, extracted as before with 1200 c.c. water, and the extract divided into two equal parts. To one (A) was added 0.196 gm. of uric acid dissolved as in the previous experiment, while the other portion (B) was left without uric acid, to ascertain whether the latter was formed from precursors in the extract under the conditions of the experiment. Another control (C) consisted of an equal quantity of extract of fresh *pig* liver, prepared in the same way, to which was added 0.2375 gm. of uric acid. These three mixtures were then kept well saturated with toluene in a water bath at 35° for forty-six hours, and air was bubbled through them con-

[10] Schittenhelm: Zeitschrift für physiologische Chemie, 1908, lvii, p. 21.

[11] An analytical study by Ritter: Methodisches zur Harnsäurebestimmung in Organauszügen. Inaugural Dissertation (Schittenhelm), Göttingen, 1905, indicates losses of 9 per cent or more in recovering added uric acid by the method here used.

stantly. Uric acid was determined as before, with the following result:

Extract from	Sycotypus. (A)	Sycotypus. (B)	Pig. (C)
Uric acid added	0.196 gm.	none	237 gm.
Uric acid recovered	0.77 gm. or 90%[12]	none	none

These experiments seem to show conclusively the absence of uricolytic enzyme in the liver of Sycotypus, and also indicate that uric acid is not formed from the purines of the Sycotypus by oxidases acting upon purines liberated by autolysis from the tissues themselves, under conditions in which uricolytic activity is easily demonstrated in mammalian tissue extracts.

The quantitative observations here recorded give further evidence that the uricolysis observed in properly conducted experiments with tissue extracts cannot be ascribed to the destructive action of the alkali solvent used, as has been claimed by some investigators[13] — otherwise a greater loss would be expected in these trials.

The extracts of the livers used in Experiments I and II (A) and (B), which had been allowed to digest (with or without the addition of uric acid) at 35° from forty-six to sixty-four hours with an abundance of air, were united and examined for purines after removal of the uric acid. The solutions were reprecipitated with ammoniacal solution of silver chloride, and after removal of the silver with hydrogen sulphide, purines were separated in the usual manner. A considerable quantity of *xanthine* was obtained, weighing 0.131 gm., and typical as regards solubilities and reactions. *No guanine* fraction (purine insoluble in ammonia) was obtained, and *no adenine* could be precipitated with picric acid. Typical hypoxanthine nitrate (0.128 gm.) was obtained in crystalline form (corresponding to 0.08 gm. *hypoxanthine*).

From this set of experiments, in which autolysis occurred in the presence of an abundant supply of air with quite the same results as when self-digestion occurred without air, it may be concluded that *xantho-oxidase* is *not present*. The presence of active nuclease and amidases is also again demonstrated.

[12] A slight loss probably occurred at one step in the analysis.

[13] AUSTIN: Journal of medical research, 1906, xv, p. 309; 1907, xvi, p. 71. See the criticism by MITCHELL: Journal of biological chemistry, 1907, iii, p. 145.

Summary.

The purines obtained by acid hydrolysis of a typical glandular invertebrate tissue — the liver of the gasteropod, Sycotypus canaliculatus — are the same as those derived from the comparable tissues of the higher animals, namely, *adenine, guanine, hypoxanthine*, and *xanthine*. It is probable, in view of the observed preponderance of the aminopurines, that the same purines are present in the nucleoprotein complexes of the mollusc as in the vertebrates.

The experiments indicate the existence of *nuclease* and *amidases* (both adenase and guanase) in the liver. Xantho-oxidase and uricolytic enzyme could not be demonstrated, but uric acid can be found in the tissues. The significance of these facts for the metabolism of the animal remains to be determined.

CONCERNING THE SUPPOSED CONNECTION BETWEEN PROTEIN COAGULATION AND THE HEAT SHORTENING OF ANIMAL TISSUES.

By EDWARD B. MEIGS.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

THE view that the shortenings to be observed in striated muscle when the tissue is gradually heated are caused by the coagulation of the various muscle proteins has been widely held. Halliburton and Brodie, who are the chief supporters of this hypothesis, have attempted to give it a general form. In a recent article [1] they show that if nerve or liver tissue be gradually heated shortenings occur at about the temperatures at which the proteins in the extracts of the tissues are precipitated.

It would be incorrect, however, to say that protein coagulation in animal tissues is always accompanied by a tendency for those tissues to shorten, or that shortening is always the result of protein coagulation. Smooth muscle, for instance, constitutes a glaring exception to such a rule. It has been shown [2] that if this tissue be heated to 50° a large proportion of its protein is coagulated at the same time that it shows a marked tendency to lengthen.

White fibrous connective tissue and elastic tissue furnish exceptions of another kind. The white fibrous tissue of mammals shows an extensive heat shortening at about 62°.[3] But Buerger and Gies [4] find only 0.22 per cent of protein coagulable by heat in this tissue, and add that they believe that a large part of this came from the contained lymph. This protein consists of a globulin which is precipitated at from 54° to 57° and an albumin precipitated at 73°. White fibrous tissue, therefore, which shortens under the influence of heat nearly as markedly as muscle, contains not more than one

[1] BRODIE and HALLIBURTON: Journal of physiology, 1904, xxxi, p. 473.

[2] MEIGS: This journal, 1909, xxiv, p. 1.

[3] BRODIE and RICHARDSON: Philosophical transactions of the Royal Society of London, 1899, cxci, p. 136.

[4] BUERGER and GIES: This journal, 1901–1902, vi, p. 228.

one-hundredth part of the coagulable protein contained in muscle, and shortens besides at a temperature at which its proteins are not precipitated.

Elastic tissue shortens on heating and lengthens on cooling for all temperatures up to 65°.[5] It is, of course, self-evident that such behavior cannot be explained by supposing it to be due to protein coagulation.

The method adopted by Halliburton and Brodie to show the causal connection between shortening and protein coagulation has been that of coincidence. The attempt has been made to show that tissues, when gradually heated, shorten at the same temperatures at which the proteins in their extracts are precipitated. The most detailed attempt of this sort is that of Brodie and Richardson [6] with striated muscle.

It is evident that the value of the method of argument used by Brodie and Richardson depends on the exactness of the coincidences demonstrated. These authors [7] make the following statement: "If we examine the tracing (Fig. 2) which we have given as a typical result of our experiments, bearing in mind the different proteids present in frog's muscle and the temperatures at which they coagulate, we immediately note an exact correspondence between the two results. At each of the three temperatures where the proteids soluble myogen-fibrin, myosin, and myogen coagulate, we find a corresponding contraction of the sartorius. And, moreover, this contraction corresponds in all its minutiæ with the range of temperature for each proteid coagulation. At a temperature at which the coagulation commences and v. Fürth obtained a clouding of his solutions, we find a contraction occurring, small in amount and slow in progress, and as the temperature rises to that at which proteid coagulation is rapid, so too our tracings show a rapid contraction, which gradually falls off as all the proteid becomes coagulated."

It seems to me that the experimental data given do not justify so strong a statement, and I propose to review those data as briefly as possible.

For the temperatures at which the various proteins in the extracts of frog's muscle are precipitated, Brodie and Richardson give the

[5] Gotschlich: Archiv für die gesammte Physiologie, 1893, liv, p. 117.

[6] Brodie and Richardson: *Loc. cit.*

[7] Brodie and Richardson: *Loc. cit.*, p. 136.

following figures,[8] which are taken from v. Fürth (I shall use v. Fürth's nomenclature for the proteins throughout):

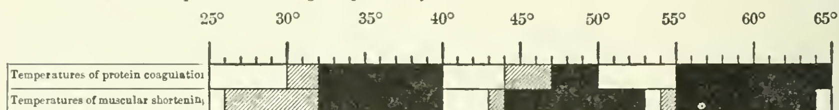
1. Soluble myogen-fibrin. Its solutions begin to cloud when a temperature of 30° is reached and give a coagulum between 32° and 40°.

2. Myosin. Its solutions become opalescent between 44° and 47° and show a precipitate between 47° and 50°.

3. Myogen. Coagulates between 55° and 65°.

TABLE I.

Diagram compiled from the data of Brodie and Richardson to show the relation between the temperatures of protein coagulation and those at which shortening occurs in a frog's sartorius. In the upper space the areas covered by diagonal lines represent the temperatures at which the protein extracts show opalescence; the blackened spaces periods of precipitation. In the lower space the same conventions represent periods of slow and rapid shortening respectively.



A short calculation from the figures given above shows that in the range of thirty-five degrees between 30° and 65°, twenty-six intervals are covered by opalescence or coagulation, and only nine intervals are left free from any kind of precipitation whatever. Every one who has worked with heat rigor curves knows that they vary widely even under similar experimental conditions. The steps in the curve vary in the temperatures at which they appear, and all except the first are often absent altogether.[9] It would not therefore prove anything in particular if from a number of such curves one could be selected in which the shortenings fell somewhere within the generous limits given by v. Fürth. The results, to have any value, should be compounded from a number of curves. Brodie and Richardson, however, simply present a certain curve which they consider "typical"; they nowhere indicate how they came to the conclusion that the curve selected was typical.

And even in the curve given the correspondence is by no means accurate. Table I was compiled from the curve in question and the coagulation figures given above.

An examination of the table shows that even in the "typical"

[8] Brodie and Richardson: *Loc. cit.*, p. 134.

[9] See Brodie and Richardson: *Loc. cit.*, Figs. 5 and 6.

curve all the contractions begin sooner than they should, and the periods of rapid contraction do not exactly correspond with the temperatures of protein coagulation. In the other curves given by Brodie and Richardson the correspondence is much more vague. Particular attention should be directed to their Fig. 4, which represents the heat shortening of a mouse's gastrocnemius. The temperature at which the first precipitation is obtained from extracts of mammalian muscle is usually given as about 47°, but this muscle had already begun to shorten at 34°, and at 44° the shortening had reached its highest rapidity. It is important to examine the curves given by Brodie and Richardson as well as the figures supposed to represent the temperatures at which the different shortenings begin.

The experimental results which have just been discussed would hardly be sufficient to prove the connection between the heat shortening of muscle and protein coagulation, even if they were in perfect agreement with those of other investigators. No such agreement, however, exists.

Vernon,[10] for instance, has studied the heat rigor of frog's voluntary muscle, and has obtained curves quite different from those of Brodie and Richardson. He suggests that some of the peculiarities of their curves may be due to their having employed cotton thread to attach their muscle to their apparatus, and he shows that this substance undergoes considerable changes in length under the influence of heat.[11] In his experiments he used fine copper wire for his attachments; I have followed this plan in my own experiments and have obtained results much more like his than like those of Brodie and Richardson. Table II is compiled from the results of two curves of Brodie and Richardson, one of Vernon's, and one of mine; it gives an idea of the extremely inconstant character of the heat rigor curve of frog's muscle.

In most of Vernon's curves and in most of mine only two marked steps make their appearance. The muscle may begin to shorten gradually at any temperature between 20° and 40°, and this gradual shortening may become rapid at any point between 30° and a little over 40°. A second shortening often makes its appearance between 50° and 60°; the temperature at which this shortening begins is somewhat more constant than in the case of the initial shortening. In summing up his results [12] Vernon speaks of the comparative con-

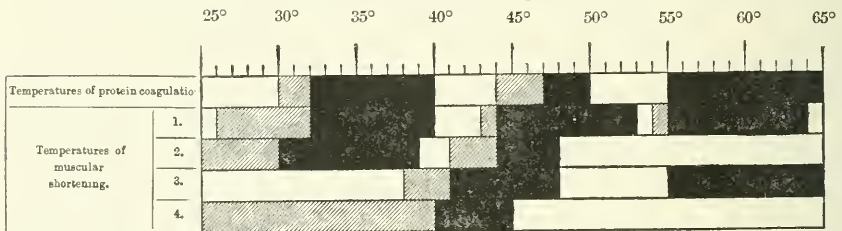
[10] VERNON: Journal of physiology, 1899, xxiv, p. 239.

[11] VERNON: *Loc. cit.*, p. 241. [12] VERNON: *Loc. cit.*, pp. 272 *sqq.*

stancy in the temperature at which this shortening begins and the inconstancy of the temperature of onset of the initial shortening. He adds that he believes the second shortening to be due to the coagulation of the muscle protein, while he thinks that the initial heat shortening has no connection whatever with protein coagulation.[13] I shall consider first the later heat shortening and afterwards the initial one.

TABLE II.

Diagram to show the relation between protein coagulation and the heat shortening of muscle. The same conventions are used as in Table I, and the first two spaces are a repetition of Table I. The space numbered 2 shows the results of another experiment of Brodie and Richardson (see Fig. 6 in their article). Space No. 3 represents one of Vernon's curves from the frog's gastrocnemius, and space No. 4 represents the results in one of my own curves made with the frog's sartorius.



It is easy to show that the shortening which occurs in striated muscle at about 55° has no connection with the coagulation of myogen, which is precipitated from the muscle extracts at the same temperature. Myogen is in time completely coagulated by alcohol, as the following experiment shows. An extract was made with 0.7 per cent NaCl solution from the muscles of a frog's leg. This was heated to 47° and the protein precipitated at that temperature was filtered off. To the filtrate was added about three times its volume of 96 per cent alcohol. In a few minutes a plentiful precipitate began to make its appearance. The mixture was allowed to stand for forty-four hours, and then most of the alcohol was evaporated off at 30°. The remaining portion of the mixture was filtered, and the filtrate gradually heated to 100°. There was no precipitate; at 70° a barely perceptible opalescence made its appearance. In forty-four hours, therefore, all the myogen and very nearly all the protein contained in the extract were precipitated by the alcohol.

If the shortening which occurs in muscle at about 55° is really the

[13] VERNON: *Loc. cit.*, pp. 275, 276.

result of protein coagulation, then coagulation of the protein by alcohol should result in shortening. But if muscles of which the irritability has been destroyed by heating to 47° be arranged to write their changes in length on a kymograph and be immersed in 96 per cent alcohol, there is no shortening even in the course of twenty-four hours. The muscle usually lengthens slightly for the first two or three hours and then maintains the same length.

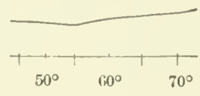


FIGURE 1. — Curve showing the heat shortening beginning at 55° in a frog's sartorius which had first been heated to 47°. The heating was carried on at such a rate that it required four minutes for the temperature to rise from 45° to 75°.

In a small muscle like the frog's sartorius most of the myogen must be coagulated after twenty-four hours' immersion in 96 per cent alcohol. But a muscle in which a large proportion of the myogen has been so coagulated is more capable of shortening at 55° than is a fresh muscle. Figs. 1 and 2 represent the heat curves of the sartorii from opposite legs of the same frog. The two muscles were heated in the same vessel to 47°. One was then arranged to

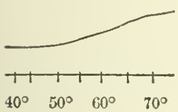


FIGURE 2. — Curve obtained from the sartorius fellow to that of which the heat shortening is recorded in Fig. 1. This muscle, after being heated along with its fellow to 47°, was immersed for twenty-four hours in 96 per cent alcohol. It was then transferred for five hours to 0.7 per cent NaCl solution, and heated in the salt solution at such a rate that it required four minutes for the temperature to rise from 40° to 75°. This curve and that of Fig. 1 were made under the same experimental conditions. In both cases the magnification of the writing lever was 5; the muscle was weighted with 0.6 gm.; and the length of muscle between attachments at the beginning of the curve was about 8 mm.

write its changes in length on a kymograph, and further heated in 0.7 per cent NaCl solution to 75°. Fig. 1 represents the curve so obtained. The other muscle was also arranged to write its changes in length on a kymograph, care being taken that the length of muscle between attachments was the same as in the first case and was immersed for twenty-four hours in 96 per cent alcohol; during this period it lengthened slightly. It was then immersed for five hours in 0.7 per cent NaCl solution, which produced no change in its length.

The muscle was treated with salt solution, because the heat shortening will not take place in tissue saturated with 96 per cent alcohol

and heated in that fluid. If 70 per cent alcohol be substituted, a shortening takes place at about 70°, but is slower and not so high as in fresh muscle. But if the alcohol be entirely substituted by water, the shortening occurs just as it does in untreated muscle.

After having remained for five hours in the salt solution, the piece of muscle was gradually heated in that fluid and gave the heat curve shown in Fig. 2. It will be observed that its heat shortening is considerably larger than that shown in Fig. 1. This is usually the case when two such muscles are compared with each other, though the difference is often less marked than in the curves given.

The following experiment shows that a muscle will still exhibit a marked heat shortening at 55° after all its proteins have been coagulated by alcohol. The muscles of a frog's leg were kept for eleven days in 96 per cent alcohol. At the end of that time they were immersed for about twenty hours in 0.7 per cent NaCl solution.

From the muscle which had been treated as described with alcohol and 0.7 per cent NaCl solution a small piece 8 mm. long was arranged to write its changes in length on a kymograph, and gradually heated in the salt solution to 80°. The curve so obtained is reproduced in Fig. 3. The other leg muscles were extracted with 0.7 per cent NaCl solution, and the extract was filtered. The filtrate, which was very slightly opalescent, was heated gradually to boiling; it showed not the slightest sign of precipitate or increase in opalescence at any temperature.

The initial shortening which occurs in striated muscle under the influence of heat is almost certainly of an entirely different nature from the shortening which occurs at about 55°. It is quite characteristic of striated muscle; no other tissue shortens 30 per cent or 40 per cent of its original length at a temperature below 40°. It has the same height as the greatest shortening which can be produced in living muscle by the tetanizing current.[14] It is not accompanied by the tendency to lose water, which has been shown to be characteristic of the shortenings which occur at about 55° in smooth and striated muscle and in catgut.[15] Finally, it is accompanied by,[16] and probably the result of,[17] the formation of a large quantity of lactic acid within the muscle. It is not surprising,

[14] Vernon: *Loc. cit.*, p. 276.

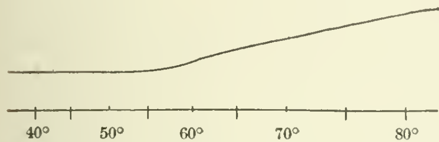
[15] Meigs: *Loc. cit.*, p. 10.

[16] Fletcher and Hopkins: Journal of physiology, 1907, xxxv, p. 247.

[17] Meigs: *Loc. cit.*, pp. 12 and 13.

therefore, that muscles which have been killed by the long-continued action of strong alcohol do not shorten when heated to 40°.

It may be shown, however, that the proteins of a fresh muscle can be coagulated gradually within the muscle without causing any shortening comparable to the initial heat shortening. An irritable



40° 50° 60° 70° 80°

FIGURE 3. — Curve showing the heat shortening of a piece of muscle, which had been kept for eleven days in 96 per cent alcohol, and then for twenty hours in 0.7 per cent NaCl solution. The heating was carried out at such a rate that it required eight minutes for the temperature to rise from 40° to 80°. Magnification of writing lever, 5; weight, 0.6 gm.; length of muscle between attachments at beginning of experiment, 8 mm.; proportional shortening about 25 per cent. The shortening is somewhat greater in this case than in Figs. 1 and 2, because this muscle had not been heated to 47° before immersion in the alcohol.

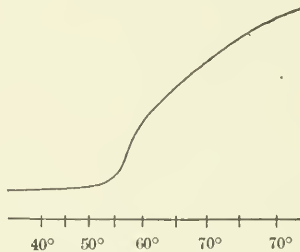


FIGURE 4. — Curve showing the heat shortening of a piece of muscle which had been treated first with weak and then with strong alcohol. The heating was carried out at such a rate that it required six minutes for the temperature to rise from 40° to 80°. Magnification of writing lever, 5; weight, 0.6 gm.; length of muscle between attachments at beginning of experiment, 14 mm.; proportional shortening, about 36 per cent.

frog's sartorius was weighted with 0.6 gm. and arranged to write its changes in length on a kymograph. It was then immersed for two hours in a mixture containing 9 parts of 0.7 per cent NaCl solution and 1 part 96 per cent alcohol. During this period it underwent no shortening. The mixture of alcohol and salt solution was then replaced by pure 96 per cent alcohol, in which the muscle remained for forty-four hours. During this period it underwent an insignificant shortening of about 4 per cent; even this could probably have been avoided by a longer treatment with the mixture of alcohol and salt solution. After the treatment with alcohol, the muscle was placed for five hours in 0.7 per cent NaCl solution, and then gradually heated in that fluid; it gave the curve shown in Fig. 4. This result shows that the proteins of muscle may, with proper precautions, be coagulated without causing any considerable

shortening, and that muscles of which the proteins have been coagulated under these conditions show the most marked shortening when heated to 55° and above.

There is, then, no reason to believe that there is any connection between protein coagulation and the heat shortening which occurs at temperatures above 50° in striated muscle, smooth muscle, and connective tissue; and these are the tissues which exhibit the phenomenon most markedly. The experiments of Brodie and Halliburton [18] with nerve and liver are hardly sufficient to demonstrate that a connection between the two phenomena exists in those tissues. These experiments are open to many of the objections which have been urged against the conclusions of Brodie and Richardson, and to some others besides. The authors, in determining the temperatures at which the various shortenings occur in nerve, select "typical" examples instead of taking the average from a number of experiments. The curves given represent the heat shortening of the sciatic nerve, while the temperatures at which the proteins of "nervous tissues" coagulate were determined in extracts of the brain. Finally, too little attention is paid to the fact that both "nerve" and "liver," as prepared by the authors, are complex structures, containing certainly connective tissue, and possibly elastic tissue also, and that the steps which they obtain in the heat shortening may be the result of simple shortenings occurring in these various tissues at different temperatures.

The facts which have been reported in this article do not, of course, preclude the possibility that the precipitation of protein from its solutions and the shrinkage of animal tissues under the influence of heat may be fundamentally more or less similar processes. They do show, however, that the shortening of striated muscle at temperatures above 50° is independent of the coagulation of myogen, and they make it seem probable that the heat shortening of most animal tissues is dependent, not on the aggregation of the particles of coagulable protein, but on some other process.

[18] Brodie and Halliburton: Journal of physiology, 1904, xxxi, p. 473.

MERCURIAL POISONING OF MEN IN A RESPIRATION CHAMBER.[1]

By THORNE M. CARPENTER and FRANCIS G. BENEDICT.

[*Nutrition Laboratory, Carnegie Institution of Washington, Boston, Massachusetts.*]

IN connection with an extended series of investigations on the respiratory exchange and heat production of man a respiration calorimeter embodying a respiration apparatus of the type Regnault and Reiset and an adiabatic constant flow calorimeter has been used for a number of years at Wesleyan University, Middletown, Connecticut. Formerly this apparatus was on the open circuit plan of Pettenkofer, although in 1902 it was modified so as to include the direct determination of oxygen.[2]

The apparatus was in constant use from March 24, 1903, to March 29, 1905, there being a total of 75 experiments with 115 days of experimenting. Thirty-one different subjects were used in these experiments in the apparatus as thus used. The air leaving the respiration chamber was forced by means of a rotary blower through a valve system which allowed it to pass through one of two sets of purifiers. The purifying system consisted, first, of a porcelain vessel filled with sulphuric acid for the removal of water; second, a silver plated can containing soda lime for the removal of carbon dioxide; and third, another sulphuric acid vessel to remove the water taken up by drawing the air as it passed through the soda lime. The air leaving the purifiers was considered anhydrous and free from carbon dioxide. It was then caused to return to the chamber, oxygen being admitted as the oxygen was consumed. In adapting the apparatus to the Regnault and Reiset plan it was absolutely necessary to secure perfect closure of all connections. Since the purifying apparatus for removing water and carbon dioxide

[1] The experiments here reported were made in the Chemical Laboratory of Wesleyan University, Middletown, Connecticut.

[2] W. O. ATWATER and F. G BENEDICT: A respiration calorimeter with appliances for the direct determination of oxygen: Publication No. 42, Carnegie Institution of Washington

from the ventilating air must be changed frequently during long experimental periods, a valve system was necessary. Recourse was had to a valve of special construction employing a mechanical seal bathed with mercury to insure perfect closure. The valves were placed at both ends of the purifying system. Since in its passage through the purifying system the air must be forced through a considerable layer of sulphuric acid, the pressure of the air as it entered the first purifying vessel was not far from 40 mm. of mercury. The purifying vessels were changed every two hours, consequently one side of the valve was under a pressure of 40 mm. of mercury, while the other side was under atmospheric pressure during the time that the absorbing system was disengaged for weighing. Under these conditions we found it exceedingly difficult to secure valves with a large enough aperture which could be absolutely tight. The type of valve finally used is described in considerable detail and shown in Fig. 10 on page 21 of Publication No. 42 of the Carnegie Institution of Washington. The closure is made by forcing a steel disk on the end of a threaded spindle against the end of the steel tube about 30 mm. in diameter. To make the closure all the more mechanically perfect a hard rubber or fibre gasket was placed in the steel disk, and as a final precaution a reservoir of mercury connected through a rubber tube with the valve chamber was raised in such a manner that mercury could flow about the mechanical closure and thus produce a mercury seal. Under these conditions the closure was perfectly satisfactory. When it was desired to open the valve, the mercury reservoir was first lowered, during which process mercury drained away from the valve chamber, and then the valve was opened by turning the spindle. In thus raising or lowering the mercury it was impossible to prevent small globules of mercury from adhering to the inside of the iron valve chamber, although all but a very small proportion of the mercury flowed into the lowered reservoir.

This valve was originally designed to meet extreme conditions of pressure at the entrance end of the absorber system, but inasmuch as it had proven absolutely tight to this high pressure it was found expedient also to use them at the exit end, where the pressure to be guarded against was very much less and amounted to only a few millimetres of mercury, *i. e.*, a pressure necessary to overcome the resistance of the air current in its passage through 9.2 metres of iron pipe 50 mm. internal diameter on its return to the respiration

chamber. It is particularly with the set of valves at the exit end of the purifying system that we have to deal.

General plan of experiments. — In order to bring the calorimeter into temperature equilibrium, the subjects usually enter the chamber some few hours before the experiment proper begins. In many instances the experiment had been previously planned to study the effects of inanition on metabolism. In nearly all cases, however, the subjects had had food but a few hours prior to being in the experiment. In those experiments in which the subject slept in the chamber over night he usually entered the apparatus about 9 P. M. and retired at 11 P. M., sleeping on the cot in the chamber. He was awakened at 7 A. M. In certain other experiments the object was simply to study the normal respiratory exchange of persons sitting quietly at rest, and the subject had no preliminary preparation other than one hour inside the chamber before the experiment proper began. Finally, some of the experiments were made with the special purpose of studying the question at hand, namely, toxic poisoning resulting from a sojourn in the chamber. The special conditions under which these experiments were made are discussed in more detail in connection with each experiment. The subjects were in most cases young men, students in the University, although two were men of more mature years.

Indices of physical conditions when subjects were inside the respiration chamber. — The complete hermetical closure of this respiration apparatus makes it practically impossible to come in physical contact with the subject. Our observations of his welfare were confined to the use of the telephone and inspection through a glass window. Certain factors, however, could be determined with as great accuracy as they could be were the subject outside of the chamber. Thus it was possible to determine with great accuracy the pulse rate, respiration rate, and body temperature. The pulse rate and respiration rate were determined by using a pneumograph.

The body temperature was at times taken in the usual way with a carefully calibrated mercurial clinical thermometer under the tongue. In general temperature observations were made with a much higher degree of accuracy by means of an electrical resistance thermometer [3] which was inserted deep in the rectum. The

[3] F. G. Benedict and J. F. Snell: Archiv für die gesammte Physiologie, 1901, lxxxviii, pp. 492–500.

variations in resistance were measured by an observer outside the calorimeter and they were recorded every few minutes. They are accurate to 0°.01 C. Since this thermometer could be worn without discomfort during sleep, the records are continuous.

Personal impressions. — In any series of experiments such as these here reported, subjects are continually influenced by personal impressions which are for the most part without scientific foundation. Thus it is a common matter for the men to state that there are marked changes in the temperature inside of the chamber and that the ventilation is better at different times, while, as a matter of fact, the temperature remains constant hour after hour and the rate of ventilation remains likewise unchanged. However, with the symptoms appearing in some of these cases, there is no question but that the men were absolutely wretched and their personal impressions were borne out by the data secured from the pulse rate, respiration rate, and body temperature.

After the subjects left the calorimeter, they were under the constant supervision of a skilled physician, and the personal impressions were again substantiated by his careful observations.

Collaborators. — The striking and characteristic symptoms appearing in these experiments attracted the attention of many scientific men in the neighborhood of Middletown. We were fortunate to have in consultation with us a number of experts whose attention to this problem we wish especially to emphasize. Dr. A. R. Diefendorf, formerly pathologist of the Connecticut Hospital for the Insane, and at present lecturer in psychiatry at Yale University, devoted considerable time to the problem, and Dr. John E. Loveland was in constant attendance on these subjects.

Appearance of toxic effects. — While, as is usual with any number of experiments with men of varying temperaments, there had been in the previous experiments occasional complaints that the subjects had slight headaches or were not thoroughly comfortable inside of the chamber, no particular attention was paid to them until one experiment in May, 1905, when a subject, who was of unusually stolid temperament, was taken violently ill with nausea and fainting several hours after the experiment. From this time on, the complaints of discomfort were very frequently observed, and actual disturbances of temperature regulation were soon noticed in a large number of experiments. These experiments, all of which were made in the spring and fall of 1905, are given below.

Experiments with Toxic Symptoms.

Experiment I. — Subject, C. R. Y., March 29–30. This experiment was originally planned to cover three days to study the influence of sleep and body position on metabolism. On the first day the subject was to lie in bed all day but not to sleep if possible, although he was expected to obtain the normal amount of sleep on the preliminary night. The experiment proper was supposed to begin at 7 A. M., March 30. The subject entered the chamber at 9 P. M., March 29, and the observations began at 11 P. M., the subject going to sleep on the cot provided inside the chamber. In the morning when he awoke at 7 A. M., he reported he had slept fairly well, waking up two or three times, and he ate breakfast consisting of cereal, crackers, and milk. During the forenoon it was noticed that the respiration was rapidly increasing, and at one o'clock the subject was nauseated and vomited, but preferred to stay inside the chamber, thinking he would feel better later on, although he said his lungs were sore at 3 P. M. It was necessary to remove him from the chamber at this time. His temperature as indicated by the clinical thermometer under his tongue was 39°.19. The electrical resistance thermometer had shown a distinct rise in temperature during the whole day. The pneumograph for recording the pulse and respiration was unfortunately not used in this experiment. After the subject left the laboratory there were no observations regarding his general condition. As a matter of fact, he was subsequently used for a number of experiments inside the chamber and showed no idiosyncrasies.

The record of body temperature as measured by the electrical resistance thermometer is as follows:

Time.	° C.	Time.	° C
1.00 A. M.	36.41	11.00 A. M.	37.38
3.00 A. M.	36.47	12.00 A. M.	37.76
5.00 A. M.	36.53	1.00 P. M.	38.23
7.00 A. M.	36.75	1.34 P. M.	38.80
9.00 A. M.	36.90	3.00 P. M.	39.19
10.00 A. M.	37.18	......	....

Experiment II. — Subject, B. F. D., May 5–7. A two-day fasting experiment was planned with this subject, who went into the respiration chamber at 7 P. M., May 5. Nothing abnormal in the experiment was noticed during the night or, indeed, the next day until late in the afternoon, when a slight hacking cough developed. At 10 P. M. in the evening there was considerable pain in the chest, and the subject began to vomit. This continued for an hour or more, and the subject was finally taken out of the chamber at 1 A. M., May 7. He complained of great shortness of breath and difficulty of respiration, with a pain in the chest and a persistent nausea. The subject remained in the laboratory too uncomfortable to leave until 6 P. M., May 7. The pain in his chest and nausea did not disappear completely until during the day of May 8. The subject took his own pulse rate inside the chamber from time to time, but no material increase was noticed, neither was there any temperature rise in this experiment. The ill effects seem to be wholly confined to respiratory disturbances and to nausea.

Experiment III. — Subject, R. D. M., May 12. In order to secure the normal heat production of man at rest, the subject, who was an editorial assistant in connection with the nutrition investigations in progress, entered the respiration chamber at 8 A. M.; the experiment proper began at 8.48 and ended at 2.48 P. M. During the experiment proper practically no abnormal conditions were noticed, but a few hours after leaving the laboratory the subject was taken violently ill. At about 4 P. M. a cough appeared, with difficulty in breathing. At 9.20 P. M. he vomited and fainted. A physician was called and a stimulant administered at 11.30 P. M. The next morning his lungs were slightly sore. No observations regarding temperature, respiration, or pulse rate were obtained other than those by the attending physician, who found the patient in a state of collapse.

Experiment IV. — Subject, H. E. B., October 19–20. A two-day fasting experiment with this subject had been planned. He entered the respiration chamber at 7 P. M., October 19, the experiment proper beginning at 9 P. M. Although sleeping as well as could be expected under for him abnormal surroundings, he experienced no discomfort until he arose from the bed at 7 A. M. Upon moving about he became dizzy and lost consciousness for a few minutes, falling upon the bed and unconsciously voiding a small amount of urine. When consciousness returned, he felt nauseated and endeavored unsuccessfully to vomit. The feeling of nausea persisted for about one hour, then after coming out of the chamber he began to feel somewhat better. He remained in the calorimeter laboratory until 3 P. M., during which time, as the table shows, there was a marked increase in the respiration rate. The subject did not complain of any cough or soreness of the lungs.

Time.	Pulse.	Respiration.	Body temperature.
Oct. 19-20.	Rate per min.	Rate per min.	° C.
9.00 P. M	..	..	36.75
11.00 P. M.	..	21	36.51
12.00 P. M.	..	22	36.58
1.00 A. M.	..	23	36.73
2.00 A. M.	..	26	37.16
3.00 A. M.	..	29	37.59
4.00 A. M.	..	33	37.92
5.00 A. M.	..	32	38.13
6.00 A. M.	..	34	38.30
7.00 A. M.	..	..	38.44
8.15 A. M.	..	..	37.39 [1]
11.00 A. M.	89	..	37.45 [1]
2.30 P. M.	90	..	37.17 [1]

[1] Sublingual.

Experiment V. — Subject, C. F. S., October 24–25. The experiment was planned to investigate the metabolism and energy production during typewriting. A man of about thirty-five years entered the chamber at 9 A. M. The experiment proper began at 10.18 A. M., ending at 2.18 P. M. At 1 P. M. he ate 450 gm. of whole milk and 142 gm. of graham crackers. During the experiment proper there was nothing abnormal noticeable, but three or four hours after leaving the chamber he was taken with nausea and vomiting. Since he had left the laboratory further observations were not obtained.

Experiment VI. — H. D. A., November 2–3. A two-day fasting experiment had been planned, and the subject entered the chamber at 3 P. M., November 2. The observations were begun at 5 P. M. The subject retired as usual at 11 P. M. and reported the next morning, when he was called at 7 A. M., that he did not sleep very well, perspiring freely during the night. On getting up in the morning a cough developed, his pulse rate increased, and the temperature continued to rise. The subject was taken out of the chamber at 2.52 P. M. On leaving the chamber a violent cough developed.

The subject was much nauseated, but did not vomit. The respiration was very painful, especially when taking a long breath. The next morning, after a comfortable night at his room, the subject still felt soreness in the chest and difficulty in taking a full breath.

Time.	Pulse.	Respiration.	Body temperature.
Nov. 2-3.	Rate per min.	Rate per min.	° C.
5.00 P. M.	..	..	36.96
7.00 P. M.	..	..	37.13
9.00 P. M.	..	..	36.70
11.00 P. M.	65	16	36.54
1.00 A. M.	75	15	36.55
2.00 A. M.	71	20	36.75
3.00 A. M.	88	22	37.05
4.00 A. M.	96	22	37.28
5.00 A. M.	88	20	37.40
6.00 A. M.	90	21	37.55
7.00 A. M.	94	21	37.46
8.00 A. M.	83	..	37.26
9.00 A. M.	85	24	37.41
10.00 A. M.	91	24	37.60
11.00 A. M.	..	..	37.70
12.00 A. M.	94	28	37.90
1.00 P. M.	..	.	38.27
1.15 P. M.	..	..	37.94 [1]
1.30 P. M.	107	..	....
5.30 P. M.	103	..	39.00 [1]
7.00 P. M.	100	..	38.56 [1]

[1] Sublingual.

Experiment VII. — Subject, F. E. S., November 4–5. This experiment was designed to study the effect of passing the oxygen used in the system through a combustion tube and thereby destroy any possible organic poisonous substance. The subject entered the chamber at 8 P. M., observations began at 9 P. M. The subject slept until 2 A. M. and woke up with a dull ache in the lower part of the lungs. Went to sleep again, but was called at 7 A. M. On rising he coughed a little and was breathing heavily. He had no appetite, eating but 7 gm. of prepared cereal, 9 gm. of bread, and 31 gm. of cream. As the temperature, pulse, and respiration all continued to increase, the subject was taken out of the chamber at 3 P. M. before more uncomfortable symptoms appeared.

Time.	Pulse.	Respiration.	Body temperature.
Nov. 4-5.	Rate per min.	Rate per min.	°C.
9.00 P. M.	74	17	37.30
11.00 P. M.	..	..	37.04
1.00 A. M.	64	18	36.70
3.00 A. M.	59	15	36.55
5.00 A. M.	73	18	36.70
7.00 A. M.	99	18	37.15
9.00 A. M.	..	..	37.41
11.00 A. M.	93	28	38.19
1.00 P. M.	..	..	38.19
2.00 P. M.	102	26	38.37
3.00 P. M.	115	31	38.30

Experiment VIII. — Subject, G. V. S., November 6–7. While the results of the preceding days were distinctly discouraging to the consummation of a two-day fasting experiment, this subject had planned for such an experiment and hence it was begun. The subject entered the chamber at 8 P. M., November 6, observations began at 9 P. M., and the subject reported at 3 A. M. a nausea and a cough every time he drew a long breath. He slept very well after 3 A. M. After rising at 7 A. M. he felt better at first, but on moving around felt very weak. Owing to the great discomfort experienced by previous subjects in these experiments, it was decided to take him out of the chamber at 7 A. M. On coming out of the chamber he was

very much nauseated and vomited. He went to his room and vomited again at 10.30 A. M. He remained in bed all day, and his lungs felt very sore. When he was lying down he could breathe fairly comfortably, but when sitting or standing he felt great pain and immediately began coughing.

Time.	Pulse.	Respiration.	Body temperature.
Nov. 6-7.	Rate per min.	Rate per min.	° C.
9.00 P. M.	74	19	37.59
11.00 P. M.	53	19	37.12
1.00 A. M.	49	16	36.34
3.00 A. M.	52	21	36.43
4.00 A. M.	66	22	36.59
5.00 A. M.	74	24	36.75
6.00 A. M.	72	31	37.04
6.52 A. M.	..	..	37.26
11.00 A. M.	90	..	37.90 [1]

[1] Sublingual.

Experiment IX. — Subject, A. H. M., November 8–9. This experiment was planned to attempt to eliminate the possible source of contamination to the air by using oxygen prepared from sodium peroxide. The subject entered at 8 P. M., observations began at 9 P. M. Upon rising at 6 A. M., November 9, the subject reported he had slept well. At 10 A. M. there was a sublingual temperature of 38°.56. The subject said he felt well. The temperature as indicated by the electrical resistance thermometer was still rising rapidly. The calorimeter window was opened at 12 noon, much to the surprise of the subject. When he stood up to come out of the chamber, he coughed severely and at the suggestion of one of the physicians he was allowed to lie inside on the cot. The window was opened, although the ventilation system was not running. He had a violent nausea and was coughing most of the time. He came out of the chamber at 4 P. M. Although the exact data regarding his subsequent condition are lacking, he felt well enough to make another experiment four days later.

Time.	Pulse.	Respiration.	Body temperature.
Nov. 8-9.	Rate per min.	Rate per min.	° C.
9.00 P. M.	..	..	36.31
11.00 P. M.	..	..	36.32
1.00 A. M.	41	15	36.06
3.00 A. M.	41	14	35.78
5.00 A. M.	41	16	35.94
5.48 A. M.	50	18	36.23
7.00 A. M.	..	..	37.15
8.00 A. M.	53	25	37.31
9.00 A. M.	59	26	38.04
10.00 A. M.	64	32	38.46
11.00 A. M.	68	25	39.27
12.00 A. M.	..	34	39.25
1.00 P. M.	..	..	39.08
1.39 P. M.	83	32	38.76
2.00 P. M.	..	..	39.17 [1]
2.19 P. M.	76	38	....
3.03 P. M.	71	34	38.89 [1]
3.46 P. M.	76	34	....

[1] Sublingual.

Variations in susceptibility and the personal equation. — During the spring of 1905, when Experiments I–III cited above were being made, there was a continuous series of experiments made with a large number of subjects in which no ill effects either during or after the experiments were observed. In the fall of 1905 there were likewise certain experiments interspersed between those here reported in which individuals also appeared immune. The most striking instances are those experiments with C. R. Y., the subject of Experiment I. On September 13 an experiment of twenty-four hours' duration was made with him with no difficulty whatever.

On October 4 and 5 the same person was used for the subject of an experiment on the digestibility of cheese, but, as he was unable to partake of the diet as planned, the experiment was stopped at eight o'clock the following morning. There were no appearances whatever of any thermal or respiratory disturbances. On October 26–29 a two-day fasting experiment was made with him without difficulty.[4]

An examination of the dates of these experiments shows that while C. R. Y. was made distinctly unwell by an experiment in March, he was not affected by several sojourns in the chamber in the fall, in spite of the fact that, judging from the frequency of the appearance of toxic symptoms in different individuals, the cause had not been removed.

On October 12–15, a two-day fasting experiment was made with another subject, H. E. S., and no abnormal results were apparent.[5]

On October 23 an experiment was made with one of us, a rest experiment of four hours' duration, with no untoward results. On the afternoon of October 24, immediately following Experiment V cited above, an experiment was made with another subject who likewise performed some typewriting. This experiment, which lasted some six hours, was without abnormal results.

It is thus apparent that individuals varied considerably in regard to their susceptibility to the toxic influences obtaining in the chamber. In at least one case, C. R. Y., it appeared that he had been affected in one experiment, but had gone through several experiments subsequently without discomfort.

Suspected sources of toxic effect. — Since this apparatus had been in constant use with almost no difficulties for several years, the appearance of these toxic effects was indeed most puzzling. To our knowledge there had been no material alteration in any step of procedure of the manipulation, and we were entirely at a loss to account for these symptoms.

Among the many suspected sources of difficulty were the possibilities of arsenic compounds in the acid and the possibility of impurities in the oxygen. With regard to the acid experiments were made with acid from different sources, and while we had been commonly accustomed to using the highest grades of commercial

[4] F. G. Benedict: Publication No. 77, Carnegie Institution of Washington, 1907, pp. 222–273.

[5] Benedict: *Loc. cit.*, pp. 222–273.

acid, we made a number of experiments with chemically pure acid, with the same result.

The oxygen supply had been obtained from the S. S. White Dental Mfg. Co., perhaps the largest manufacturers of oxygen for medical use in the United States. A visit to their works by one of us resulted in a most careful examination of their method of manufacture, and no possible sources of contamination were found there. As an added precaution, the oxygen in certain experiments before being admitted to the chamber was passed through a heated glass tube containing copper oxide, and in one experiment oxygen prepared over sodium peroxide was used. In all the experiments the same results were obtained.

Among the large number of experts consulted on this problem, it was suggested a number of times that there might have been some bacterial infection which, on account of the closed connections of the chamber, might have been communicated from subject to subject. To prevent this as far as possible, the chamber was fumigated with formaldehyde candles repeatedly without avail. Furthermore, it was impossible to conceive of air passing through strong sulphuric acid twice and then through a filter of cotton containing sodium bicarbonate and not have bacteria practically removed.

While the possible presence of mercury vapor in the system had always been recognized, it was felt that the surface of mercury exposed to the air current was so small as to almost preclude any possible material volatilization of the mercury, but when other sources of difficulty failed to be detected, it was finally decided to remove the mercury valves at the rear of the system and simultaneously replace the old piping which conducted the air from the valve to the chamber with new galvanized iron pipe of like size. Temporary changes were made by substituting rubber hose in place of the galvanized iron piping formerly used. The mercury valves were replaced with brass valves commonly used in steam and water piping.

The mercury valves at the entrance end of the absorber system were not removed, because here the pressure to be guarded against was very large, greater than at the exit end, and it was assumed that any mercury vapor would be completely removed from the air current by passing twice through the acid in the porcelain absorbers.

Experiments following the Changes in the Valve System of the Respiration Chamber.

It is exceedingly fortunate for the success of these experiments that two of the gentlemen who had been seriously affected by their previous sojourn in the respiration apparatus willingly volunteered to subject themselves to the further experiments with the apparatus as altered. It is a great pleasure here to express our appreciation of the high scientific interest that actuated both these gentlemen, Mr. A. H. Middlemass and Mr. H. D. Allen, to expose themselves to the possibilities of another experience as distressing as they had formerly gone through. Fortunately both these experiments were without unpleasant results.

Experiment X. — Subject, A. H. M., November 13–14. The subject of the experiment entered the chamber at 2 P. M., November 13; the experiment proper began at 5 P. M., and as no unpleasant conditions developed, the next day, at 12.15 P. M., the experiment ended. During this time the pulse, respiration, and temperature all indicated nothing abnormal.

Experiment XI. — Subject, H. D. A., November 14–15. This experiment began two hours after Experiment X ended. The experiment proper began at 3 P. M., and the next morning, as no untoward results appeared, the experiment was concluded.

Subsequent experiments. — Following Experiments X and XI, the respiration calorimeter was used for a large number of metabolism experiments during the winters of 1905–1906 and 1906–1907. Some 109 experiments with 14 individuals were made, and in no instance did the sojourn in the chamber produce any uncomfortable results.

The complete disappearance of all feelings of discomfort with the removal of the mercury valves and old piping seems to prove conclusively that these were the chief causes of the toxic symptoms noticed in the earlier experiments. While we were far from being able to carry out a careful toxicological investigation of this problem, we utilized as much time as we could spare from our other metabolism experiments to attempt to throw more light upon the exact cause of toxic symptoms.

Experiments on dogs. — Two experiments were made with dogs in which the dogs were confined in a metal box through which air

was drawn, and the air before entering the chamber was passed through the suspected mercury valve and piping which had been removed from the respiration chamber. In one of these experiments the dog used was very old, somewhat over twenty years, and while he refused to eat during the experiment, he was so very feeble at the conclusion it was decided most humane to chloroform him. It was impossible to draw any deductions from this experiment.

A much younger and more active dog lived in this small metal box with air passing through these pipes most of the time for two weeks without any ill effects. It should be stated, however, that the immunity of this particular dog to mercurial poisoning must have been very marked, as for several days it was arranged to have him sleep on a wire screen over a large number of dishes containing mercury. Even under these conditions the dog exhibited absolutely no indications of mercurial poisoning.

Experiments on man using the original mercury valve and piping. — A second attempt was made in December, 1906, to secure more definite evidence regarding this case by repeating an experiment with man in the respiration chamber using the mercury valve and piping that had formerly been removed and using one of the subjects, A. H. M., who formerly exhibited toxic symptoms. The subject volunteered to make this experiment. The experiment continued for three days, and, as a matter of fact, on the early morning of the second day there was a marked temperature rise. He did not, however, reach any actual febrile state, and as it did not rise any farther and he himself made no complaint and there being no respiratory disturbances, the experiment continued for some thirty hours after the temperature rise was noticed. The pulse also showed no increase. Unfortunately again this experiment does not leave any clear evidence regarding the case.

Test for mercury on the inner walls of the piping. — Since all the experiments pointed towards a gradual absorption of mercury vapor by the layer of zinc inside of the galvanized iron pipe, an attempt was made to demonstrate the presence of mercury. A section of the pipe was placed in the lathe and one chip was taken off for some distance. This material was subjected to tests for mercury, which, however, were unsatisfactory. There was no visible appearance of amalgamation, and the tests for mercury were by no means satisfactory, so that we could not state definitely whether there was mercury present or not. The important point was that there was no appearance of amalgamation on the zinc itself.

Conclusion.

While there lacks a definite scientific demonstration that the causes of toxic poisoning during these experiments were due to poisoning by mercurial vapor, there seems to be no other possible conclusion. The examination of the literature shows but very little evidence regarding cases of mercurial poisoning presenting symptoms of the type noticed here, although Rubner [6] states that in mercurial poisoning sleep is disturbed and there is fever which can be more or less pronounced. Of special interest, however, in considering the question of poisoning inside the respiration chamber is the discussion of mercurial poisoning brought out by Krogh.[7] Krogh employed mercury in his admirable apparatus used in his experimental researches on the expiration of free nitrogen from the body. In these researches he found that there was a great mortality in the experiments with eggs, and he also is inclined to believe that the classic experiments of Seegen and Nowak were also complicated by mercurial poisoning.

Bing [8] reports nine cases of poisoning of patients in a hospital due to the escape of steam through a mercury reduction valve. The patients exhibited a temporary rise of temperature, increase of respiration, increase of pulse rate, cyanosis, vomiting, and diarrhea. Two of them (infants) died, but the others recovered in a few days.

[6] Rubner: Lehrbuch der Hygiene, p. 761.

[7] Krogh: Skandinavisches Archiv für Physiologie, 1906, xviii, p. 399.

[8] Bing: Archiv für Hygiene, 1903, xlvi, pp. 200–223

PRELIMINARY OBSERVATIONS ON METABOLISM DURING FEVER.

By THORNE M. CARPENTER and FRANCIS G. BENEDICT.

[*Nutrition Laboratory, Carnegie Institution of Washington, Boston, Massachusetts.*]

Introduction.

THE typical picture of fever, presenting as it does high temperature, flushed skin, rapid respiration and pulse rate, has led to the assumption that during febrile process there is a marked increase in heat elimination.

The temperature rise is fundamentally to be considered as a disturbance of the delicate adjustment between thermogenesis and thermolysis ordinarily obtaining, and accordingly such disturbance can be accounted for in several ways. There may be an increased thermogenesis, or there may be a decreased thermolysis, and finally both factors may exert an influence.

In considering the methods used to study metabolism during fever, it is seen that they have been confined to two, namely, direct calorimetry, in which the heat elimination from the whole or a part of the body has been measured, and indirect calorimetry, in which the heat production has been computed from a study of the gaseous exchange. Assuming that the gaseous exchange is a direct measure of the heat production in the body, an assumption that in the light of more recent investigations is very much to be doubted, it is seen that this method can be used only in studying cases in which the temperature rise can be ascribed solely to increased thermogenesis. If the production of heat remains constant and there is a decreased loss of heat, a rise in temperature will occur, but this will not be shown in the method of indirect calorimetry. Obviously, then, if the temperature rise is a resultant of both of the above-mentioned factors, indirect calorimetry can throw but little light on such complicated processes. Since we must rely upon

evidence furnished by direct calorimetry to solve this important problem, any observations involving direct calorimetry which contribute to our knowledge of metabolism in the febrile state can reasonably be considered as of value.

In connection with an extensive series of investigations with the respiration calorimeter in Wesleyan University, Middletown, Conn., an accidental toxic condition of the ventilating air in the respiration apparatus resulted in a number of marked cases of respiratory disturbance accompanied by fever. All the subjects were men, and usually the experiments were designed to study some special problem in metabolism, such as the influence of inanition and the influence of the ingestion of various kinds of food. The toxicological features of these cases have been collected and presented in the preceding article.[1] Since at the time we were using every possible means to eliminate the toxic conditions, but little time was spared for studying the metabolism in the febrile condition, and hence the observations we have to report are more or less fragmentary, and pending a more comprehensive investigation of a number of typical fevers by means of the respiration calorimeter, they are to be looked upon as distinctly preliminary.

Inasmuch as these investigations are of so incomplete a nature, it hardly seems desirable in this place to enter into a long review of the literature of the subject of metabolism during fever. Such reviews have been worked out admirably by Kraus,[2] Krehl,[3] and especially has the literature been well reviewed by Likhatscheff and Avroroff.[4] This last review is unfortunately in Russian and hence not accessible to most readers.[5]

[1] T. M. Carpenter and F. G. Benedict: This journal, 1909, xxiv, p. 187.

[2] Kraus: Von Noorden's Metabolism, Physiology and Pathology, 1907, ii, p. 90, W. T. Keener & Co., Chicago.

[3] Krehl: Zeitschrift für allgemeine Physiologie, 1902, i, p. 29.

[4] Likhatscheff and Avroroff: Investigations of gas and heat exchange in fevers, Reports of the Imperial Military Academy, St. Petersburg, 1902, v, Nos. 3 and 4.

[5] A short account of the original experiments of these authors on a case of malarial fever has been published by them: Likhatscheff et Avroroff, Comptes rendus de XIII Congress International de Medicin, Paris, 1900. Section, Pathologie générale et pathologie expérimentelle. The original Russian monograph has been translated for use in the Nutrition Laboratory, where the original and the translation are always accessible.

Experimental Part.

The apparatus used in these experiments was a respiration calorimeter of the closed circuit type which permitted the simultaneous determination of the carbon dioxide production, water vapor elimination, oxygen absorption, and heat production.

While in the course of many years' experimenting with this apparatus, there had been various personal impressions recorded by the subjects as to their well being while inside the chamber, some having complained of slight headaches, others of temperature changes and minor body discomforts, there were no serious interruptions to the experiments until the spring of 1905, when one or two men were taken with violent nausea after the experiment had ceased. In the fall of 1905 a number of subjects in a short space of time were taken with violent nausea, respiratory disturbances, and marked temperature rise while inside of the respiration chamber, the discomfort being so great that in most instances it was necessary to remove the subject from the chamber. The subjective impressions of these men, together with a discussion of the toxicological condition in all probability causing this disturbance, are to be found in the preceding paper.[6] Suffice here to state that the cause of the disturbance was in all probability the use of mercury valves in the ventilating system. On the removal of these valves all disturbance ceased, and we have evidently here to do with a rather remarkable instance of mercurial poisoning which presented a very striking picture. Some ten men were thus affected by the mercurial poisoning. Of these, however, the febrile condition was reached while inside the chamber with only six, and hence these six alone can be used to furnish fragmentary evidence regarding the metabolism during a febrile state.

In order to study the metabolism during fever with these men, it is highly desirable that control experiments should have been made with the same subject under as near like conditions of bodily activity as possible. Unfortunately this could not be done, as other more pressing work had been delayed by reason of the experience with the mercurial poisoning. It transpired, however, that in a few instances we had other experiments with these same subjects which permitted of at least a reasonable comparison, and we also

[6] T. M. Carpenter and F. G. Benedict: This journal, 1909, xxiv, p. 187.

have from a large number of unpublished experiments selected one or two which permit of reasonable comparison with the other experiments, although with different individuals. In making these selections it was our effort in so far as possible to select individuals of the same body weight and general build.

In the last analysis, however, we must lay the greatest stress in our comparison of the experiments themselves on the metabolism before and during the state of fever. Some of these experiments were designed especially to aid in solving the problem of the source of the toxic influences in the chamber, no other plans being involved in their arrangement, and consequently the data secured were not obtained with the frequency that would have been desired had it been planned at that time to study metabolism during fever with special reference to the time relations.

In all the experiments the subjects were inside the chamber one or two hours before the experiment proper began to become accustomed to the surroundings and prepare for the longer experiment. Provisions were made inside the chamber to permit a comfortable night's sleep, and the subject usually undressed, with the exception of a union suit of underclothing, covered himself with a blanket, and retired at 11 P. M.

Some experiments were made after fasting and some after food, the febrile condition being only an incident and not a part of the plan itself. Usually the subject slept until 7 A. M., when he was called to collect the urine, and the body weight was taken at this time.

Body temperature. — The body temperature, which is of the utmost importance in the study of fever, was determined in all of these experiments by means of an electrical resistance thermometer. This thermometer permits the measurement of temperature deep in the rectum to 0.01° C. and, indeed, without any discomfort to the subject, so that continuous observations during the night can be obtained. The apparatus is in constant use in the laboratory and has proven eminently satisfactory.

In certain of these experiments recourse was had to the usual clinical thermometer and the sublingual temperature recorded. In connection with these sublingual temperatures it should be stated that the subjects had not engaged in any muscular work and that the temperature of the surrounding air in the chamber remained constant during the whole experiment. There is then no doubt

but that the sublingual temperatures recorded were reasonably accurate.

Respiration and pulse rate. — In certain of the experiments the subjects were asked to take their own pulse, counting it for two minutes and making their own records. The respiration rate was obtained by means of a pneumograph which was connected, through a metal tube passing the wall of the chamber, with a tambour. This tambour could be used to draw a curve on the kymograph paper, and in some cases the vibrations of the tambour were striking enough without drawing the curve. The respiration movements were easily obtained, and while in some experiments it was possible to obtain the pulse rate by counting the minor vibrations of the tambour, in other instances it could not be obtained with sufficient accuracy. When obtained, it was with great accuracy and considerable frequency. These observations were likewise made without the knowledge of the subject.

Fever Experiment I. — Subject, C. R. Y. The subject, whose body weight without clothing was 67.8 kilos, entered the chamber the evening before and went to bed at the usual time. He woke up two or three times during the night, which was not at all unusual during the first experience in abnormal surroundings. He arose at 7 A. M. and ate breakfast consisting of 200 gm. of milk, shredded wheat, graham crackers, gluten, cereal and 80 gm. of sugar. The total diet furnished about 4.6 gm. of nitrogen and 502 calories of energy. He lay down again at 7.48 A. M., as the experiment was primarily designed to study the influence of the metabolism while the subject was lying down as compared with sitting up. About 1 P. M. he drank a little water, but immediately vomited. He was removed from the chamber at 3 P. M.[7] During this experiment it was possible to study in two-hour periods throughout practically the whole experiment the metabolism so far as indicated by the carbon dioxide production, water vapor elimination, oxygen consumption, and heat production. The results are given in Table I.

The relatively large carbon dioxide elimination and oxygen absorption between 7 A. M. and 9 A. M. has a natural explanation in that during this period there was unusual muscular activity incidental to rising from bed, dressing, collecting the urine, and minor preparations for the day.

It was impracticable to secure the pulse or respiration rate.

[7] For further details regarding this experiment, see this journal, 1909, xxiv, p. 191.

TABLE I.

METABOLISM OF C. R. Y. DURING FEVER. EXPERIMENT I. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.
March 30, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.
							36.41
1 A. M.– 3 A. M.	29.4	58.2	22.8	.94	78.7	79.6	36.47
3 A. M.– 5 A. M.	22.9	46.2	20.4	.82	73.3	74.4	36.53
5 A. M.– 7 A. M.	24.9	44.2	23.6	.77	73.4	79.2	36.75
7 A. M.– 9 A. M.	37.7	38.0	36.9	.74	92.4	97.1	36.90
9 A. M.–10 A. M.	31.2 (9 A. M.–11 A. M.)	42.9 (9 A. M.–11 A. M.)	26.3 (9 A. M.–11 A. M.)	.87 (9 A. M.–11 A. M.)	90.8	108.0	37.18
10 A. M.–11 A. M.					92.8	103.8	37.38
11 A. M.–12 M.	33.4 (11 A. M.–1 P. M.)	40.1 (11 A. M.–1 P. M.)	31.3 (11 A. M.–1 P. M.)	.77 (11 A. M.–1 P. M.)	89.9	111.4	37.76
12 M. – 1 P. M.					84.6	112.4	38.23
1 P. M.– 3 P. M.	38.1	44.8	31.4	.88	79.6	108.0	39.19

Fever Experiment II. — Subject, H. E. B. The subject, whose body weight without clothing was 56.7 kilos, entered the chamber at 7 P. M., October 19, and the observations began at 9 P. M. After a reasonably comfortable night's sleep, in which, however, he reports he was awake more or less and felt warm and perspiration was free, he arose at 7 A. M. On rising he became dizzy and unconscious and fainted for a few moments. Nausea continued for about one hour, and although he was removed from the calorimeter chamber, he remained in the laboratory until 3 o'clock in the afternoon. The results of the metabolism are shown in Table II.

During the experiment proper the pulse rate could not be obtained, but after coming out of the chamber the pulse rate was found to be, at 11 A. M., 89, and, at 2.30 P. M., 90.

The data as here tabulated require certain explanations, particularly with reference to the heat elimination and production, which is apparently very large during the period from 11 P. M. to 12 P. M. when the subject was asleep, while during the next period from 12 to 1 A. M. it falls off enormously. This apparently large heat production is in part false in that it has been found necessary to make a correction for the amount of heat required to warm up the bed

and bedding after the subject goes to sleep. At present the very unsatisfactory usage is to assume that 30 calories of heat were required in warming up the bed and bedding to the temperature at which they were during the greater part of the night, consequently to the heat measured by the calorimeter we have added 30 calories. This correction was determined on a large number

TABLE II.

METABOLISM OF H. E. B. DURING FEVER. EXPERIMENT II. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Respiration rate per minute.
Oct. 19-20, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.	
							36.75	
9 P. M.–11 P. M.	28.5	37.2	22.6	.92	103.3	96.7	36.51	..
11 P. M.–12 P. M.	27.9	43.4	21.1	.96	90.0	92.8	36.58	21
12 P. M.– 1 A. M.					54.1	61.0	36.73	22
1 A. M.– 2 A. M.	26.8	39.9	20.5	.95	52.5	73.5	37.16	24
2 A. M.– 3 A. M.					58.9	79.9	37.59	27
3 A. M.– 4 A. M.	30.2	41.9	21.8	1.01	85.0	100.9	37.92	32
4 A. M.– 5 A. M.					74.8	84.6	38.13	33
5 A. M.– 6 A. M.	31.8	43.0	24.6	.94	78.1	85.9	38.30	33
6 A. M.– 7 A. M.					76.4	82.6	38.44	..

of experiments where men completely undressed and had heavier clothing than was used by this man, and consequently the correction is at best tentative.

On the other hand, during the period between 7 A. M. and 8 A. M. in the morning there is likewise an addition of heat to that produced during this hour by the amount of heat lost by bed and bedding as they are cooled by the surrounding temperature to that of the room, and it has been our custom to subtract 30 calories from the heat as measured by the calorimeter in the period from 7 A. M. to 8 A. M. When the total metabolism for twenty-four hours is involved, this correction simply balances in that there is an addition of heat to the measured amount between 11 P. M. and 12 P. M. and there is a deduction from the amount measured between 7 A. M.

and 8 A. M., the correction being only to aid in subdividing the day into periods. In this experiment the heat production from 11 P. M. to 12 midnight is very much larger than that during the following hours of the night. In explanation of this large heat

TABLE III.

METABOLISM OF H. D. A. DURING FEVER. EXPERIMENT III. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per minute.	Respiration rate per minute.
Nov. 2-3, 1905.	gm.	gm.	gm.		Cal.	Cal.	° C.		
							36.70		
9 P. M.–11 P. M.	29.3	49.3	22.0	.97	111.8	105.4	36.54	65	15
11 P. M.– 1 A. M.	29.3	49.1	26.7	.80	86.7	86.2	36.55	70	14
1 A. M.– 2 A. M.	27.7	54.7	24.8	.81	79.7	90.3	36.75	74	18
2 A. M.– 3 A. M.					78.8	95.1	37.05	83	21
3 A. M.– 4 A. M.	30.0	53.4	27.4	.79	85.3	97.6	37.28	88	23
4 A. M.– 5 A. M.					90.5	96.5	37.40	91	23
5 A. M.– 6 A. M.	28.9	56.1	26.4	.80	82.8	90.4	37.55	91	19
6 A. M.– 7 A. M.					100.6	94.5	37.46	93	23
7 A. M.– 8 A. M.	38.6	49.8	38.8	.72	131.5	117.5	37.26	84	..
8 A. M.– 9 A. M.					94.1	100.0	37.41	81	24
9 A. M.–10 A. M.	31.5	59.4	28.9	.79	105.5	115.5	37.60	85	24
10 A. M.–11 A. M.					92.8	97.6	37.70	91	24
11 A. M.–12 M.	33.7	39.5	29.8	.82	110.8	120.8	37.90	94	28
12 M. – 1 P. M.					70.7	90.3	38.27	..	..
1 P. M.–1.52 P. M.	...	..	..	..	99.1	109.8	38.46	107	..

production it should be stated that in this experiment the subject did not make up his bed and undress until some time after 11 P. M., and hence the extra heat may have been because of this extraneous muscular activity during a period in which subjects are usually resting quietly in bed.

Fever Experiment III. — Subject, H. D. A. The subject, whose body weight without clothing was 65.4 kilos, entered the chamber on the afternoon of

November 2. The observations extended from 9 P. M., November 2, to 1.52 P. M., November 3. Shortly after entering the chamber the subject defecated after an enema. He spent the afternoon and evening in sitting quietly reading, although between 9 and 11 P. M. he made a dozen tests with the hand dynamometer. He went to bed shortly after 11 P. M. and did not spend a particularly quiet night, as he felt somewhat warm and woke up rather early in the morning. After getting up at 7 A. M. he spent most of the forenoon lying down, reading and sleeping a part of the time. The subject was much nauseated during the day, but did not vomit. The results of his metabolism are given in Table III.

In addition to the pulse records above noted it was found at 5.30 P. M., November 3, on coming out of the chamber that the pulse was 103, and, at 7 P. M., 100.

The large heat production from 9 P. M. to 11 P. M. is probably in great part accounted for by making strength tests with the hand dynamometer.

TABLE IV.

METABOLISM OF F. E. S. DURING FEVER. EXPERIMENT IV. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per minute.	Respiration rate per minute.
Nov. 4–5, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.		
							37.30		
9 P. M.–11 P. M.	28.2	42.5	22.2	.92	112.1	104.2	37.04	74	17
11 P. M.– 1 A. M.					76.5	66.9	36.70	61	18
1 A. M.– 3 A. M.					65.5	61.1	36.55	61	16
3 A. M.– 5 A. M.					61.0	64.1	36.70	64	17
5 A. M.– 7 A. M.					59.3	70.0	37.15	74	18
7 A. M.– 9 A. M.					105.6	111.8	37.41	99	18
9 A. M.–11 A. M.	37.8	41.2	34.1	.81	111.6	130.4	38.19	..	..
11 A. M.– 1 P. M.					108.9	108.4	38.19	93	28
1 P. M.– 2 P. M.					71.2	79.5	38.37	..	..
2 P. M.– 3 P. M.					102.0	97.9	38.30	109	27

Fever Experiment IV. — Subject, F. E. S. The subject, whose body weight without clothing was 56.6 kilos, entered the chamber during the evening

of November 4, and the observations continued from 9 P. M., November 4, until 3 P. M., November 5. After the preliminary weighing of the man with his bed clothing, etc., he sat reading from 9.30 until bedtime at 11 P. M. He awoke at 2 A. M., but went to sleep again. He arose at 7 A. M. and ate a light breakfast consisting of cream, cereal, bread and butter, containing 0.4 gm. nitrogen and 143 calories. Most of the forenoon was spent in reading until 12.43, after which he lay down and was probably asleep until 2.30 P. M., when he arose and sat reading until the end of the experiment. The results of the metabolism are given in Table IV.

The respiratory gases were studied in this experiment in long periods, since the special object of the experiment was to test the effect of the passing of the oxygen used in the experiment over heated copper oxide and thus oxidize any possible poisonous ingredients. The records of the heat measurements, however, are taken so frequently that it is possible to apportion the heat elimination and heat production with reasonable accuracy into two-hour periods, although it is necessary in this apportionment to assume that the amounts of vaporized water per hour remained constant throughout the whole period. Thus in the long period from 9 P. M., November 4, to 9 A. M., November 5, there were 42.5 gm. of water vaporized per hour. The amount of water vaporized was taken into consideration in computing the heat elimination, as the evaporation of 1 gm. of water at 20° required 0.586 calories. Since the experiment was in two long periods and there was a very close agreement in amounts of water vaporized per hour, this assumption is probably not far out of the way.

Fever Experiment V. — Subject, G. V. S. The subject, whose body weight without clothing was 56.0 kilos, entered the chamber at 8 P. M., November 6, and the observations were continued from 9 P. M., November 6, to 6.52 A. M., November 7, when it was necessary to take him out of the chamber. He reported that he slept well until 3 A. M., but experienced nausea with coughing afterwards and had but little sleep during the rest of his sojourn in the calorimeter.

The absence of a marked temperature rise in this experiment may throw doubt on the legitimacy of including it in this discussion of fever, but the rapidly increasing pulse and respiration rates justify the assumption that the body temperature was inclined to rise, as at 11 A. M., November 7, the temperature sublingual was 37.°90.

TABLE V.

METABOLISM OF G. V. S. DURING FEVER. EXPERIMENT V. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Respiration rate per min.
Nov. 6–7, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.		
							37.59		
9 P. M.–11 P. M.					110.9	99.3	37.12	74	19
11 P. M.– 1 A. M.					83.9	63.4	36.34	52	17
1 A. M.– 3 A. M.					62.9	64.5	36.43	51	18
3 A. M.– 4 A. M.	25.6	49.8	22.6	.82	73.4	80.7	36.59	57	22
4 A. M.– 5 A. M.					57.3	64.6	36.75	64	25
5 A. M.– 6 A. M.					82.5	96.2	37.04	73	27
6 A.M.– 6.52 A.M.					65.2	74.8	37.26	..	..

The pulse rate at 11 A. M., November 7, four hours after coming out of the chamber, was 90.

Fever Experiment VI. — Subject, A. H. M. The subject, whose body weight without clothing was 62.8 kilos, entered the chamber at 8 P. M., November 8, and observations continued from 9 P. M. until 12 noon on November 9. The subject reported an excellent night's sleep, but was very drowsy on the evening before. He ate breakfast consisting of cream, cereal, and milk with an estimated nitrogen content of about 3 gm. and 790 calories of energy. Most of the forenoon was spent lying down on the bed, reading or sleeping. The results of his metabolism are given in Table VI.

In this experiment, as in the preceding one, the special object being to test the toxic influences inside the chamber, the gaseous exchange was studied only in long periods, and the same method of apportionment of the heat of vaporization of water in the computation of the heat production and elimination was used in this experiment as in Experiment V.

EXPERIMENTS FOR COMPARISON.

Two of these subjects, H. D. A. and A. H. M., who were made wretchedly ill by mercurial vapor in the air current, had sufficient

interest in the success of the respiration chamber to volunteer to be the subjects of experiments made after the removal of the mercury valve, although at that time it was by no means certain that the removal of the valves would insure the absence of the

TABLE VI.

METABOLISM OF A. H. M. DURING FEVER. EXPERIMENT VI. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Resp. rate per min.
Nov. 8-9, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.		
							36.31		
9 P.M.–11 P.M.					102.3	102.0	36.32	..	..
11 P.M.– 1 A.M.					72.6	65.0	36.06	43	14
1 A.M.– 3 A.M.					57.3	49.2	35.78	42	14
3 A.M.– 5 A.M.	25.9	36.7	22.9	.82	59.4	63.3	35.94	42	14
5 A.M.– 5.48 A.M.					67.1	86.4	36.23	44	17
5.48 A.M.– 7 A.M.					83.2	124.6	37.15	..	..
7 A.M.– 8 A.M.					86.6	94.8	37.31	52	26
8 A.M.– 9 A.M.					76.2	115.5	38.04	..	..
9 A.M.–10 A.M.					68.6	91.0	38.46	59	26
10 A.M.–11 A.M.	30.3	33.3	27.9	.79	62.3	105.9	39.27	64	29
11 A.M.–12 M.					89.7	88.1	39.25	68	30
12 M. – 1 P.M.	..	..	..	..	..	..	39.08	..	..
1 P.M.– 2 P.M.	..	..	..	..	..	..	39.17	83	32
2 P.M.– 3 P.M.	..	..	..	..	..	..	38.89	76	36
3 P.M.– 4 P.M.	..	..	..	..	..	..	..	73	34

toxicological symptoms. Consequently, with these two experiments we have direct control. One of the other subjects, C. R. Y., was also used in another experiment inside the chamber, and the results of this experiment can be used with a certain amount of accuracy for comparison. The remaining experiments must unfortunately be compared with experiments made on other individuals of like body weight and general build. This last method of comparison is ad-

mittedly very unsatisfactory, but it is possible to predict with considerable accuracy the metabolism from people of similar age, weight, and general build, provided there is like muscular activity in both instances. Unfortunately, again, in these comparative experiments it was in most instances impossible to exactly duplicate the muscular activity in the febrile experiments. On the other hand, since the fever subjects were lying down most of the time, the muscular work in the control experiments was almost invariably somewhat greater than in the fever experiments, and this fact is of unusual interest, as will be seen in the general discussion.

Control Experiments.

Control Experiment I. — Subject, C. R. Y., October 27, 1905. In this experiment the subject was undergoing a two-days fast, which he completed successfully with no abnormal indications. As a matter of fact, for a few days before this experiment and for two or three days after this experiment, the respiration calorimeter was used for experiments with other subjects in which they showed marked toxic symptoms as a result of this mercurial poisoning; but this subject who six months before had also given strong evidence of mercurial poisoning, here passed through the experiment without the slightest difficulty. For purposes of comparison, only that portion of the experiment which covers the same time of day as is covered in Experiment I is here presented. The metabolism during this experiment was studied in considerable detail, and the experiment as a whole is described completely elsewhere.[8]

The subject went to sleep at the usual time, but said he did not sleep very well, waking up occasionally; rose at 7 A. M. and dressed and spent practically the rest of the morning sitting quietly reading. About 12 noon, he lay down on the bed and read lying down. Then in the afternoon until 3 o'clock he was lying down on the bed reading and probably was asleep from 2 o'clock until 3. The body weight without clothing at the time of this experiment was 67.1 kilos. So much of the data regarding the metabolism studied with this man as are required for comparison with the fever experiments are given in Table VII herewith.

These data may be taken as indicating the metabolism of this man under normal conditions. The last food was eaten at 6 P. M. on October 26.

[8] F. G. Benedict: Publication No. 77 of the Carnegie Institution of Washington.

The usual increase in heat production attendant upon rising, adjusting bed and clothes for the day is noticed between the hours of 7 A. M. to 9 A. M.

The time covered by the preliminary night and a portion of the day's fast agrees with the time covered by the experiment showing

TABLE VII.

Metabolism of C. R. Y. Control Experiment I. (Quantities per Hour.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Respiration rate per min.
Oct. 27, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.		
							36.44		
1 A. M.–3 A. M.	23.8	41.6	20.2	.86	74.5	72.4	36.39	66	15
3 A. M.–5 A. M.	25.1	38.5	19.6	.93	75.0	79.6	36.57	57	14
5 A. M.–7 A. M.	26.2	37.8	..	..	56.0	63.7	36.86	60	14
7 A. M.–9 A. M.	36.8	34.3	35.4	.76	104.8	107.2	37.03	..	17
9 A. M.–10 A. M.	29.4	24.5	22.0	.97	87.6	85.8	36.89	..	16
10 A. M.–11 A. M.							36.98	..	16
11 A. M.–12 M.	27 6	42.8	23.2	.87	77.4	71.1	36.97	..	17
12 M. – 1 P. M.							36.78	..	14
1 P. M.–3 P. M.	24.5	37.9	19.7	.91	67.1	66.7	36.83	..	15

fever on March 30, 1905. The activity for the night was very much the same in the two experiments compared. The activity for the following day was different for the two experiments, but it is thought that the difference in activity will be offset by the fact that during the experiment showing fever the man ate a certain amount of food (energy of this food was 502.3 calories and the nitrogen, 4.16 gm.). Following the eating of this food in the experiment showing fever the man was lying down most of the time, whereas during the day of October 27, which is used for comparison, the man was sitting up and there was some activity until he lay down later in the day.

Control Experiments II and III. — Subject, H. R. D., December 4–5, 1905, and May 9–10, 1906. Data for portions of these two experiments have

been selected for comparison with the fever experiment with H. E. B. made on October 19–20, 1905. The two men were of about the same body weight. H. E. B. weighed 60.7 kilos with clothing. The weight of H. R. D. on December 4–5 was about 60 kilos, and, on May 9–10, 61.1 kilos. The data for all three experiments are for the night, when the men were asleep most of the time. Neither subject slept all night, sleep for

TABLE VIII.

METABOLISM OF H. R. D. CONTROL EXPERIMENT II. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per minute.	Respiration rate per min.
Dec. 4–5, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.		
							36.65		
11 P. M.–12 P. M.	24.7	23.6	22.9	.78	68.5	63.9	36.67	79	20
12 P. M.– 1 A. M.							36.49	67	18
1 A. M.– 2 A. M.	18.3	36.5	16.2	.82	64.1	57.1	36.24	65	17
2 A. M.– 3 A. M.							36.22	63	17
3 A. M.– 4 A. M.	20.1	31.2	21.0	.70	61.7	59.3	36.18	64	16
4 A. M.– 5 A. M.							36.14	65	16
5 A. M.– 6 A. M.	21.3	30.2	16.3	.95	59.4	69.4	36.31	66	17
6 A. M.– 7 A. M.							36.58	63	17

H. R. D. on December 4–5 being perhaps somewhat better than for his other night and than that obtained by H. E. B. The two nights, October 19–20 with H. E. B. and December 4–5 with H. R. D., were in preparation for a regular fast for a day or two, while the night of May 9–10 with H. R. D. was a continuation of an experiment already under way, the subject having eaten at 9.10 A. M. on May 9 about 100 gm. of gluten bread and 220 gm. of skim milk, the energy being 621 calories and the nitrogen 15.39 gm. Except for the quality of sleep the conditions of the three nights were about the same with the usual preparations at bedtime.

In Control Experiment II the subject went to bed at 11 P. M., December 4, and slept very well, waking for a few minutes at 4 A. M. At 6.30 he woke up but lay quietly in bed until called at 7 A. M.

The metabolism during this experiment is given in Table VIII herewith.

In Control Experiment III the subject went to bed at 9.45 P. M., May 9,

1906. He did not sleep as well as in Control Experiment II, owing to a sense of discomfort attending the inserting of the rectal thermometer. Usually the subjects do not notice the presence of the thermometer five minutes after it has been inserted.

The data for the metabolism are given in Table IX.

TABLE IX.

Metabolism of H. R. D. Control Experiment III. (Quantities per Hour.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Resp. rate per min.
May 9-10, 1906.	gm.	gm.	gm.		Cal.	Cal.	°C.		
9.30 P.M.–11.30 P.M.	24.8	25.5	..	..	65.9	53.1	36.78 36.30	52	16
11.30 P.M.–12.30 P.M.	18.8	23.5	20.5	.67	61.1	54.3	36.30	51	15
12.30 P.M.– 1.30 A.M.							36.31	53	15
1.30 A.M.– 2.30 A.M.	20.8	22.8	21.1	.72	60.5	69.5	36.28	52	14
2.30 A.M.– 3.30 A.M.							36.41	53	14
3.30 A.M.– 4.30 A.M.	21.0	22.2	19.4	.79	61.8	61.0	36.36	53	15
4.30 A.M.– 5.30 A.M.							36.39	54	15
5.30 A.M.– 6.30 A.M.	21.0	21.1	17.4	.88	63.0	76.4	36.65	56	18
6.30 A.M.– 7.30 A.M.							36.93	66	16

This experiment began on the half hour, so the subject did not get up until called at 7.30 A. M., while in the other experiments the subjects usually rose at 7 A. M. Obviously the metabolism is in no wise affected, so far as its use for comparison is concerned, by this schedule.

These two experiments are also to be compared with the fever experiment with G. V. S., November 6–7, 1905. H. R. D. and G. V. S. were men of about the same body weight. G. V. S. weighed 60 kilos, and H. R. D. on December 4–5 weighed about 60 kilos and, on May 9–10, 61.1 kilos. The experiment with G. V. S. was a night of sleep in preparation for a fast. He slept well until 3 A. M., when he had trouble with his stomach and coughed and did not sleep much afterwards. The experiments, December 4–5, 1905, and May 9–10, 1906, with H. R. D. show somewhat the same activity

conditions, the one for December 4–5 perhaps a little nearer to the conditions of the experiment with G. V. S., November 6–7, in that H. R. D. on that night awoke about the same time in the early morning that G. V. S. did and his sleep was likewise broken afterwards.

Control Experiment IV. — Subject, H. R. D., April 20–21, 1906. The data given here are from an experiment made on April 20–21, 1906, when the subject fasted during the day of April 20, made his preparations for bed

TABLE X.

METABOLISM OF H. R. D. CONTROL EXPERIMENT IV. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Resp. rate per min.
April 20–21, 1906.	gm.	gm.	gm.		Cal.	Cal.	°C.		
9.00 P.M.–11 P.M.	22.7	22.8	17.6	.94	74.7	..	36.08 ..	56	16
11.00 P.M.– 1 A.M.	21.0	22.2	20.1	.76	64.6	..	..	52	16
1.00 A.M.– 3 A.M.	20.9	21.3	18.4	.83	65.4	..	..	67	17
3.00 A.M.– 5 A.M.	21.7	21.8	21.0	.75	65.9	..	36.23	60	16
5.00 A.M.– 7 A.M.	23.5	22.4	24.0	.71	67.9	75.9	36.55	67	16
7.00 A.M.–8.10 A.M.	36.9	26.9	41.0	.65	94.7	123.7	36.97	85	18
8.10 A.M.–10.10 A.M.	31.1	26.4	27.4	.82	92.6	90.2	36.93	79	19
10.10 A.M.–12.10 P.M.	34.0	27.4	28.3	.87	92.4	92.9	37.01	82	20
12.10 P.M.– 1.10 P.M.	32.2	26.6	23.6	.99	89.7	84.8	36.92	83	21
1.10 P.M.– 2.10 P.M.							36.88	77	19
2.10 P.M.– 3.10 P.M.	29.1	24.6	21.6	.98	85.2	82.2	36.86	78	19
3.10 P.M.– 4.10 P.M							36.84	70	18

at the usual time, and slept during the night until about 6.30 A. M. on April 21. After 7 A. M. he ate 1171 gm. of bananas and 103 gm. of sugar, the energy being 1581 calories and the nitrogen 2.10 gm. After eating his breakfast he sat idle or reading during the remainder of the time. These data are used for comparison with data obtained in the experiment with H. E. S. on November 4–5, 1905, in which the subject went to bed at the usual time. The subject reported that he was awake at 2 A. M., but went to sleep again. In the morning he got up at 7 o'clock and in the next

hour ate 308 gm. of cream, 7 gm. of breakfast cereal, 9 gm. of bread, and 4 gm. of butter, the energy being about 143 calories and the nitrogen about 0.40 gm. After breakfast the subject was weighed, as were also the chair and clothes. He then walked from 10.12 until 10.52 A. M. He was more or less active until about 12.45 P. M., when he lay down and must have slept for an hour or so. The experiment of April 20–21 with H. R. D. has been chosen for comparison with the experiment on November 4–5 with H. E. S., because the men were of about the same body weight. H. E. S. weighed 60.7 kilos and H. R. D. weighed 62.5. Both weights were with clothing. H. R. D. ate considerably more food, and the energy and nitrogen of the food were considerably greater than eaten in the H. E. S. experiment. H. R. D. was not nearly so active immediately following the eating of the food as was H. E. S., who weighed himself and chair and clothes and was otherwise more or less active, besides walking about for 40 minutes during the two hours succeeding the breakfast period.

The data for the metabolism are given in Table X herewith.

Owing to a defective connection, the electrical rectal thermometer did not give readings during the early part of the night, and hence the heat production cannot be accurately computed. It is evident that the changes in body temperature must have been small during this time, and hence the heat elimination is probably a very close index of the true heat production.

Control Experiment V. — Subject, H. D. A., November 14–15, 1905. The data included under this head are for the afternoon and night of November 14 and the morning of November 15 to compare with the corresponding period of a fever experiment made on November 2–3 with the same subject. The subject ate supper at about the accustomed time in the calorimeter on November 2. After supper he sat reading during the evening and then prepared to retire at the usual time and slept until morning, but not very well. The morning following the night of sleep was spent in a certain amount of activity for a couple of hours in the midst of which he slept perhaps 20 minutes. The remainder of the forenoon he was lying down mostly and slept into the early afternoon. At 6 P. M. on November 14 the subject ate supper consisting of 60 gm. of shredded wheat and 245.4 gm. of cream; the energy being about 739 calories and the nitrogen about 2.00 gm. Following supper, he spent the evening sitting reading, writing, and some studying. Then he prepared for bed at the usual time and retired and, so far as known, slept well until morning. The forenoon following his night of sleep was spent with a certain amount

of activity for an hour or so, and then the subject lay on the bed most of the remainder of the time.

The metabolism in the control experiment is given in Table XI herewith.

TABLE XI.

METABOLISM OF H. D. A. CONTROL EXPERIMENT V. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Respiration rate per min.
Nov. 14-15, 1905.	gm.	gm.	gm.		Cal.	Cal.	°C.		
							37.23		
3 P. M.– 5 P. M.	38.3	38.4	33.7	.83	97.7	94.8	37.15	70	16
5 P. M.– 7 P. M.	37.1	39.6	34.7	.78	105.9	112.4	37.37	70	19
7 P. M.– 9 P. M.	34.9	39.5	32.9	.77	105.2	100.1	37.21	73	17
9 P. M.–11 P. M.	29.5	33.6	25.4	.85	99.4	93.5	37.02	71	15
11 P. M.– 1 A. M.	26.7	35.2	27.8	.70	85.7	89.9	37.23	70	13
1 A. M.– 2 A. M.	25.3	38.6	23.9	.77	73.6	77.6	37.25	68	13
2 A. M.– 3 A. M.							37.39	67	14
3 A. M.– 4 A. M.	27.4	49.8	..	..	84.9	84.5	37.41	67	15
4 A. M.– 5 A. M.							37.41	67	13
5 A. M.– 6 A. M.	29.8	53.2	27.8	.78	84.6	79.2	37.51	..	..
6 A. M.– 7 A. M.							37.26	..	..
7 A. M.– 8 A. M.	38.5	53.7	40.0	.70	94.5	84.6	37.18	..	..
8 A. M.– 9 A. M.							37.02	..	..
9 A. M.–10 A. M.	28.6	51.7	30.0	.69	78.9	82.7	37.11	..	..
10 A. M.–11 A. M.							37.18	..	..

The pneumograph failed to operate satisfactorily after 5 A. M., so the pulse and respiration rates are wanting.

Control Experiment VI. — Subject, A. H. M., November 13–14, 1905. The subject entered the chamber at 4 P. M. and at 6 ate supper consisting of bread, 190.2 gm.; cheese, 40.3 gm.; milk, 256 gm.; and butter, 12.7 gm.; the energy being about 1031 calories and the nitrogen about 5.78 gm. Following his supper the subject spent the evening reading or sitting idly, and becoming drowsy he prepared for bed and probably went to sleep

immediately after 11 o'clock and had a good night's sleep. In the morning he arose at 7 o'clock and ate 101 gm. of bananas, 226.5 gm. of milk, and 86.8 gm. of bread for his breakfast. The energy of the food was about 522.1 calories and the nitrogen about 2.69 gm. After breakfast he was lying down until noontime except that he got up occasionally.

TABLE XII.

METABOLISM OF A. H. M. CONTROL EXPERIMENT VI. (QUANTITIES PER HOUR.)

Period.	Carbon dioxide exhaled.	Water vaporized.	Oxygen consumed.	Respiratory quotient.	Heat eliminated.	Heat produced.	Body temperature.	Pulse rate per min.	Respiration rate per. min.
Nov. 14-15, 1905.	gm.	gm.	gm.		Cal.	Cal.	° C.		
5 P.M.– 7 P.M.	33.5	37.1	27.7	.88	104.7	98.3	37.00 36.69	59	21
7 P.M.– 9 P.M.	30.4	39.9	30.7	.72	101.7	..	..	..	22
9 P.M.–11 P.M.	23.8	31.9	23.8	.73	87.7	..	..	44	23
11 P.M.– 1 A.M.	21.5	35.9	22.2	.71	75.4	..	..	41	15
1 A.M.– 3 A.M.	17.4	32.5	20.2	.63	54.5	..	..	40	16
3 A.M.– 5 A.M.	20.1	30.7	19.8	.74	55.2	..	..	41	17
5 A.M.– 6 A.M. 6 A.M.– 7 A.M.	22.8	31.1	25.3	.65	56.5	..	.. ..	42 44	16 20
7 A.M.– 8 A.M. 8 A.M.– 9 A.M.	35.9	33.0	36.6	.71	82.5	..	.. 36.98	.. 65	.. 23
9 A.M.–10 A.M. 10 A.M.–11 A.M.	29.6	35.7	26.3	.82	85.3	89.2	37.12 37.16	49 48	24 25
11 A.M.–12.15 P.M.	24.3	31.2	20.6	.86	81.6	81.8	37.25	43	24

This experiment is used for comparison with an experiment made with the same subject on November 8–9, 1905, during which the subject spent the evening of November 8 in reading or lying on the bed following supper (amount eaten unknown). He adjusted his bed to lie down about half an hour before bedtime, then prepared for bed and retired at 11 o'clock. He said he slept well all night. Following his breakfast he lay down and was lying down most of the time until he was taken from the chamber along in the afternoon. He slept some of the time, was up occasionally, and later he coughed a great deal. The activity during the two experi-

ments was much the same, except that during the forenoon of November 14 the subject did not sleep, but on the other hand he was not quite so active as he was when awake during the morning of November 9.

The metabolism in this experiment is given in Table XII herewith.

In this experiment of November 13–14, unfortunately the rectal thermometer did not operate properly, and it was impossible to get accurate temperature observations between 7 P. M. and 9 A. M., and hence the heat production cannot be satisfactorily computed. The probable difference between heat production and heat elimination may, however, be reasonably estimated when the regular curve for body temperature is taken into consideration. Usually there is a marked fall in temperature immediately after the subject goes to bed, persisting for an hour or more. The result would be that during this period there would be a loss of heat from the body and apparently a greater heat elimination, and the heat production would be somewhat smaller than the heat elimination. On the other hand, beginning with about 5 o'clock in the morning, there is a noticeable temperature rise, which increases rapidly after the subject gets out of bed, so that during the periods from 5 to 8 the heat elimination is somewhat less than the heat production, as part of the produced heat is used to warm the body. Under the conditions obtaining inside the respiration chamber when the muscular activity is very slight, these temperature fluctuations are not very noticeable, and hence for purposes of comparison it is reasonable to assume that the heat production does not differ greatly from the heat elimination. As a matter of fact, in two other experiments made with this same subject, covering the same periods of time, the heat production did not differ from the heat elimination more than 4 calories in any period, thus substantiating this view.

Discussion of Results.

The most striking feature regarding these experiments is the marked and rapid temperature rise in certain of them. This was in almost every instance accompanied by a marked increase in the respiration rate. On the other hand, in some experiments, namely, with G. V. S., where the temperature rise was not very marked, the respiration rate increased noticeably, thus indicating a respiratory disturbance.

The pulse rate also increased during the febrile stage. Unfortunately the pulse rate could not be obtained in the first and second experiments, as the technique was not perfected at that time for securing this information.

Carbon dioxide production. —The carbon dioxide production is commonly considered as a general index of the total metabolism, although obviously with variations in the proportions of fat and carbohydrates burned, the energy accompanying this combustion will vary per gram of carbon dioxide. However, as a general index of metabolism, the carbon dioxide may still be taken as fairly accurate. In these experiments the carbon dioxide determinations in both fever and control experiments have been combined in Table XIII for purpose of comparison. Bearing in mind the difficulties of comparing experiments of this nature, particularly when the control experiments must be made with a subject other than that used in the fever experiments, the evidence is still very striking to show that the carbon dioxide per hour during fever is somewhat greater than that during the control. With C. R. Y. this is shown in practically all periods in which there was a marked temperature increase. Unfortunately the long periods of observation in the experiments with F. E. S., G. V. S., and A. H. M. could not permit of the careful apportionment over the short periods as do the others, but nevertheless the carbon dioxide excretion is apparently greater during the fever than during the control.

Oxygen consumption. — While there are variations in the amount of energy per gram of carbon dioxide depending upon whether the combustion is of fats or carbohydrates, these differences disappear in large part when measurements of oxygen are taken into consideration, as the number of calories per gram of oxygen is not widely different whether the substance burned be fat, carbohydrate, or protein. Accordingly we would expect to find in experiments of this type the oxygen consumption as especially indicative of the total metabolism. Before considering the figures for these experiments in both fever and control, it is necessary to bear in mind that the determinations of oxygen with the respiration apparatus here used, while extremely accurate for experiments of twenty-four hours' duration, are by no means so accurate for shorter periods. The large volume of residual air, some 4500 litres, is subject to considerable temperature variations, particularly when there are differences in the bodily activity at the beginning and end of the

experimental period. In determining the oxygen according to this method, the absolute volume of gas inside the respiration chamber

TABLE XIII.

CARBON DIOXIDE PER HOUR DURING FEVER AND CONTROL.

Subject.	1 A. M. to 3 A. M.	3 A. M. to 5 A. M.	5 A. M. to 7 A. M.	7 A. M. to 9 A. M.	9 A. M. to 11 A. M.	11 A. M. to 1 P. M.	1 P. M. to 3 P. M.	3 P. M. to 5 P. M.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
C. R. Y. Fever 1 . .	29.4	22.9	24.9	38.7	31.2	33.4	38.1	
C. R. Y. Control I .	23.8	25.1	26.2	36.8	29.4	27.6	24.5	
H. E. B. Fever 2 . .	26.8	30.2	31.8	...	...	...	..	..
H. R.D. Control II .	18.3	20.1	21.3	...	...	...	..	..
H. D. A. Fever 3 . .	27.7	30.0	28.9	38.6	31.5	33.7	...	...
H. D. A. Control III.	25.3	27.4	29.8	38.5	28.6	...	...	...
F. E. S. Fever 4 . .	28.2				37.8			
H. R. D. Control IV.	20.9	21.7	23.5	36.9[1]	31.1	34.0	32.2	29.1
G. V. S. Fever 5 . .	25.6							
H. R. D. Control II .	18.3	20.1	21.3	...	...	...	...	...
A. H. M. Fever 6 . .	25.9				30.3[2]			
A. H. M. Control VI	17.4	20.1	22.8	35.9	29.6	24.3[3]	...	...
Nov. 21[4] " "	17.4	19.0	20.0	...	...	...	...	...
Mar. 23[4] " "	...	...	...	...	26.5[5]	25.4	..	...

[1] 7.00 A. M.–8.10 A. M. The remaining figures for this experiment are for two-hour periods following 8.10 A. M.
[2] 9.00–12.00 noon. [3] 11.00 A.M.–12.15 P.M.
[4] Control experiment made with same subject and introduced for further comparison.
[5] 8.30 A. M.–9.30 A. M.

must be known with great accuracy, and hence the temperature fluctuations can influence the accuracy of this calculation in a marked degree. This feature of the determination of oxygen consump-

TABLE XIV.

OXYGEN CONSUMED PER HOUR IN FEVER AND CONTROL.

<table>
<tr><th>Subject.</th><th>1 A.M. to 3 A.M.</th><th>3 A.M. to 5 A.M.</th><th>5 A.M. to 7 A.M.</th><th>7 A.M. to 9 A.M.</th><th>9 A.M. to 11 A.M.</th><th>11 A.M. to 1 P.M.</th><th>1 P.M. to 3 P.M.</th><th>3 P.M. to 5 P.M.</th></tr>
<tr><td></td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td></tr>
<tr><td>C. R. Y. Fever 1 . .</td><td>22.8</td><td>20.4</td><td>23.6</td><td>36.9</td><td>26.3</td><td>31.3</td><td>31.4</td><td>...</td></tr>
<tr><td>C. R. Y. Control I .</td><td>20.2</td><td>19.6</td><td>16.5</td><td>35.4</td><td>22.0</td><td>23.2</td><td>19.7</td><td>...</td></tr>
<tr><td>H. E. B. Fever 2 . .</td><td>20.5</td><td>21.8</td><td>24.6</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>H. R. D. Control II .</td><td>16.2</td><td>21.0</td><td>16.3</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>H. R. D. Control III</td><td>21.1</td><td>19.4</td><td>17.4</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>H. D. A. Fever 3 . .</td><td>24.8</td><td>27.4</td><td>26.4</td><td>38.8</td><td>28.9</td><td>29.8</td><td>...</td><td>...</td></tr>
<tr><td>H. D. A. Control V .</td><td>23.9</td><td>...</td><td>27.8</td><td>40.0</td><td>30.0</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>F. E. S. Fever 4 . .</td><td colspan="4">22.2</td><td colspan="3">34.1</td><td></td></tr>
<tr><td>H. R. D. Control IV</td><td>18.4</td><td>21.0</td><td>24.0</td><td>41.0[1]</td><td>27.4</td><td>28.3</td><td>23.6</td><td>21.6</td></tr>
<tr><td>G. V. S. Fever 5 . .</td><td colspan="3">22.6</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>H. R. D. Control II .</td><td>16.2</td><td>21.0</td><td>16.3</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>H. R. D. " III</td><td>21.1</td><td>19.4</td><td>17.4</td><td>...</td><td>...</td><td>...</td><td>..</td><td>...</td></tr>
<tr><td>A. H. M. Fever 6 . .</td><td colspan="4">22.9</td><td colspan="2">27.9[2]</td><td></td><td></td></tr>
<tr><td>A. H. M. Control VI</td><td>20.2</td><td>19.8</td><td>25.3</td><td>36.6</td><td>26.3</td><td>20.6[3]</td><td>...</td><td>...</td></tr>
<tr><td>Nov. 21[4] " "</td><td>14.1</td><td>15.5</td><td>16.1</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>Mar. 23[4] " "</td><td>...</td><td>...</td><td>...</td><td>...</td><td>23.2[5]</td><td>20.0</td><td>...</td><td>...</td></tr>
</table>

[1] 7.00 A.M.–8.10 A.M. The remaining figures for this experiment are for two-hour periods following 8.10 A.M.
[2] 9.00–12 noon.
[3] 11 A.M.–12.15 P.M.
[4] Control experiment made with same subject and introduced for further comparison.
[5] 8.30 A.M.–9.30 A.M.

tion with this apparatus has been discussed at considerable length elsewhere.[9]

In the particular experiments here under discussion, however, the bodily activity aside from going to bed at night and getting up in the morning was in the majority of the periods reasonably constant. It is possible, therefore, in these experiments to use the data for the oxygen with a reasonable feeling of confidence that they are fairly typical of the exact oxygen consumption during the periods under discussion.

The data for oxygen absorption both during fever and control have been compiled in Table XIV for comparison.

The oxygen consumption during fever is in practically all cases noticeably greater than during control. The only exception to this is in the case of the experiment with H. D. A.

Respiratory quotient. — It is conceivable that during fever there may be a greater draft upon previously stored glycogen, and hence one might expect a variation in the respiratory quotient to correspond to this increase in carbohydrate burned during the febrile state. As a matter of fact, the respiratory quotients as determined in these experiments have not been tabulated, especially as it is believed that the possible errors in the oxygen determination would render any deductions from them liable to error. They have been given in connection with each experiment, and while the data show a slight tendency for the respiratory quotient to increase during fever, the complications attending the ingestion of food, variations in muscular activity, and errors in oxygen determination do not warrant any sweeping deductions from these data.

Water vaporized. — The special significance of the determination of water vapor in these experiments was to add to the heat brought away by the water current of the respiration calorimeter the heat of vaporization of water, as this amounts to 0.586 calories per gram of water. Hence in these experiments no particular study was made of water vaporized other than for this purpose. The data have been collected and summarized in Table XV herewith.

The figures show that in general there was an increase in the water of vaporization during fever over that during the control period. Since, however, the control experiments showed marked

[9] F. G. Benedict: Publication No. 77 of the Carnegie Institution of Washington, p. 451, and F. G. Benedict and R. D. Milner: Bulletin 175 of the Office of Experiment Stations, U. S. Department of Agriculture, pp. 28–30.

variations when compared with the fever experiments during periods when there was no appreciable fever, it is obvious that here again we cannot draw any sweeping deductions regarding this point.

TABLE XV.

Water Vaporized per Hour in Fever and Control.

<table>
<tr><th>Subject.</th><th>1 A.M. to 3 A.M.</th><th>3 A.M. to 5 A.M.</th><th>5 A.M. to 7 A.M.</th><th>7 A.M. to 9 A.M.</th><th>9 A.M. to 11 A.M.</th><th>11 A.M. to 1 P.M.</th><th>1 P.M. to 3 P.M.</th><th>3 P.M. to 5 P.M.</th></tr>
<tr><td></td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm.</td><td>gm</td></tr>
<tr><td>C. R. Y. Fever I . .</td><td>58.2</td><td>46.2</td><td>44.2</td><td>38.0</td><td>42.9</td><td>40.1</td><td>44.8</td><td>...</td></tr>
<tr><td>C. R. Y. Control I .</td><td>41.6</td><td>38.5</td><td>37.8</td><td>34.3</td><td>24.5</td><td>42.8</td><td>37.9</td><td></td></tr>
<tr><td>H. E. B. Fever 2 . .</td><td>39.9</td><td>41.9</td><td>43.0</td><td>...</td><td>...</td><td>...</td><td>..</td><td>...</td></tr>
<tr><td>H. R. D. Control II.</td><td>36.5</td><td>31.2</td><td>30.2</td><td>...</td><td>...</td><td>...</td><td>..</td><td>...</td></tr>
<tr><td>H. R. D. " III</td><td>22.8</td><td>22.2</td><td>21.1</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>H. D. A. Fever 3 . .</td><td>54.7</td><td>53.4</td><td>56.1</td><td>49.8</td><td>59.4</td><td>39.5</td><td>...</td><td>...</td></tr>
<tr><td>H. D. A. Control V</td><td>38.6</td><td>49.8</td><td>53.2</td><td>53.7</td><td>51.7</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>F. E. S. Fever 4 . .</td><td colspan="4">42.5</td><td colspan="3">41.2</td><td></td></tr>
<tr><td>H. R. D. Control IV</td><td>21.3</td><td>21.8</td><td>22.4</td><td>26.9 [1]</td><td>26.4</td><td>27.4</td><td>26.6</td><td>24.6</td></tr>
<tr><td>G. V. S. Fever 5 . .</td><td colspan="3">49.8</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>H. R. D. Control II</td><td>36.5</td><td>31.2</td><td>30.2</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>H. R. D. " III</td><td>22.8</td><td>22.2</td><td>21.1</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>A. H. M. Fever 6 .</td><td colspan="4">36.7</td><td colspan="2">33.2 [2]</td><td></td><td></td></tr>
<tr><td>A. H. M. Control VI</td><td>32.5</td><td>30.7</td><td>31.1</td><td>33.0</td><td>35.7</td><td>31.2 [3]</td><td>...</td><td>...</td></tr>
<tr><td>Nov. 21 [4] " "</td><td>30.0</td><td>27.8</td><td>27.0</td><td>...</td><td>...</td><td>...</td><td>...</td><td>...</td></tr>
<tr><td>March 23 [4] " "</td><td>...</td><td>...</td><td>...</td><td>...</td><td>28.7 [5]</td><td>28.8</td><td>...</td><td>...</td></tr>
</table>

[1] 7.00 A.M–8.10 A.M. The remaining figures for this experiment are for two-hour periods following 8.10 A.M.
[2] 9.00–12 noon.
[3] 11 A.M.–12.15 P.M.
[4] Control experiment made with same subject and introduced for further comparison.
[5] 8.30 A.M.–9.30 A.M.

An increased water of vaporization output might be expected as a result of the increased respiration rate during fever in that more air would be drawn into the lungs, there saturated with water and expelled again. This is, however, on the assumption that the total ventilation of the lungs is greater during these fever experiments than during the control. It is greatly to be questioned, however, whether or not the increased respiration rate was not in part compensated by a diminished volume, for, as noted elsewhere,[10] a number of the subjects complained of difficulty in taking long breaths and the respiration was of a decidedly shallow type. Hence we are not sure that there was a greater total ventilation during the febrile period.

The mere fact that the body temperature is increased some 1 or 2 degrees C. would not in itself lead us to expect any marked increase in the amount of water vaporized from the surface of the body, but obviously more elaborate experiments to study this particular point should be made before any accurate deductions can be drawn. The present data indicate that a greater amount of water is vaporized from the lungs and skin during fever than under normal conditions.

Heat eliminated. — These experiments permit of an interesting comparison of the heat elimination during fever and during control. The results have been tabulated for comparison in Table XVI herewith.

An examination of the results shows an interesting contradiction. In all experiments but that with A. H. M. there is a noticeable increase in the heat elimination during the fever periods. In the experiment with A. H. M., on the other hand, there is in some instances an actual decrease. This can, however, in part at least, be accounted for by the fact that during the fever experiments this subject spent most of the forenoon lying on the bed reading or sleeping, and hence the conditions are not ideal for comparison. If we consider particularly those periods during the night when the subjects were in bed and presumably asleep, the evidence is all in favor of the assumption that there was a considerable increase in the heat elimination, the only exception to this being the experiment with F. E. S., controlled by the experiment with H. R. D., another subject. Here the contrary is indicated, as the heat elimination was somewhat larger in the control period.

[10] T. M. Carpenter and F. G. Benedict: This journal, 1909, xxiv, p. 194

TABLE XVI.

HEAT ELIMINATED PER HOUR IN FEVER AND CONTROL.

Subject.	1 A. M. to 2 A. M. Cal.	2 A. M. to 3 A. M. Cal.	3 A. M. to 4 A. M. Cal.	4 A. M. to 5 A. M. Cal.	5 A. M. to 6 A. M. Cal.	6 A. M. to 7 A. M. Cal.	7 A. M. to 8 A. M. Cal.	8 A. M. to 9 A. M. Cal.	9 A. M. to 10 A. M. Cal.	10 A. M. to 11 A. M. Cal.	11 A. M. to 12 A. M. Cal.	12 A. M. to 1 P. M. Cal.	1 P. M. to 2 P. M. Cal.	2 P. M. to 3 P. M. Cal.	3 P. M. to 4 P. M. Cal.
C. R. Y. Fever 1	78.7		73.3		73.4		92.4		90.8	92.8	89.9	84.6	79.6		...
C. R. Y. Control II	74.5		75.0		56.0		104.8		87.6		77.4		67.1		...
H. E. B. Fever 2	52.5	58.9	85.0	74.8	78.1	76.4	...	...	...	...	...	...	...	...	...
H. R. D. Control II	64.1		61.7		59.4		...	...	...	...	...	...	...	...	...
H. R. D. " III	60.5		61.8		63.0		...	...	...	...	...	...	...	...	...
H. D. A. Fever 3	79.7	78.8	85.3	90.5	82.8	100.6	131.5	94.1	105.5	92.8	110.8	70.7	99.1	...	...
H. D. A. Control V	73.6		84.9		84.6		94.5		78.9		...	...	...	...	...
F. E. S. Fever 4	65.5		61.0		59.3		105.6		111.6		108.9		71.2	102.0	
H. R. D. Control IV	65.4		65.9		67.9		94.7	92.6		92.4		89.7		85.2	
G. V. S. Fever 5	62.9		73.4	57.3	82.5	65.2	...	...	...	...	...	...	...	...	...
H. R. D. Control II	64.1		61.7		59.4		...	...	...	...	...	...	...	...	...
H. R. D. " III	60.5		61.8		63.0		...	...	...	...	...	...	...	...	...
A. H. M. Fever 6	57.3		59.4		67.1	83.2	86.6	76.2	68.6	62.3	89.7	...	...	...	...
A. H. M. Control VI	54.5		55.2		56.5		82.5		85.3		...	...	...	...	...
Nov. 21 [1] " "	52.6		54.1		53.8		...	...	...	...	...	...	...	...	...
March 23 [1] " "	...	...	...	...	...	...		85.6		79.4		...	...	...	...

[1] Control experiments made with same subject and introduced for further comparison.

In view of the necessarily tentative nature of all deductions made from these experiments, it has not been deemed advisable to attempt to discuss the influence of fever on the various paths of heat elimination. As an extensive investigation into the total metabolism and heat production of the various types of fevers is soon to be undertaken, such discussion can profitably be left until greater and more accurate and positive data have been accumulated.

Heat production. — The marked temperature rise in many of these experiments results in the storage of a considerable amount of heat within the body, and accordingly the heat elimination as measured by the heat given off from the body supplemented by the heat required to vaporize the water does not give a correct impression of the heat actually produced. It is necessary, therefore, to correct the heat elimination for changes in body temperature.[11]

The results for these fever and control experiments have been computed and compared in Table XVII herewith.

Whatever doubt may exist with regard to the increase of carbon dioxide production, oxygen consumption, water vaporization, and heat elimination, there can be no doubt that during these experiments there was a marked increase in heat production. In practically every instance we find, during the periods when fever was at its highest, a very noticeable increase in the heat production. In considering this table it is important to distinguish between those periods which are averaged together. Thus, in the period from 1 P. M. to 2 P. M. with F. E. S. the heat produced was 79.5 calories, while apparently in the control period with H. R. D. it was 84.8 calories. However, if we average the period from 12 noon to 1 P. M., and 1 P. M. to 2 P. M., we obtain evidence of distinct heat increase. This is but another indication of the disadvantage of comparing two experiments on two different individuals. When we compare the experiments made with C. R. Y., H. D. A., and A. H. M., in which the control was made with the same person, the figures show conclusively that during the febrile stage there is a marked increase in the total heat production.

Unfortunately the data do not throw any light upon the heat production during the period when the body temperature remains constant nor during defervescence of the fever. To be sure, in the

[11] For a complete discussion of this point, see Publication No. 77 of the Carnegie Institution of Washington, pp. 46–50.

TABLE XVII.

HEAT PRODUCED PER HOUR IN FEVER AND CONTROL.

Subject.	1 A. M. to 2 A. M. Cal.	2 A. M. to 3 A. M. Cal.	3 A. M. to 4 A. M. Cal.	4 A. M. to 5 A. M. Cal.	5 A. M. to 6 A. M. Cal.	6 A. M. to 7 A. M. Cal.	7 A. M. to 8 A. M. Cal.	8 A. M. to 9 A. M. Cal.	9 A.M. to 10 A.M. Cal.	10 A. M. to 11 A. M. Cal.	11 A. M. to 12 noon Cal.	12 noon to 1 P. M. Cal.	1 P.M. to 2 P.M. Cal.	2 P. M. to 3 P. M. Cal.	3 P. M. to 4 P. M. Cal.
C. R. Y. Fever 1 . .	79.6		74.4		79.2		97.1		108.0	1	111.4	112.4	108.0		...
C. R. Y. Control I .	72.4		79.6		63.7		107.2		85.8		71.1		66.7		...
H. E. B. Fever 2 . .	73.5	79.9	100.9	84.6	85.9	82.6	...	...	...	...	...	...	...	...	...
H. R. D. Control II .	57.1		59.3		69.4		...	...	...	...	...	...	...	...	...
H. R.D. Control III	69.5		61.0		76.4		...	...	...	...	...	...	...	...	...
H. D. A. Fever 3 . .	90.3	95.1	97.6	96.5	90.4	94.5	117.5	100.0	115.5	97.6	120.8	90.3	109.8	...	...
H. D. A. Control III	77.6		84.5		79.2		84.6		82.7		...	...	...	...	...
F. E. S. Fever 4 . .	61.1		64.1		70.0		111.8		130.4		108.4		79.5	97.9	...
H. R. D. Control IV	...		...		75.9		123.7	90.2		92.9		84.8		82.2	
G. V. S. Fever 5 . .	64.5		80.7	64.6	96.2	74.8	...	...	...	...	...	...	...	...	...
H. R. D. Control II	57.1		59.3		69.4		...	...	...	...	...	...	...	...	...
H. R. D. " III	69.5		61.0		76.4		...	...	...	...	...	...	...	...	...
A. H. M. Fever 6 . .	49.2		63.3		86.4	124.6	94.8	115.5	91.0	105.9	88.1	...	...	...	...
A. H. M. Control VI	...		...		...		...		89.2		81.8	...	...	...	...
Nov. 21[1] " "	54.6		54.5		57.9		...	...	...	...	...	...	...	...	...
March 23[1] " "	...	...	...	...	...	...	...	84.9		78.3		...	...	...	...

[1] Control experiments made with same subject and introduced for further comparison.

case of F. E. S., the body temperature remained practically constant from 12 noon to 3 P. M. and there was an indication of a noticeable falling off in the heat production. Similarly in the case of A. H. M. the temperature had reached a maximum at 11 A.M. and from 11 to 12 noon, the heat production decreased noticeably. From these few observations we might infer that the heat production after the body temperature had ceased rising was considerably less than during the period of temperature rise, but further experiments on fever will be planned to include observations on this point.

THE VARIATIONS IN THE ENZYME CONCENTRATION WITH THE VARIATION IN THE BLOOD SUPPLY TO THE SECRETING GLAND.

By J. G. RYAN.

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

I. Introductory.

THE experiments reported in this paper are a continuation of the studies of Carlson, Greer, and Becht, Carlson and McLean,[1] on the variations in the composition of the saliva that follow the variation in the blood supply to the glands. The work was undertaken under Professor Carlson's direction and assistance. In some of the experiments I was aided by Mr. A. B. Luckhardt.

The main aim of the work was the determination of the relative concentration of the ferments in the saliva produced by stimulation of Jacobson's nerve, or reflexly, and that produced by the stimulation of the cervical sympathetic; and, secondly, to ascertain whether these differences can be duplicated by variations in the blood supply to the active gland. The studies of Carlson have shown that the concentration of the organic solids in the saliva increases *pari passu* with the diminution in the blood supply to the gland. If it should be found that the enzyme concentration runs parallel with that of the organic constituents, this might afford a clew to the structure of the enzyme itself.

The mechanism of secretion of ferments and enzymes, their chemical composition and relation to organic substances, particularly the proteids, have long been matters of interest and research. Quite a number of the well-known enzymes, *e. g.*, ptyalin[2] and lipase,[3] although not yet isolated in absolutely pure form, have been found not to give any of the proteid reactions; so it seems

[1] Carlson, Greer, and Becht: This journal, 1907, xix, pp. 360, 408, xx, p. 180; Carlson and McLean: This journal, 1908, xx, p. 457, xxii, p. 279.

[2] Cohnheim: Virchow's Archiv für pathologische Anatomie, 1863, xxviii, p. 241.

[3] Nichol: Personal communication.

that, in case of part of the enzymes at least, the proteid reactions formerly obtained represented impurities and not the true chemical nature of the enzymes themselves.

There is some evidence in favor of the idea that enzymes are derivatives of the substances upon which they act; *e. g.*, yeast growing on galactose does not ferment it at first, but will after some time has elapsed, — the conditions during the growth of the yeast having become such as to favor the formation of an enzyme or ferment from the galactose itself. This view, however, could not be true in the strict sense in the case of ptyalin, since the ptyalin is produced within the salivary gland cells, and in the absence of the substance upon which it exerts its action, — starch.

II. Literature.

Becher and Ludwig[4] found that the submaxillary corda saliva grows poorer in organic solids as the gland approaches fatigue. This was confirmed by Heidenhain,[5] who also showed that stimulation of Jacobson's nerve in the dog gives watery saliva from the parotid, while stimulation of the cervical sympathetic nerve gives a viscid saliva.

Langley,[6] by microscopic examination of the parotid of the rabbit, observed that a more rapid change takes place in the gland cells during stimulation of the cervical sympathetic than during stimulation of Jacobson's nerve. None of these observers, however, took occasion to test the amylolytic activity of the saliva collected under the different conditions of secretion.

Hofbauer[7] demonstrated that the activity of human mixed saliva fluctuates during the course of twenty-four hours, being greater before breakfast than after, — that is to say, the amylolytic activity of the saliva was diminished after a period of activity of the glands. He did not attempt to show any relation between the amylolytic power and the chemical composition of the saliva.

Chittenden and Ely[8] carried out a series of experiments on

[4] Becher and Ludwig: Zeitschrift für rationelle Medizin, 1851, i, p. 278.

[5] Heidenhain: Archiv für die gesammte Physiologie, 1878, xvii, p. 28; Hermann's Handbuch, v, p. 55.

[6] Langley: Journal of physiology, 1880, ii, p. 261.

[7] Hofbauer: Archiv für die gesammte Physiologie, 1897, lxv, p. 503.

[8] Chittenden and Ely: American chemical journal, 1883, iv, p. 329.

human mixed saliva collected between 9 and 10 A. M. in order to show the relation of alkalinity (the alkaline salts contained in the saliva) to the amylolytic power. They obtained fairly constant results for the same individual, but the results for different individuals fluctuated greatly, showing no corresponding differences nor relations between the amylolytic power and the alkalinity.

Langley [9] states that the alkalinity of mixed human saliva is least when fasting (as before breakfast), and increases, reaching its maximum during or after eating. On the other hand, Chittenden and Richards,[10] while studying the variations in amylolytic power and chemical composition of human saliva, found the alkalinity, acidity, and amylolytic power to be greater before breakfast than after breakfast, — the same being true for dinner, but to a less marked degree. This suggests some degree of relationship between the amylolytic power and the alkaline salts contained in the saliva. However, in view of the fact that ptyalin acts best in neutral solutions,[11] we would expect a greater amylolytic power after breakfast, when the alkalinity and acidity are reduced. Chittenden and Richards call attention to these points, and their results show that the differences in acidity and alkalinity before and after breakfast bear no close parallelism with the amylolytic power. They are forced to the conclusion that the greater activity of saliva before breakfast means simply a higher concentration of ptyalin. In the same series of experiments they also determined the relation of the amylolytic power to the organic and inorganic solids in the saliva. Their results, although variable for different individuals, showed, on the whole, more organic and inorganic matter in the saliva before meals, and the stronger amylolytic power nearly always corresponded to the greater amount of organic and inorganic solids.

III. Experimental Methods.

Large and medium sized rabbits were used in all experiments, and the various samples of saliva collected under different conditions of secretion were tested comparatively as to their relative

[9] Langley: Text-book of physiology, 1898, i, p. 504.

[10] Chittenden and Richards: This journal, 1898, i, p. 461.

[11] Langley and Eves: Journal of physiology, 1883, iv, p. 18; Chittenden and Smith: Studies in physiological chemistry, Yale Univ., 1885, i, p. 8.

amylolytic powers. The concentration of the ptyalin was determined (1) by the rate of clearing of the starch solution and (2) by the complete disappearance of erythrodextrin. A 1 per cent solution of arrowroot starch was used, and the saliva and starch were always mixed in the proportion of 1 c.c. to 250 c.c. In a majority of the experiments where series of samples were collected it was impossible to obtain more than 0.5 to 1 c.c. in each sample, thus making it necessary to use small amounts of saliva in order that several tests could be made from each sample to check up the results and eliminate experimental errors. Therefore, to maintain the ratio of 1 c.c. to 250 c.c. as mentioned above, 0.1 c.c. of pure undiluted saliva from the various samples was carefully measured out into very small specially prepared glass receptacles of uniform size and depth and dropped simultaneously into narrow-necked 100 c.c. flasks containing 25 c.c. of starch, and after shaking the mixtures for the same length of time and with the same intensity, they were allowed to stand at room temperature, — the rate of clearing and the disappearance of the erythrodextrin being taken as evidence of the rate of action. After the first general comparative tests were completed, a second test was always made to obtain more accurately the relative percentages of ptyalin in the different samples collected under different conditions of secretion. At first this was tried by diluting the highly active samples with physiological salt solution to various strengths and comparing these dilutions with the less active ones. But finding it extremely difficult to get uniform mixtures with such small amounts of saliva, and to obviate the possibility of any retarding or accelerating effect that the salt solution might have upon the rate of action of the ptyalin, this source of error was eliminated by taking a certain amount (0.1 c.c.) of the highly active samples and comparing it with 0.2, 0.3, 0.4, 0.5, and 0.6 c.c. of the less active ones. By this method it could be determined how much of the weaker saliva was necessary to equal 0.1 c.c. of the stronger, and by applying Schütz's Law their relative percentages of ptyalin could be quite accurately measured.

The methods of obtaining the saliva were as follows: (1) under ether anæsthesia and (2) without anæsthesia.

(1) **During ether anæsthesia.** (*a*) *Submaxillary saliva.* — In the first few experiments cannulas were placed in Wharton's ducts, and samples of 0.5 c.c. each of submaxillary saliva collected by alternate

stimulation of the chorda and cervical sympathetic, and lastly by injection of pilocarpin when the glands would no longer respond to electrical stimulation of the nerves.

(*b*) *Parotid saliva.* — Parotid saliva was obtained by placing cannulas in Stenson's ducts and collecting saliva under the following conditions: (1) By stimulation of Jacobson's nerve, the electrodes being placed in the middle ear after rupture of the tympanic membrane and filling the tympanum with physiological saline solution. (2) By simultaneous stimulation of the cervical sympathetic and Jacobson's nerve alternating with stimulation of Jacobson's nerve alone. (3) By stimulating of Jacobson's nerve, alternating the samples with and without anæmia of the gland produced by clamping of both common carotid and both vertebral arteries. In the majority of cases the anæmia after ligation was so complete as to necessitate artificial respiration, and in most instances the carotid artery on the same side as the gland had to be released occasionally in order to get any flow at all, even during stimulation of Jacobson's nerve. In ligating the vertebral arteries care had to be taken to keep the animal warm and avoid too much manipulation of the brachial plexus, otherwise the animal would invariably die of shock. Several of the animals seemed to be particularly susceptible to the shock produced by placing the electrodes in the middle ear, and for this reason in part of the experiments pilocarpine was substituted in place of electrical stimulation of Jacobson's nerve.

(2) **Without anæsthesia.** (*a*) *Parotid saliva.* — In this series of experiments without anæsthesia a permanent fistula was established in each cheek of a large white rabbit, and with proper care the one animal served to complete the entire series. Saliva was obtained from these fistulas as follows: (1) The animal fasted over night and then normal or reflex saliva was collected by allowing the rabbit to eat cabbage, carrots, etc., several samples of 0.5 c.c. each being obtained at each feeding. (2) Two samples were collected by feeding cabbage. Then the cervical sympathetic nerves were isolated (by local anæsthesia with ethyl chloride) and stimulated for ten minutes, during which time there was never any appreciable flow from the gland except at one time 1 c.c. was obtained. After this period of stimulation of the sympathetic, the animal was allowed to eat again and the saliva collected as before. (3) Owing to the difficulty of keeping the animal alive after clamping of the carotid

and vertebral arteries, anæmia of the glands was produced as follows: (*a*) Two samples of reflex saliva were obtained and then 30 c.c. of blood drawn from the ear. After allowing the animal to rest for a few minutes it was fed more cabbage and the saliva collected as before. If the animal refused to eat after bleeding, the saliva was obtained by injection of pilocarpin. (*b*) The animal was bled every fifth day from the ear for three experiments, and then after a week's rest a fourth test was made by drawing 25 c.c. of blood directly from the heart with a sterile needle. (4) Numbers 1, 2, and 3 were all repeated, using pilocarpine instead of letting the rabbit eat cabbage.

The methods of collecting saliva without anæsthesia were used to check up our previous results by approaching as nearly as possible to pure physiological conditions, thus obviating the errors that might be introduced by changes in blood pressure, heart action, osmotic pressure of the blood,[12] etc., produced by the anæsthetic.

IV. Results.

I. **During anæsthesia.** (1) *Submaxillary saliva.* — The submaxillary as obtained in the first few experiments was found to be entirely devoid of amylolytic power. After testing a number of samples, some of them standing several days at thermostat temperature, we were convinced that the submaxillary saliva of the rabbit exerts no appreciable diastatic action on starch, and consequently it was not considered in the remainder of the experiments.

(2) *Parotid saliva.* — The parotid saliva, on the other hand, is highly active, equal to and in some instances greater than pure parotid human saliva collected by inserting a cannula into Stenson's duct at its opening into the vestibule of the mouth. In one case, however, in a perfectly healthy rabbit the parotid saliva showed absolutely no action on starch, and the serum from the same animal was also found to be entirely free from diastase. We offer no explanation of this peculiar phenomenon.

(*a*) *Gradual decrease in the ptyalin during the secretion of the gland.* — Table I is a typical example of the results of eight experiments, in four of which series of samples of 1 c.c. each were obtained by stimulation of Jacobson's nerve till the gland became

[12] Carlson and Luckhardt: This journal, 1908, xxi, p. 162.

fatigued, and in the other four by the use of pilocarpine. The saliva of different animals varied considerably in the rate of action, but the same essential features are always present. From

TABLE I.

The Decrease in the Ptyalin Concentration of the Rabbit's Parotid Saliva during the Period of Activity. Results of Experiments 1 (Stimulation of Jacobson's Nerve) and 5 (Hypodermic Injection of Pilocarpine).

Saliva samples of 1 c.c. each	Results of Experiment 1.		Results of Experiment 5.	
	Rate of clearing.	Disappearance of erythrodextrin.	Rate of clearing.	Disappearance of erythrodextrin.
	min. sec.	min.	min. sec.	min. sec.
No. 1	0 15	2	0 12	2 00
No. 2	0 30	5	0 40	6 00
No. 3	0 37	6	0 45	7 30
No. 4	0 42	8	0 52	9 00
No. 5	0 60	10	1 8	14 00
No. 6	1 30	16	2 00	22 00
No. 7	4 00	30	5 00	50 00
No. 8	15 00	2 hours	12 00	2 hours
No. 9	Scarcely any action	..	No action after 1 hour	....

this table we see that the amylolytic power decreases rapidly at first, then gradually, and as the gland approaches fatigue there appears to be another sudden decrease, and finally the ptyalin almost entirely disappears. It would thus seem that *the concentration of the ptyalin during a period of activity runs the same course as that of the organic solids.*

(*b*) *The increase in ptyalin produced by stimulation of cervical sympathetic.* — In six experiments upon the effect produced by simultaneous stimulation of Jacobson's nerve and the cervical sympathetic, alternating with stimulation of Jacobson's nerve alone, uniform results were obtained, as shown in Table II, which represents one typical experiment.

(*c*) *The increase in the ptyalin produced by clamping the arteries supplying the gland.* — The six experiments were carried out using anæmia produced by clamping of both common carotids and both vertebral arteries instead of stimulation of the cervical sympathetic, and the results were practically identical with those on the sympathetic saliva. This shows that the con-

TABLE II.

Detail of Experiment 2 in the Series Showing the Increase in the Ptyalin Concentration Produced by the Stimulation of the Cervical Sympathetic.

Saliva samples of 1 c.c. each.	Action.		Comparative action.	Relative concentration of ptyalin calculated according to Schütz's Law.
	Rate of clearing.	Disappearance of erythrodextrin.		
		min.		
(1) Jacobson's N.		5		
(2) Sym. & J. N.	$2 > 1$	1	0.1 c.c. of (2) ≎ 0.6 c.c. of (1)	No. 2 : No. 1 :: 36 : 1
(3) J. N.	$3 < 2$	10	0.1 c.c. of (2) ≎ 0.3 c.c. of (3)	No. 2 : No. 3 :: 9 : 1
(4) Sym. & J. N.	$4 > 3$	2	0.1 c.c. of (4) ≎ 0.4 c.c. of (3)	No. 4 : No. 3 :: 16 : 1
(5) J. N.	$5 < 4$	20	No test made	
(6) Sym. & J. N.	$6 > 5$	15	0.1 c.c. of (6) 0.2 c.c. of (5)	No. 6 : No. 5 :: 4 : 1

ditions (vaso-constriction during stimulation of the sympathetic and clamping the arteries) producing a reduction in oxygen supply to the parotid gland cause an increase in the concentration of ptyalin in the parotid saliva. A corresponding change in the percentage of organic solids is produced by these same conditions of secretion, but to a less marked degree. The work of Heidenhain and Carlson, Greer and Becht, and Carlson and McLean on the salivary glands of the dog, cat, and rabbit shows the parotid sympathetic saliva to be much richer in organic solids than saliva obtained by stimulation of Jacobson's nerve or by injection of pilocarpine, but in no instance did they find the great difference shown in the relative amylolytic powers of the two salivas. This marked difference as shown by Schütz's Law seems almost incredible, but, on the other hand, even if Schütz's Law would not hold good, there is still a greater difference than has been obtained

in the relative percentages of the other organic constituents. In these experiments it was not possible to measure the degree of anæmia during sympathetic stimulation or compression of the arteries to the gland, and for this reason the same differences in concentration of ptyalin were not always obtained. In two instances the changes were only slight, probably due to faulty stimulation or to an inactive sympathetic nerve. The rate of secretion during anæmia of the gland (however produced) is always very slow, but in these two cases the slowing was scarcely perceptible, and we were led to believe that marked diminution in the blood supply had not been secured. A rapid secretion of saliva as during vaso-dilation means a lessening of the concentration; that is to say, the more rapidly the fluid passes through the gland cells the less specific constituents seem to be carried away in solution from them.

II. **Normal or reflex saliva under different conditions of secretion (sympathetic stimulation, anæmia) shows the same variations as in the previous experiments.** — The results of the experiments conducted without anæsthesia in every way confirmed the previous experiments. When the rabbit was allowed to eat till the rate of secretion was greatly reduced, there was always the same gradual decrease in percentage of ptyalin as was obtained under anæsthesia on injection of pilocarpine and by stimulation of Jacobson's nerve. When the first two samples of reflex saliva were followed by stimulation of the cervical sympathetic under local anæsthesia, the first cubic centimetre of saliva following the stimulation showed the same marked increase in ptyalin and then the gradual diminution till the nerve was again stimulated. The same was true for pilocarpine saliva.

During anæmia of the glands produced by the two methods of bleeding the animal, the changes in the concentration of ptyalin in reflex and pilocarpine saliva, in every particular confirmed those experiments in which diminution in the blood supply was secured by clamping of the arteries supplying the glands. But the increase in the ptyalin was not so great, obviously because by the bleeding process the anæmia secured in the gland was not so marked.

Summary and Conclusions.

1. The submaxillary saliva of the rabbit contains no diastase. The parotid saliva contains ptyalin in about the same concentration

as the human parotid saliva, but in apparently normal rabbits diastatic ferments may be entirely wanting both in the parotid saliva and in the blood serum.

2. The saliva secreted during and immediately following the stimulation of the cervical sympathetic nerve is much richer in ptyalin than normal reflex saliva, pilocarpine saliva, or saliva secured by direct stimulation of Jacobson's nerve. The same increase in the ptyalin concentration is produced by artificially diminishing the blood supply to the gland, and is, therefore, probably due to the diminished blood and oxygen supply rather than to any difference in the character of the cranial and the sympathetic secretory nerves. The above facts support Professor Carlson's view that the traphic theory of Heidenhain is superfluous.

3. There is a gradual diminution in the ptyalin concentration during the period of secretion, and towards the end of a prolonged period of secretion the ptyalin may entirely disappear from the saliva.

4. Under different conditions of gland activity the variation in the concentration of ptyalin takes the same course as that of the other organic constituents, but the former may greatly exceed the latter.

5. It is not probable that the dependence of the ferment concentration on the blood supply to the gland and on the rate of secretion is peculiar to the salivary gland. It is in all probability a factor in some of the variations in ferment concentration observed in the gastric and pancreatic juice under different conditions of secretion, as well as in the variations in the concentration of the dog's saliva on variation of the character of the stimulus in the mouth, as recorded by Pawlow.[13]

[13] CARLSON: Personal communication.

ON RHEOTROPISM. — II. RHEOTROPISM OF FISH BLIND IN ONE EYE.

By E. P. LYON.

[*From the Physiological Laboratory of St. Louis University and the Marine Biological Laboratory, Woods Hole, Mass.*] [1]

THE tropism theory reduced to its simplest terms is *one-sided response to one-sided stimulation.* Theoretically the response can be either excitation or inhibition. The increase or decrease of activity can be on the same side as the stimulus or on the opposite side. In any case the inequality of response, if it takes place in locomotor elements, is bound to lead to orientation of the organism in such a way that stimulation and, consequently, response will be equal on the two sides of the organism. Such orientation in the case of bilaterally symmetrical organisms would usually be with the plane of symmetry in the direction of the stimulating force.

Verworn [2] words the whole idea thus: "Only unsymmetrical stimulation can control the direction of motion."

In the case of simple organisms one may predicate a direct action of the stimulus on the locomotor apparatus of one side of the body. Thus has been explained the response of ciliate infusoria to the galvanic current. In higher animals, however, where the stimulus acts on afferent nerve terminals and only indirectly affects the locomotor elements and produces orientation, one is obliged to imagine a simple nervous connection between sense organs and muscles. It makes no difference, theoretically, whether the relation be crossed or homolateral. The essential condition for a tropic response must be conceived of as a more intimate relation of a given sense organ with the muscles of one side of the body than with those of the other side. A diagrammatic formulation of nerve connections of

[1] The thanks of the writer are due to the Director of the Missouri Botanical Garden, Professor Trelease, for the use of the ponds and streams in the garden.

[2] VERWORN: General Physiology; translated by Lee, 1899, p. 429.

somewhat the character under discussion will be found in Loeb and Maxwell's [3] paper on Galvanotropism in *Palemonetes.* The forced character of the orientation, determined by the fixed anatomical relations of the receiving, conducting, and responding elements is an essential part of the tropism theory as applied to the higher forms. Jennings says in this connection: "In order to retain any of its value for explaining movements of organisms, it would have to be held at least that the connections between the sense organs and the muscles are of a perfectly definite character, so that when a certain sense organ is stimulated a certain motor organ moves in a certain way." He believes the theory of little value.

It seems to the writer that the tropism theory is valid, and that many cases of animal behavior, to say nothing of numerous cases among plants, conform to the theory. To admit that in the higher forms the underlying reaction may also be classified as a reflex does not destroy the valuable conception of one-sided response to one-sided stimulation. To admit a degree of variability in the response does not destroy the value of this conception any more than the recognition of inhibition and reinforcement of reflexes in the higher animals destroys the general conception of reflexes.

I believe, however, that the tropism formula has been applied too widely and indiscriminately. As in the case of other valuable discoveries and theories, there has been too much inclination on the part of investigators to explain the most diverse phenomena according to the new mode of interpretation. Too often the gathering of organisms under the influence of a stimulus was called a tropism. It is now manifest that gatherings may take place in several ways, and that the mere fact that a collection of organisms occurs is no criterion of tropic action. Even when orientation can be demonstrated, it is evident that one must study the mechanism of response before he can safely classify the behavior as belonging to the tropic type. Each example must be studied by itself, and only by such detailed study, applied to many forms of life, can the extent to which the tropism theory applies to animal reactions be ascertained. Meanwhile one may continue to use the names which have come into common use, detaching from them all significance as explanations of phenomena. When a much larger body of facts has accumulated, a new nomenclature may perhaps be attempted.

[3] Loeb and Maxwell: Archiv für die gesammte Physiologie, 1896, lxiii, p. 121.

In the case of rheotropism in fishes it was shown in an earlier paper [4] that the stimulation is due to the fixed points of the environment. The animal reacts to the objects on the bottom or sides of the stream against or by which it is passively borne by the current. The current stimulates only in the sense that it causes relative movement between the fish and the fixed environment.

The stimulus may be visual, as when the fish orients itself in a quiet stream at any point up from the bottom. Or it may be tactile when the organism touches and reacts to the bottom along which it is dragged by the force of the current. Likewise the stimulus is apparently tactile or muscular when the fish reacts to a rapid and unequal current, which it can do to some extent without touching or seeing solid objects.

That the fixed, solid environment is the essential element of stimulation in rheotropism is shown by the inability of blind fish to orient themselves unless they touch bottom. It is further proven by the fact that normal fish orient themselves as well in a body of water whose sides and bottom move but which itself stands still as if the usual reverse condition prevailed. If the water of a river should suddenly cease to flow and the banks and bottom start to move up stream with the velocity previously held by the water, every fish would retain its orientation. There would be no change in its behavior, and in fact it would never be aware of any change whatever in its surroundings. Nor, for that matter, would a man be any better able to appreciate such a change, provided there were no other points of reference than river and river bank.

Parker [5] has shown that the same rule of stimulation holds good in the rheotropic response of amphioxus, the stimulus being tactile.

Hadley [6] demonstrated that young lobsters orient themselves to a moving environment, the eye being the sense organ here brought into use.

Jennings [7] has expressed the belief, with which my own observations agree, that the orientation of paramœcia in a current of water is due to their being drawn along the solid wall or between layers

[4] LYON: This journal, 1904, xii, p. 149.

[5] PARKER: Proceedings of the American Academy of Arts and Sciences, 1908, xliii, p. 415.

[6] HADLEY: Journal of comparative neurology and psychology, 1908, xviii, p. 199.

[7] JENNINGS: Behavior of the lower organisms, 1906, p. 74.

of water of unequal velocities. In other words, this case of rheotropism resembles that of blinded fish.

The question arises whether any or all of these responses to currents of water correspond to the tropism scheme.

The explanation of some of them on such a basis would from the start appear doubtful or impossible. For example, a blind fish in a stream is unoriented until it touches bottom. The stimulation is applied to its ventral side. It responds, not by turning toward the source of stimulation (ventrally) or away from it (dorsally), but by turning to the right or left, as its structure demands. Similarly a fish facing down a narrow stream where it can see both banks would appear to be stimulated equally on both sides. The field of vision is travelling in the same direction and with the same velocity across both retinas and yet it turns to one side. Unless it inhibits or abolishes in some way one of the fields of vision and responds only to the other, we cannot explain its reaction as the result of one-sided stimulation. Moreover, the disregard of one field of vision would imply processes more complicated than those recognized as lying at the basis of a tropic response. Further, a normal fish, on being subjected to a gentle current moving in the direction of its body (*i. e.*, postero-anteriorly), often fails to turn against it, but maintains its relation to fixed objects by swimming backward. I have observed this repeatedly among fish in tide streams. After a few moments, if the current continues, the fish usually turn about and head up stream.

It was to test this matter further that the experiments described here were performed. They were prompted by Holmes'[8] paper on *Ranatra*, some of whose reactions to light he believes to be more complicated than simple reflexes. For example, these animals go to the light even when one eye and all of the other except a small posterior segment are covered with black paint.

This behavior was quite different from that of certain other insects investigated by Holmes,[9] and from that of the butterfly, *Vanessa antiopa*, described by Parker.[10] Here the blackening of one eye causes the animal to creep or fly in circles with the uncovered eye directed toward the axis of rotation. This experiment constitutes,

[8] Holmes: Journal of comparative neurology and psychology, 1905, xv, p. 305.

[9] Holmes: This journal, 1901, v, p. 211.

[10] Parker: Mark anniversary volume, 1903, p. 455.

I believe, a definite proof of the tropic nature of the response these organisms make to light.

Another striking instance is recorded by Barrows.[11] The pomace fly, *Drosophila ampelophila,* is attracted by certain odors. If one of the olfactory antennæ be removed, the fly appears perfectly normal until subjected to the odor emitted by its usual food. It then moves in circles toward the normal antenna. This appears to be a perfect example of positive chemotropism. This same fly, however, on being investigated recently by Carpenter,[12] failed, except occasionally, to show circus movements on blackening one eye. On the contrary, such one-eyed flies "crept in a fairly direct path toward the light."

These examples emphasize what was said above relative to the necessity of investigating each organism, and, indeed, each response of that organism, by itself. They further make it apparent that if the connection between the eye and the locomotor muscles of a fish is of the simple, definite character demanded by the tropism theory, then fish having only one eye should always react to a current of water by turning toward one side only.

Several species of marine bony fishes, of which the scup was particularly satisfactory, and also the fresh-water perch and sunfish were used in testing this idea. Young fish display a more stereotyped rheotropic response than old ones and were chiefly used on that account.

Instead of a real current of water it is more convenient to experiment with a box with solid sides and bottom, and whose ends are filled in with wire screen. The bottom is covered with pebbles, sand, etc. The sides are hung with water plants. When the box is immersed and drawn through the water, the fish orient themselves in the direction of movement of the box and against the stream of water which *appears to pass* through the box. The direction of the box and consequently of the pseudo-current can instantly be reversed.

An effort was first made to see whether normal fish had a tendency to turn to one side more than to the other. Many counts were made. All individuals which were not disturbed by the presence of the operator seemed to turn as readily to the right as to the left. *Fundulus* and some other species are so wary of the experimenter as to be unsatisfactory.

[11] Barrows: Journal of experimental zoölogy, 1907, iv, p. 515.

[12] Carpenter: Journal of comparative neurology, 1908, xviii, p. 483.

In blinding fish for the subsequent experiments several methods were used, such as cutting the optic nerve, cutting out the entire eye, taking out the lens, corrosion of the cornea by acid or heat, smearing the cornea with a mixture of vaseline and lampblack, etc. The results were identical so far as rheotropism is concerned.

On blinding one eye many fish showed a decided tendency to move in circles with the normal eye toward the centre. This lasts only a short time. It is less marked when one covers the eye with non-irritant paint than when a cutting operation is performed.

In addition to this limited effect of unilateral blindness another peculiarity was noticed. All these fish, after being blinded on one side, failed to keep the median plane of their bodies in the vertical. They always tipped the dorsi-ventral axis more or less, the uninjured side being down. I kept such fish as long as three months and they never recovered the normal position. Smearing one eye produced the same disorientation so long as the paint remains over the cornea. No explanation is at hand. The observation should be further investigated. For the present I will merely mention as suggestive the intimate relation between the eyes and the geotropic organs which has been before observed. I [13] found some years ago, for example, that the responses of the crayfish to gravity were diminished after blinding. I also mentioned in that paper the interesting fact that certain plants lose their geotropic irritability in the dark. It is well known, too, that the slight involuntary movements by which the vertical position of the human body is maintained are increased on closing the eyes.

The fish blinded in one eye by any of the methods mentioned were tried as to their rheotropic responses in the manner already described, either immediately after operation or, more usually, after some hours or days had elapsed. *The universal finding was that they showed no more tendency to turn toward one side than toward the other.* For example, out of 50 trials, a certain individual turned 26 times toward the uninjured (left) eye, 24 times toward the blinded (right) side.

Presently it was observed that, if the fish were located somewhat across the line of direction when the current started, *it always turned the nearest way* so as to head against the stream. It was further noted that even the slightest angle of deviation from the direction

[13] Lyon: This journal, 1899, iii, p. 86.

of the current was sufficient to determine the direction of turning. If there is the slightest observable deviation of the animal's longitudinal axis from the line of the current, the direction of turning can be prophesied correctly in more than 90 per cent of the trials.

These one-eyed fish also occasionally react to a current by swimming backward. I am unable to bring these observations into accord with the simple tropism theory.

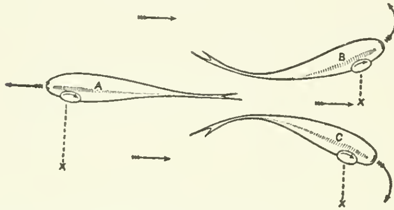


FIGURE 1. — Showing direction of turning and swimming of fish blinded in one eye, with reference to a current of water. Showing also that these fish tip the dorsal fin downward on the side of the uninjured eye. Direction of current indicated by long arrows. Direction of movement of images on retina by arrows in the eyes.

So far as I can see, the visual stimulations of the fish indirectly due to the current fall under two types. If the animal is at rest and a current is started in the same direction that the animal is headed, the fish will be passively carried by the current, and the visual field will cross the retina from posterior to anterior (Fig. 1, *B, C*), in other words, in the opposite direction to the apparent motion of the solid objects of the sides and bottom of the stream. If the water and fish are at rest and the solid environment is moved antero-posteriorly, the movement across the visual field is, as before, from behind forward. In either of these cases (which are in reality one), the fish turns about so that the motion of the visual field is reversed. It then moves forward with such a velocity that the field of vision remains approximately fixed on the retina.

If the animal is at rest and the current starts or the environment moves in such direction that the field of vision moves across the retina antero-posteriorly (Fig. 1, *A*), the animal does not turn about. It is stimulated to move forward at such a rate that the field of vision remains approximately stationary on the retina.

If the animal were more or less crosswise to the current, nevertheless the motion of the visual field across the retina would nearly always have a component of the character indicated. This component in a given eye would be sightly different according to whether the long axis of the fish deviated to one side or the other of the current lines. Nevertheless, I find it impossible to conceive a simple reflex connection between the retina and the muscles by which

the turning to one side or the other under such conditions could be brought about.

Summary.

Fish with one eye blinded react to currents of water like normal fish. The usual form of stimulation is visual. The fish turn the nearest way to face the current, whether in so turning the motion be toward or from the injured side. Like normal fish, they sometimes compensate the motion of a gentle current by swimming backward. It seems to the writer impossible to bring these observations into accord with the tropism scheme of one-sided response to one-sided stimulation.

HYDROLYSIS OF CRYSTALLIZED ALBUMIN FROM HEN'S EGG.[1]

By THOMAS B. OSBORNE, D. BREESE JONES, AND CHARLES S. LEAVENWORTH.

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

ANALYSES of the products of hydrolysis of ovalbumin have been made by Abderhalden and Pregl[2] and by Hougounenq,[3] but the results which they report differed so widely that we have thought it desirable to hydrolyze this important food protein again. This seemed the more desirable because it was hoped that by so doing we would have an opportunity of comparing the results of our analyses with those of others, and thereby obtain more data than are now available as to the agreement to be expected between different chemists employing Fischer's ester method when working wholly independently of one another.

The material which we have employed for this hydrolysis was obtained from perfectly fresh eggs, was recrystallized at least six successive times, and no material was used which contained any protein coagulating below 70°. Our preparation, therefore, may be considered to be practically free from conalbumin. Whether it was equally free from ovomucoid cannot well be determined, for we have no means for judging of the presence of very small quantities of this substance when mixed with ovalbumin. The method of preparation, however, was such as to make it highly improbable that more than insignificant quantities of adsorbed ovomucoid were present in our material. The ovomucin of Eichholz[4] was separated

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

[2] ABDERHALDEN and PREGL: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 24.

[3] HOUGOUNENQ: Comptes rendus de l'Académie des Sciences, 1906, cxlii, p. 173.

[4] EICHHOLZ: Journal of physiology, 1898, xxiii, p. 163.

by first removing all the protein precipitable from the egg white at half saturation with ammonium sulphate. After separating the ovalbumin by repeated crystallization from ammonium sulphate solution, it was coagulated by pouring a solution of the crystals, freed as far as possible from adherent sulphate by pressing between filter paper, into a large volume of boiling water. The coagulum was then washed with hot water, dehydrated with absolute alcohol, and extracted with ether.

Hydrolysis of Ovalbumin.

Four hundred and fifty grams of the ovalbumin, just described, equivalent to 396.27 gm. ash and moisture free, were dissolved in 900 c.c. of hydrochloric acid, specific gravity 1.1, by heating on a water bath for three hours. The hydrolysis was then completed by boiling in an oil bath for twenty hours.

The hydrolysis solution, which contained considerable insoluble material, was concentrated under diminished pressure to a thick syrup, and freed from water by repeatedly evaporating under diminished pressure with absolute alcohol. The amino-acids were then esterified in the usual manner, and the free esters liberated, shaken out with ether, and dried over anhydrous sodium sulphate. The aqueous layer was freed from inorganic salts and again esterified.

Ether was removed from the esters by distilling off at 760 mm. pressure, and 346 gm. of esters obtained, which were distilled as follows:

Fraction.	Temperature of bath up to	Pressure.	Weight.
I	100°	10.00 mm.	48.00 gm.
II	107°	0.50 "	49.00 "
	107°	0.40 "	21.19 "
III	152°	0.50 "	62.70 "
IV	200°	0.40 "	51.00 "
	214°	0.70 "	10.29 "
Total			242.18 gm.

The undistilled residue weighed 56.00 gm.

Fraction I. — This fraction was saponified by boiling with ten volumes of water for ten hours, the solution evaporated to dryness under diminished pressure at 50°, and proline extracted from the

residue by boiling with alcohol. The part insoluble in alcohol, which weighed 32.44 gm., was separated into thirteen fractions by systematic crystallization, and the thirteenth fraction, which contained all of the remaining substance, weighed only 3.02 gm. Analysis showed this to contain 38.77 per cent of carbon and 7.52 per cent of hydrogen, indicating the presence of very little glycocoll if any. By further systematic crystallization of these thirteen fractions, 13 gm. leucine, 9.91 gm. of valine, and 8.81 gm. of alanine were obtained.

The leucine, when recrystallized once, gave the following analysis:

Carbon and hydrogen, 0.1437 gm. subst., gave 0.2891 gm. CO_2 and 0.1279 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . . . = C 54.87; H 9.89 " "

The valine crystallized in homogeneous characteristic plates which gave the following analysis:

Carbon and hydrogen, 0.1207 gm. subst., gave 0.2280 gm. CO_2 and 0.1022 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.28; H 9.40 per cent.
Found . . . . . . = C 51.52; H 9.40 " "

When dissolved in 20 per cent HCl, it showed a specific rotation of $(a)\frac{20^\circ}{D} = +23.10^\circ$. For further identification the valine was racemized by heating for twenty-four hours with baryta in an autoclave at 170°–175° and converted into the phenylhydantoic acid derivative which crystallized from water in characteristic hexagonal plates melting sharply at 159°.

Carbon and hydrogen, 0.1308 gm. subst., gave 0.2936 gm. CO_2 and 0.0816 gm. H_2O.

Calculated for $C_{12}H_{16}O_3N_2$ = C 60.96; H 6.83 per cent.
Found . . . . = C 61.22; H 6.98 " "

The alanine, when recrystallized from water, formed large, dense prisms which decomposed at 290°.

Carbon and hydrogen, 0.1798 gm. subst., gave 0.2667 gm. CO_2 and 0.1283 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.45; H 7.86 per cent.
Found . . . . . . = C 40.45; H 7.93 " "

Fraction II. — This fraction was saponified by boiling with water, evaporated to dryness, and the proline extracted from the residue with absolute alcohol. The amino-acids insoluble in alcohol weighed 38.79 gm., of which 27.77 gm. were leucine. When recrystallized once from water, the leucine gave the following analysis:

Carbon and hydrogen, 0.1375 gm. subst., gave 0.2752 gm. CO_2 and 0.1212 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . . . = C 54.59; H 9.79 " "

From the filtrate from the leucine were isolated 2.65 gm. copper aspartate and 4.02 gm. of the copper salt of leucine. The copper aspartate crystallized in the characteristic sheaves of fine needles which gave the following analysis:

Copper, 0.1297 gm. air-dried subst., gave 0.0372 gm. CuO.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07 per cent.
Found . . . . = Cu 22.91 " "

The leucine copper salt on analysis gave the following results:

Copper, 0.1190 gm. air-dried subst., gave 0.0288 gm. CuO.

Calculated for $C_{12}H_{24}O_4N_2Cu$ = Cu 19.50 per cent.
Found . . . . = Cu 19.34 " "

From the alcoholic extracts of Fractions I and II the proline was separated in the usual way. By converting the proline into its copper salt and separating the lævo from the racemic salt by solution in alcohol, a quantity of each of these forms was obtained which corresponded to 13.65 gm. of *l*-proline and 0.46 gm. of *r*-proline. The phenylhydantoine of the *l*-proline crystallized in characteristic prisms which melted sharply at 142°.

The air-dried racemic proline copper salt gave the following analysis:

Water, 0.1371 gm. subst., lost 0.0155 gm. H_2O.

Calculated for $C_{10}H_{16}O_4N_2Cu\ 2\ H_2O$ = H_2O 10.99 per cent.
Found . . . . = H_2O 11.30 " "

Copper, 0.1206 gm. subst., dried at 110°, gave 0.0330 gm. CuO.

Calculated for $C_{10}H_{16}O_4N_2Cu$ = Cu 21.81 per cent.
Found . . . . = Cu 21.86 " "

Fraction III. — The phenylalanine was extracted with ether and converted into the hydrochloride, which weighed 9.37 gm. The free phenylalanine gave the following analysis:

Carbon and hydrogen, 0.1222 gm. subst., gave 0.2930 gm. CO_2 and 0.0748 gm. H_2O.

Calculated for $C_9H_{11}O_2N$ = C 65.45; H 6.66 per cent.
Found . . . . . . = C 65.44; H 6.80 " "

The aqueous layer was saponified with baryta, and 3.05 gm. aspartic acid obtained in the form of the barium salt. The free aspartic acid reddened without decomposing at about 300°.

Carbon and hydrogen, 0.2271 gm. subst., gave 0.2997 gm. CO_2 and 0.1099 gm. H_2O.

Calculated for $C_4H_7O_4N$ = C 36.09; H 5.26 per cent.
Found . . . . . . = C 35.99; H 5.38 " "

The filtrate from the barium aspartate yielded 6.32 gm. glutaminic acid hydrochloride and 7.33 gm. copper aspartate. The copper aspartate gave the following analysis:

Copper, 0.1496 gm. subst., gave 0.0433 gm. CuO.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07 per cent.
Found . . . . = Cu 23.13 " "

The filtrate from the copper aspartate was freed from copper with hydrogen sulphide and an attempt made to isolate serine. From the mixture of substances contained in the solution 0.3 gm. of aspartic acid and 0.29 gm. of phenylalanine were isolated, but no serine was obtained even after long-continued efforts to bring it to crystallization.

Fraction IV. — This fraction, when treated in the same way as described for Fraction III, yielded 11.48 gm. of phenylalanine hydrochloride. By freeing the mother liquor from this from the excess of hydrochloric acid, decolorizing with animal charcoal, and neutralizing with ammonia, 3 gm. of the free acid were isolated which decomposed at 273°. The total phenylalanine thus obtained from the esters was equal to 20.08 gm. of free phenylalanine, or 5.7 per cent of the ovalbumin. The aqueous solution from which the phenylalanine had been removed by shaking with ether yielded 29.33 gm. of glutaminic acid hydrochloride and 1.2 gm. of copper

aspartate. The free glutaminic acid decomposed with effervescence at 202°.

Carbon and hydrogen, 0.1649 gm. subst., gave 0.2463 gm. CO_2 and 0.0901 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81; H 6.12 per cent.
Found . . . . . . = C 40.74; H 6.07 " "

The Residue after Distillation.

This yielded 1.60 gm. of glutaminic acid hydrochloride, which decomposed at 200°.

From the total products of this hydrolysis were isolated 29.85 gm. glutaminic acid, or 7.53 per cent of the ovalbumin, or about 82 per cent of the quantity obtained by Osborne and Gilbert [5] by a direct determination, namely, 9.10 per cent.

Cystine.

Cystine was not obtained, although a persistent effort was made to separate it from a solution of the products of hydrochloric acid hydrolysis of 100 gm. of the ovalbumin. Under similar conditions Abderhalden and Pregl isolated 0.2 gm. of cystine, which shows the presence of this amino-acid in the albumin. We have no doubt that our failure to obtain cystine was entirely due to our inability to establish favorable conditions for its separation, for, as Moerner [6] says, it is an accident if the best possible output is obtained.

Tyrosine.

A quantity of ovalbumin equivalent to 43.87 gm. ash and moisture free substance was hydrolyzed by boiling for twenty-four hours in an oil bath with a mixture of 150 gm. of sulphuric acid and 300 c.c. of water. The sulphuric acid was then removed from the diluted solution with an equivalent quantity of baryta, and after thoroughly washing the barium sulphate by repeatedly boiling with water, the filtrate and washings were concentrated to crystallization. The sub-

[5] Osborne and Gilbert: This journal, 1906, xv, p. 333.
[6] Moerner, K. A. H.: Zeitschrift für physiologische Chemie, 1902, xxxiv, p. 215.

stance which separated after twenty-four hours was dissolved in water, the solution decolorized by boiling with bone black, and the tyrosine crystallized by concentrating and cooling. The tyrosine, thus obtained, weighed 0.7805 gm. equal to 1.77 per cent, and gave the following result on analysis:

Nitrogen, 0.2183 gm. subst., required 1.68 c.c. $\frac{5}{7}$ N—HCl.
Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.
Found . . . . = N 7.69 " "

The filtrate and washings of the tyrosine were used for determinations of the basic amino-acids according to the method of Kossel and Patten.

Histidine.

The solution of the histidine = 500 c.c.

Nitrogen, 50 c.c. sol. required 2.04 c.c. $\frac{5}{7}$ N—HCl = 0.2040 gm. N in 500 c.c. = 0.7500 gm. histidine = 1.71 per cent.

The histidine was converted into the dichloride for identification.

Chlorine, 0.1153 gm. subst., gave 0.1453 gm. AgCl.
Calculated for $C_6H_{11}O_2N_3Cl_2$ = Cl 31.14 per cent.
Found . . . . = Cl 31.16 " "

Arginine.

The solution of the arginine = 1000 c.c.

Nitrogen, 50 c.c. sol. required 3.3 c.c. $\frac{5}{7}$ N—HCl = 0.6600 gm. N in 1000 c.c. = 2.0506 gm. arginine + 0.1026 gm. = 2.1532 gm. = 4.91 per cent.

The arginine was converted into the copper-nitrate double salt for identification.

Copper, 0.1154 gm. subst., air dry, gave 0.0160 gm. CuO.
Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2$ 3 H_2O = Cu 10.79 per cent.
Found . . . . = Cu 11.08 " "

Lysine.

The lysine picrate weighed 4.2407 gm. = 1.6509 gm. lysine = 3.76 per cent.

The lysine picrate gave the following analysis:

Nitrogen, 0.3000 gm. subst., dried at 100°, required 5.62 c.c. $\frac{5}{7}$ N—HCl.
Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$ = N 18.67 per cent.
Found . . . . = N 18.73 " "

Carbohydrate.

Very conflicting statements are to be found in the literature respecting not only the amount, but even the existence of carbohydrate in ovalbumin. Much of the confusion has unquestionably arisen from a lack of purity of the ovalbumin used in many of the experiments, for some of those who were careful to separate their ovalbumin from ovomucoid overlooked the presence of ovomucin. Since Eichholz has shown that ovomucin yields an osazone, a part of the contradictory data that are on record may be explained by the presence of this substance in some of the preparations of ovalbumin which have been examined.

Abderhalden[7] has found that the glucosamine content of once recrystallized ovalbumin is 7 per cent; of thrice recrystallized, 4 per cent; and seven times recrystallized is 2.5 per cent, as indicated by the weight of the crude osazone. The latter proportion is in close agreement with the quantity similarly estimated by Osborne and Campbell[8] from the weight of the crude osazone obtained from carefully purified preparations of ovalbumin.

From the six times recrystallized ovalbumin used for this hydrolysis, in one experiment we obtained a similar quantity of crude osazone, but on recrystallizing this we found that it contained a not inconsiderable quantity of tyrosine. After separating the tyrosine too little of the osazone remained to permit of its purification by further recrystallization.

In another experiment 10 gm. of the ovalbumin were boiled with

[7] Abderhalden: Lehrbuch der physiologischen Chemie, 1909, p. 217.

[8] Osborne and Campbell: Journal of the American Chemical Society, 1900, xxii, p. 422.

200 c.c. of 5 per cent sulphuric acid for three hours, and after removing the sulphuric acid the solution was concentrated, under diminished pressure at a low temperature, to about 150 c.c. A mixture of 6 c.c. of phenylhydrazine and 6 c.c. of 80 per cent acetic acid and 20 gm. of sodium acetate was then added, and the solution heated on the water bath for three hours. After twenty-four hours the substance which separated was filtered out, washed with water and with alcohol, and dried at 100°. By concentrating and cooling the filtrate, from this first separation, a further small quantity was obtained which was added to the first. The crude product, which weighed 1.1033 gm. was dissolved, as far as possible, in boiling absolute alcohol, the filtered solution concentrated, and water added until a precipitate formed. After heating until all redissolved, the solution was allowed to cool over night. The substance which separated in balls of needles was again recrystallized, as before, and obtained in the form characteristic of glucosazone. When dried at 100°, this weighed 0.0860 gm. By concentrating the filtrate a second crop, weighing 0.0500 gm., was obtained, making the total pure glucosazone 0.1360 gm., which melted at 202°. From 0.5000 gm. of pure crystallized glucose, by similar treatment, 0.6470 gm. of osazone was obtained. If the osazone was yielded by the glucosamine from the ovalbumin in the same proportion as by the glucose, this quantity would correspond to 0.1136 gm. of glucosamine, or 1.23 per cent of the moisture and ash-free ovalbumin. It is not probable that this result gives any fair measure of the glucosamine which ovalbumin yields on hydrolysis, for we have no evidence that the brief hydrolysis employed is sufficient to liberate the whole of this substance. It is possible that much of the glucosamine may have been removed with the barium sulphate, for Müller [9] found that his solutions lost much in reducing power after removing sulphuric acid with baryta. He also found that glucosazone separates from a solution of glucosamine more slowly than from a solution of glucose, and from 3 gm. of glucosamine hydrochloride he obtained only 1.0240 gm. of glucosazone.

An attempt was made to isolate the glucosamine as the phenylisocyanate derivative according to the method proposed by Steudel,[10] but without success. Attempts to estimate the carbohydrate from the copper oxide reducing power of the hydrolysis solutions also

[9] Müller: Zeitschrift für Biologie, 1901, xlii, p. 468.

[10] Steudel: Zeitschrift für physiologische Chemie, 1902, xxxiv, p. 353.

failed, for, in harmony with the previous experience of Osborne and Campbell,[11] no reduction could be obtained, even after repeated and persistent efforts.

The amount of glucosamine which we have found can therefore be considered to be of only qualitative value, for approximately quantitative determinations evidently cannot yet be made by any of the methods now available.

The results of the several hydrolyses of ovalbumin which have been made are as follows:

	Osborne and Jones. per cent.	Abderholden and Pregl. per cent.	Hougounenq and Morel. per cent.
Glycocoll	0.00	0.00	0.00
Alanine	2.22	2.10	8.40
Valine	2.50	?	...
Leucine	10.71	6.10	15.20
Proline	3.56	2.25	1.10
Phenylalanine	5.07	4.40	5.20
Aspartic acid	2.20	1.50	1.70
Glutaminic acid	9.10	8.00	3.50
Serine	?	?	...
Tyrosine	1.77	1.10	0.99
Cystine	?	0.20	...
Histidine	1.71	...	...
Arginine	4.91	...	...
Lysine	3.76	...	0.27
Ammonia	1.34	...	...
Glucosamine	1.23	...	...
Tryptophane	present	...	...
Total	50.08		

Hougounenq's analysis was made on the products of hydrolysis with baryta, and is therefore not comparable with those of Abderhalden and Pregl or the authors'. In a note published later by Abderhalden[12] he gives as corrected figures for leucine 7.1 and for alanine 8.1 per cent, but gives no data concerning the way in which this result was reached. It is to be noted that the sum of the leucine, valine, and alanine in the hydrolysis by Abderhalden

[11] Osborne and Campbell: Journal of the American Chemical Society, 1900, xxii, p. 422.

[12] Abderhalden: Zeitschrift für physiologische Chemie, 1906, xlviii, p. 518.

and Pregl, namely, 15.2 per cent, agrees closely with the sum of the leucine, valine, and alanine which we have found, namely, 15.43 per cent. As we weighed only practically pure substances which were subjected to a strict chemical identification, it would seem probable that Abderhalden and Pregl's earlier figure for alanine was more nearly correct than the revised figure given by Abderhalden later. In other respects these two independent hydrolyses are in good agreement, and show that essentially the same results can be obtained by experienced workers if sufficient care is taken in separating the different amino-acids.

The low summation shown by our hydrolysis is not due to any defect in carrying out the processes incident to the isolation of the amino-acids, for throughout the entire analysis the separations were effected with unusually small losses. The unusual deficiency, in our opinion, is rather to be attributed to the presence of some non-protein complex which, in combination with protein, constitutes this albumin. Possibly some complex similar to chondroitin-sulphuric acid may here occur, which is suggested by the fact that the amount of sulphide sulphur to be obtained from ovalbumin indicates that one half of its total sulphur belongs to some other complex than cystine. Further evidence of this is given by the fact that when boiled with dilute sulphuric acid a volatile acid is yielded in small quantity, which Seemann [13] found to be acetic acid. We, also, observed the presence of a volatile acid, but its amount was too small to permit of its identification.

[13] SEEMANN: Archiv für Verdauungsheit, 1898, iv, p. 275.

THE EFFECTS OF CHLORIDE, SULPHATE, NITRATE, AND NITRITE RADICLES OF SOME COMMON BASES ON THE FROG'S HEART.

By F. C. COOK.

[*From the Laboratory of Physiology of the George Washington University Medical School, Washington, D. C.*]

THE question of the relation of the inorganic salts of the blood and lymph to the contractility of the heart and other muscles has been under discussion for many years. Investigators have concerned themselves chiefly with the action of the chlorides of sodium, potassium, and calcium, but the actions of other salts of these bases and of the salts of other bases have been but sparingly studied.

A review of the literature on this subject up to the year 1898 is given by Howell,[1] who concludes that the calcium in calcium chloride is the stimulus responsible for heart contraction, while the rhythmic contractions and relaxations are brought about by the addition of a certain proportion of potassium. In mixed solutions, such as Ringer's, sodium chloride seems to be essential, chiefly for preserving the osmotic relations between the tissues and the surrounding liquid. Similar work was continued by Greene,[2] who dealt with the relations of the inorganic salts of blood to the automatic activity of strips of ventricular muscle. Greene also gives a review of the literature on this subject.

Among more recent investigators in this country may be mentioned Loeb,[3] Meltzer and Auer,[4] Howell,[5] Guenther,[6] Carlson,[7] Matthews,[8] Denis,[9] and Hunt.[10] The latter investigator studied the

[1] Howell: This journal, 1898, ii, p. 47.
[2] Greene: *Ibid.*, 1898, ii, p. 82.
[3] Loeb: Studies in general physiology, University of Chicago, 1905.
[4] Meltzer and Auer: This journal, 1908, xxi, pp. 400 and 449.
[5] Howell: *Ibid.*, 1901, vi, p. 191.
[6] Guenther: *Ibid.*, 1905, xiv, p. 73.
[7] Carlson: *Ibid.*, 1904, xii, pp. 55, 67, 471.
[8] Matthews: *Ibid.*, 1907, xix, pp. 5, 20, and 323
[9] Denis: *Ibid.*, 1906, xvii, p. 35.
[10] Hunt: *Ibid.*, 1899, ii, p. 395.

nervous mechanism of the heart. The earlier work of Ringer, Locke, and others is well known.

The object of this work was to study the actions of dilute solutions (1 per cent) of the chlorides, sulphates, nitrates, and nitrites of sodium, ammonium, strontium, magnesium, copper and iron, as well as those of the dilute acids themselves, on the frog's heart. It was considered quite probable that the acid radicle might have an important bearing in determining the influence of the base on the frog's heart. Another object was to determine the difference, if any, in the relative actions of the nitrates and nitrites on the heart. The extensive use of nitrates in the preservation of meat is well known. At the present time the general action of nitrate and nitrite radicles on the body as a whole and on special physiological functions is of considerable interest and must soon be carefully investigated.

In the experiments which are reported in this paper the procedure was as follows: A frog was pithed and tied to a frog board. No curare or other drugs were used. Care was taken not to injure more structures than necessary and to prevent unnecessary loss of blood. All curves were made with the heart *in situ* and with nerves, etc., intact. A delicate wire attached the apex of the heart to a fine counterbalanced heart lever, and the beats were recorded on a slowly moving kymograph on which the time curve was recorded simultaneously from a contact metronome beating seconds. No records of the heart beats were made until several minutes after pithing the frog in order to allow time for recovery from "shock." Twenty to thirty seconds were allowed for the recording of normal heart curves. Then 2 c.c. of solution were allowed to flow over the heart from a finely drawn-out pipette. The time of flow was approximately one minute. Immediately after the application of the dilute solutions of the salts or acids, the excess was removed with filter paper and the heart was bathed with normal saline solution (0.6 per cent) from a pipette in the same manner as the testing solutions. One per cent solutions of the salts and twenty-five hundredths of one per cent solutions of the acids were used. The curves for each experiment were divided into sections of twenty-second periods, and the number and strength of the beats were determined for these periods before, during, and after the treatment with each solution.

The results of the experiments are recorded in the tables below. The effects of the solutions on the rate of the heart will be found

in Table I. and those on the force of the beat in Table II. In each case the results indicate the specific action as determined by counts

TABLE I.

THE ACTION OF SOME COMMON SALTS ON THE RATE OF THE BEAT OF THE FROG'S HEART.

	No_3	No_2	So_4	Cl
Cu_3	Inc. (brief)	....	Inc. (slight)	Inc. (slight)
Sr	Inc.	....	Inc.	Inc.
Mg.	Dec.	....	Inc.	Inc.
NH_4	Inc.	....	No change	Inc.
Na	Inc. (slight)	Inc. (slight)	Inc. (irreg.)	Inc.
K	Inc. (irreg.)	Inc.	Inc.	Dec. & inc.
Fe_3	Dec. (slight)	....	Dec. (irreg.)	No change
H	Inc.	....	Dec.	Inc. (slight)

TABLE II.

THE ACTION OF SOME COMMON SALTS ON THE FORCE OF THE BEAT OF THE FROG'S HEART.

	No_3	No_2	So_4	Cl
Cu_3	No change	....	Inc.	Inc.
Sr	Inc.	....	Inc.	Dec.
Mg	Inc. (slight)	....	Inc. (slight)	No change
NH_4	Inc.	....	Inc. (slight)	Inc. (slight)
Na	Inc.	Inc. (slight)	Inc.	Inc. (slight)
K	Inc. (slight)	Dec. & inc.	Dec. & inc.	Dec. (slight)
Fe_3	Dec.	....	No change	Inc. (slight)
H	Dec. (strong)	....	Inc.	Dec. & inc.

and measurements. Since the heart rate varied so greatly in the different frogs, no comparisons of the average rates for the different salts could be made.

All the salts of copper which were studied acted as stimulants by increasing both the rate and the force of the heart beat under the conditions of the experiment. In the case of the nitrate this action was not so marked on the rate, and on the force there was so slight a change in all the experiments that it could not be considered of any value. That dilute solutions of copper sulphate act as stimulants to the heart is well known, and this stimulating action is shown here both on the rate and on the force of the action.

With the exception of the chloride, the salts of strontium increased both the rate and the force, while in the case of the chloride the rate was increased, the force being decreased. These results, it will be noted, are not strictly in accord with the statements of Wood,[11] who says that the strontium salts produce at first a stimulation of the heart muscle followed by a slowing and paralysis. These primary and secondary actions were not found in my experiments, but it is, of course, possible that the long-continued action of strontium is accompanied by the later depressing effects noted by that author.

The salts of magnesium showed a varied action. The sulphate and chloride increased the rate, the sulphate increased the force slightly. With the nitrate the rate was decreased, the force being slightly increased.

The experiments of Macnider and Matthews[12] showed that the chloride or sulphate of magnesium injected into the circulation of the dog had a depressing effect on the heart, slowed the heart rhythm, and decreased the contractions. Many other investigators have reported similar results, but in their experiments there was a much greater effect in these directions, possibly because of the more intimate relation of the magnesium salt with the heart muscle and nervous tissues through the feeding of the heart by the magnesium-loaded blood.

From the accounts of various experimenters the salts of ammonium are considered to be cardiac stimulants, but the results are discordant. It is said that large doses produce a fall of blood pressure, that they arrest the heart in diastole, and that ammonia acts on the heart directly. All the results in the present study have been in the direction of stimulation, although no appreciable change

[11] WOOD: Therapeutics, its principles and practice, 14th ed., Philadelphia, 1908.

[12] MACNIDER and MATTHEWS: *Loc. cit.*

in rate was noted when ammonium sulphate was used. The importance of the ammonium salts of the blood in regulating or affecting the heart beat is worth noting, for so far as the author is aware little has been done to indicate the value or function of these important blood salts in regulating the rhythm of the heart.

In these experiments the salts of sodium had a slight stimulating action, increasing both the rate and the force of the heart. This increase was not marked on either the rate or the force, as will be observed from an examination of the tables. Sodium is the largest single mineral constituent of the blood, and it is doubtful if small doses have any appreciable effect on the normal tissue of animals. It certainly does not depress the system as potassium is said to do.

The salts of potassium, as noted in the tables above, are rather irregular in their action. The nitrite markedly increased the rate, but at first decreased and later increased the force. The other salts were irregular in respect to increasing the rates, while their actions on the force were varied. There was often found an initial decrease followed by a secondary increased action. The results obtained in these experiments are in accord with the statements of Wood[13] that there is some evidence to show that potassium has a slight stimulating effect on the heart when given in small doses. Wood concludes that such an action is not proven, but it is difficult to explain the results otherwise. On the other hand, we know, however, that in full doses the action of potassium is depressive, both on the heart and the blood vessels.

The salts of iron have been little studied in respect to their action on the heart, although in this case there is also the probability, noted above in the case of ammonium, that this constituent of the blood may have an important bearing upon the heart action. In the cases of two of the salts here studied the rate was decreased, and in the case of the chloride so slight a change was noted that it was within the limit of experimental error. With the nitrate a decided decrease in the force was found, while with the chloride a slight increase was noted. The sulphate had no effect upon the strength of the beat.

The dilute acids showed a diversity of action. The nitric acid increased the number of beats and decreased the force. The sulphuric acid decreased the number of beats but increased the force.

[13] Wood: *Loc. cit.*

The hydrochloric acid slightly increased the number of beats; the force showed an initial decrease followed by an increase.

Summary.

The action of nitrites on the heart has been sparingly studied, and their action is not well established. With the small doses which were used a slight stimulating action was noted. Large doses, however, are said to depress the cardiac muscles as well as the vaso-motor system.

From the limited number of experiments here recorded the indications are that nitrates, with the exception of magnesium and iron, increase the rate of the heart beats, and that the force of the heart beat is increased save in the case of iron and hydrogen.

All the sulphates, with the exception of iron and hydrogen, increased the rate, and in no case was a decreased force noted.

Of the chlorides all increased the rate, although potassium was found to be irregular in action. All but strontium and potassium increased the force; in these two cases there was a decrease.

This work was begun in conjunction with Mr. E. W. Boughton, but the results recorded in this paper were obtained after the pressure of other duties had compelled his withdrawal. The writer wishes to express his indebtedness to Professor Shepherd Ivory Franz for suggestions and assistance throughout the progress of the work.

A QUANTITATIVE STUDY OF FARADIC STIMULATION. — III. THE MEASUREMENT OF "MAKE" SHOCKS.

By E. G. Martin.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

In the first paper of this series[1] I set about the task of developing a plan whereby the physiological efficiencies of faradic stimuli might be expressed in terms of stimulation units. In that paper were stated the various factors which determine the physiological intensities of induction shocks. The factors of primary importance were shown to be the construction of the inductorium, the position of the secondary coil with respect to the primary, and the intensity of the primary current. Inasmuch as Helmholtz[2] had shown that the relationship of these factors to "break" shocks is a comparatively simple one, the first step was the development of a scheme of calibration for "break" stimuli which should take into account the influence of these three fundamental factors. This calibration was presented in the second paper of the series.[3] The present paper contains the second step in the general plan, a method for measuring the influence of these same three factors on "make" shocks.

From the outset I desired to base the method of measuring "make" stimuli, if possible, upon the calibration which had been proposed for "break" shocks. From observations reported in the first paper of the series[4] it was clear that that calibration would not express directly the relationships existing between "make" shocks as it does those between "breaks." A general consideration of the physical principles underlying the production of the two sorts of induced currents led me, however, to believe that a simple mathematical relationship between the intensities of "break" stimuli and

[1] Martin: This journal, 1908, xxii, p. 61.

[2] Helmholtz: Poggendorf's Annalen der Physik und Chemie, 1851, lxxxiii, p. 505.

[3] Martin: *Loc. cit.*, p. 116.

[4] Martin: *Ibid.*, p. 68.

of " make " stimuli might be shown to exist, and a formula deduced for expressing the latter in terms of the calibration already proposed for the former. Finding myself unable to make any progress toward such a formula through study of the principles of electromagnetic induction, I turned to experimentation in the hope that an empirical formula could be established which should fulfil the purpose desired.

The method of experimentation was essentially the same as that used in the study of " break " shocks,[5] the index of the value of the stimulus being the minimal contraction of a frog's gastrocnemius muscle uncurarized. With the onset of hot weather a change in the method of applying the stimulus to the muscle was adopted in the hope of improving the ability of isolated muscles to retain uniform irritability. Instead of stimulating by means of platinum needles thrust directly through the stripped muscle a specially arranged nerve-muscle preparation was used. The entire leg with skin intact was removed from the body. The gastrocnemius was separated in its skin according to the usual laboratory method. A longitudinal incision was then made through the skin and muscles of the ventral surface of the thigh exposing the femur, which was then cut through as high as possible and drawn outward through the skin incision to serve, when placed in a clamp, as support for the entire preparation. A little blunt dissecting in the cavity left by the withdrawal of the bone revealed the sciatic nerve. This was slipped across the terminals of a pair of small shielded electrodes. The thigh muscles were then returned as nearly as possible to place, care being taken that they should protect the nerve completely from exposure to air. The leg with shielded electrodes in place was supported in a moist chamber in such fashion as to allow the femur to be placed in a clamp and the gastrocnemius to be attached to a recording lever. This preparation when made with care could usually be depended on to maintain uniform irritability for two or three hours in the hottest weather. In most instances the threshold value of the stimulus applied thus through the nerve was lower than the average obtained from direct stimulation of the muscle. The difference, however, was not so marked as might have been expected. The results given by these preparations were perfectly concordant with those obtained from muscles stimulated directly.

[5] MARTIN: *Loc. cit.*, p. 117.

Application of the "Break" Calibration to "Make" Stimuli.

The problem before me was to determine by experiment whether under a given set of conditions for generating induced currents a definite mathematical relationship exists between the physiological intensities of the "break" and "make" shocks. In a former paper[6] it was shown that for equal "break" stimuli the product $\frac{M}{L} \times I$ is constant, $\frac{M}{L}$ being the "calibration number" whose value depends on the position of the secondary coil with respect to the primary, and I being the intensity of the primary current in amperes. This constant represents the value of the stimulus and will be called Z,[7] the general formula for "break" shocks then being:

$$Z_b = \frac{M}{L} I. \qquad (1)$$

My first procedure was to obtain a series of equal "make" stimuli with the secondary coil at various distances from the primary. The "calibration number" for each secondary position was then multiplied by the intensity of primary current employed at that position, and the products for each experiment were set down in a table. Three such experiments are quoted in Table I. For the inner positions of the secondary coil, positions which have relatively large values of $\frac{M}{L}$, the product $\frac{M}{L} \times I$ is nearly constant; as the secondary coil is moved out into the parts of the field where the values of $\frac{M}{L}$ are small, the product $\frac{M}{L} \times I$ is progressively larger the farther out the secondary coil is pushed, and consequently the smaller are the values of $\frac{M}{L}$. Numerous repetitions of the experiment gave precisely similar results.

These experiments indicated quite clearly the existence of a comparatively simple relationship between "make" and "break" stimuli, and also suggested a method for expressing the relationship mathe-

[6] Martin: *Loc. cit.*, p. 132.

[7] To distinguish between "break" stimuli and "make" stimuli the former will be represented by Z_b, the latter by Z_m.

matically in the simplest possible fashion, namely, through the introduction of a single factor into the "break" shock formula (1), which when introduced would cause it to give equal values for Z for equal "make" stimuli. It is obvious, from a study of Table I,

TABLE I.

VALUES OBTAINED WHEN THE PRIMARY CURRENTS GIVING EQUAL "MAKE" STIMULI ARE MULTIPLIED BY THEIR CORRESPONDING CALIBRATION NUMBERS. COIL B.

Position of secondary in cm.	Value of $\frac{M}{L}$	Exp. of April 18, 1907. E. M. D. P. of primary current = 8.8 volts.		Exp. of Nov. 5, 1907. E. M. D. P. of primary current = 4 volts.		Exp. of Nov. 18, 1907. E. M. D. P. of primary current = 5 volts.	
		Val. of I. in amperes.	$\frac{M}{L} \times$ I.	Val. of I. in amperes.	$\frac{M}{L} \times$ I.	Val. of I. in amperes.	$\frac{M}{L} \times$ I.
0	3920	......	....	0.0005	1.96	0.00053	2.08
4	3600	0.00185	6.66	......	....	......	....
8	2600	0.00250	6.50	0.00075	1.95	0.00080	2.08
12	1100	0.00603	6.64	0.0020	2.20	......	....
14	530	0.0138	7.32	......	....	......	....
16	250	0.0297	7.42	0.0090	2.25	0.0090	2.25
20	91	0.110	10.00	0.027	2.46	0.027	2.46
24	46	0.335	15.40	0.070	3.22	0.064	2.94
25	40	0.475	19.00	......	....	......	....
28	27	....	....	0.20	5.40	0.175	4.90

that the factor to be introduced must be relatively larger the smaller is the value of $\frac{M}{L}$ and must tend to diminish $\frac{M}{L}$. A constant number has this effect if it is subtracted from $\frac{M}{L}$. Formula (1) modified in accordance with this idea becomes

$$Z_m = \left(\frac{M}{L} - \mathrm{K}\right) I. \qquad (2)$$

It was found that in practically every experiment of a large series some number could be selected to be substituted for K in formula (2) with a fairly constant value of Z resulting. For each experiment the value of K had to be determined empirically, and it was

found to vary widely in different experiments. In all the earlier experiments the values of K were negligibly small in comparison with the values of $\frac{M}{L}$ for secondary positions of 12 cm. or less. Therefore, in order to save time, most of the later experiments were begun with the secondary coil at 12 cm. Some typical experiments illustrating the application of formula (2) to equal "make" stimuli are given in Table II.

TABLE II.

Experiments showing that Equal "Make" Stimuli give Equal Values of Z_m when the Latter is computed according to the Formula $Z_m = \left(\frac{M}{L} - K\right) I$. Coil B.

Position of secondary in cm.	Value of $\frac{M}{L}$.	Exp. of Nov. 5, 1907. Pri. voltage = 20. K = 5.5.		Exp. of Oct. 14, 1908. Pri. voltage = 4. K = 18.		Exp. of Oct. 29, 1908. Pri. voltage = 10. K = 12.		Exp. of Jan. 4, 1909. Pri. voltage = 2. K = 22.	
		Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.
12	1100	0.00205	2.25	0.00125	1.36	0.0023	2.50	0.0012	1.29
16	250	0.0086	2.10	0.0059	1.37	....	....	0.0053	1.21
18	145	....	....	0.0108	1.37	0.0195	2.60	....	....
20	91	0.0235	2.00	0.0185	1.35	....	....	0.018	1.24
22	64	....	....	0.0285	1.34	0.0475	2.47	....	....
24	46	0.049	2.03	0.048	1.34	0.074	2.52	0.052	1.25
26	35	....	....	0.080	1.36	0.110	2.53	....	....
28	27	0.095	2.04	....	....	0.170	2.55	0.27	1.35
30	21	....	....	....	....	0.268	2.41	....	....
32	16.5	0.21	2.31	....	....	....	....	....	....
36	11.5	0.34	2.04	....	....	....	....	....	....

Development of a General Formula for "Make" Shocks.

The discovery of formula (2) is a decided step toward the ultimate solution of the problem of measuring "make" shocks, but it is not a complete solution, since it offers no means of determining

in advance what the value of K will be under any given set of conditions. The next thing done was to study a large series of experiments with reference to the conditions upon which the values of K depend.

That the voltage of the primary current has great influence upon the stimulating values of "make" shocks was stated in a former paper [8] and is demonstrated in Table III, where it is shown that to

TABLE III.

EXPERIMENTS SHOWING THAT TO OBTAIN EQUAL "MAKE" STIMULI WITH VARYING PRIMARY VOLTAGES CONSIDERABLE COMPENSATORY CHANGES IN PRIMARY AMPERAGE ARE NECESSARY.

EXPERIMENT OF NOV. 19, 1907. SECONDARY COIL AT 16 CM. $\frac{M}{L} = 250$.					
Primary E. M. D. P. in volts.	1	2	4	8	21
Primary current in amperes.	0.07	0.027	0.0208	0.0199	0.0188
EXPERIMENT OF NOV. 2, 1908. SECONDARY AT 22 CM. $\frac{M}{L} = 64$.					
Primary E. M. D. P. in volts.	2	4	6	8	10
Primary current in amperes.	0.097	0.048	0.042	0.038	0.0365

obtain equal "make" stimuli with varying primary voltages considerable compensatory changes in primary current intensities are necessary.

In order to eliminate for the moment the voltage factor, all the experiments with "make" shocks were divided into groups, each group containing all the experiments at any single primary voltage. The values of K for the different experiments of any group still differed widely, but it was now noticeable that wherever the value of K was large the value of Z was also large and *vice versa*. This suggested at once a possible dependence of the value of K upon that of Z. To test this possibility the experiments of each group were plotted, values of K against values of Z. The resulting curve in each case is a straight line having the simple equation

$$K = aZ. \qquad (3)$$

[8] MARTIN: *Loc. cit.*, p. 72.

Fig. I gives the curve for coil *B* obtained by plotting the experiments at 2 volts. The value of *a* given by this curve is 18. Substituting in equation (2) the value of *K* given in equation (3), we have

$$Z_m = \frac{M}{L} I - aZI. \qquad (4)$$

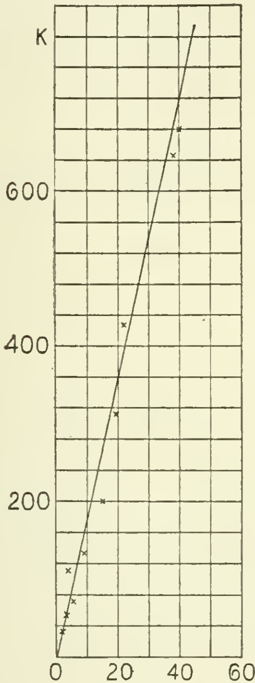


FIGURE I. — Curve obtained when the values of *Z* given by the application of the formula $Z = \left(\frac{M}{L} - K\right) I$ to the experiments performed with a primary voltage of 2 are plotted against the values of *K* used in these experiments. Ordinates represent values of *K*; abscissæ represent values of *Z*. The equation for this curve is $K = 18\ Z$.

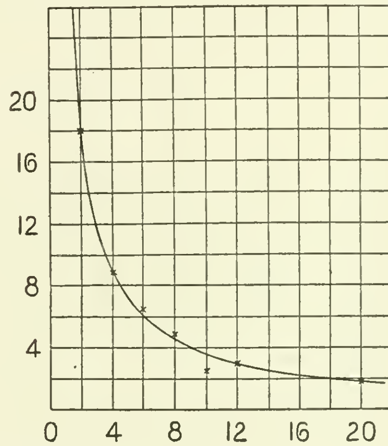


FIGURE II. — Curve obtained by plotting against the different primary voltages used in these experiments the values of *a* obtained from curves plotted as in Fig. 1. The equation for this curve is $E \times a = 36$. Ordinates represent values of *a*; abscissæ represent primary voltages.

This solved for Z_m and simplified gives

$$Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + a}. \qquad (5)$$

an equation which enables us to determine the value of "make" stimuli at any given primary voltage, for which the value of *a* is known.

There remains now for the completion of the "make" shock formula only the establishment of a definite relationship between the values of a at various primary voltages and the voltages themselves. To determine whether such a relationship exists another curve was plotted, primary voltages against values of a previously determined. This curve is represented in Fig. 2. It has the simple equation

$$E \cdot a = C, \qquad (6)$$

in which C represents a constant. Substituting in equation (5) the value of a given by equation (6), we have

$$Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + \frac{C}{E}},$$

which is the general equation for "make" induction shocks. The value of C is fixed for each inductorium. For the one with which this equation was developed, coil B, its value is 36.

The equation here presented is based upon eighty-five concordant experiments in which were used 16 different primary voltages ranging from 1 to 49 volts. Some representative experiments are given in Tables IV and V.

Application of the general formula for "make" stimuli to another inductorium. — As an additional assurance of the correctness of the general formula above presented and also to learn how much experimentation is required for the empirical determination of the value of C, some experiments were carried on with an inductorium which in this work will be known as coil A. This inductorium is a recent one, made by Bischhausen Brothers of Berne. It is provided with the Kronecker graduation, which, however, is unfortunately quite useless for quantitative purposes, because the makers of the inductorium have taken pains to secure the iron core of the primary coil firmly in place. In a former paper [9] it was emphasized that under this circumstance the relationships indicated by the Kronecker graduation do not hold. For that matter the graduation was not intended by its originator to apply to this condition.[10] This inductorium I calibrated for "break" shocks according to the method previously

[9] Martin: *Loc. cit.*, p. 64.

[10] Cyon: Methodik der physiologischen Experimente, Giessen, 1876, p. 381.

TABLE IV.

Representative Experiments showing the Application of the Formula $Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + \frac{36}{E}}$ to the Measurement of "Make" Shocks,

Coil B. In these Experiments the Voltage of the Primary Current was unaltered during Individual Experiments.

Position of Secondary in cm.	Value of $\frac{M}{Z}$.	June 27, 1908. E=2 volts.		October 23, 1908. E=4 volts.		Nov. 18, 1907. E=5 volts.		July 18, 1908. E=8 volts.		October 30, 1908. E=10 volts.		Jan. 6, 1908. E=20 volts.	
		Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.
0	3920	......	....	......	....	0.00053	2.07	....	....	......	....	......	....
8	2600	......	....	......	....	0.0008	2.07	....	....	......	....	......	....
12	1100	......	....	0.00075	0.82	......	....	0.0024	2.61	......	....	......	....
14	530	0.0046	2.25	......	....	......	....	0.0057	2.95	......	....	......	....
16	250	0.0096	2.05	......	....	0.009	2.13	0.0114	2.72	0.0027	0.67	0.0135	3.06
18	145	0.0198	2.12	0.0065	0.89	......	....	......	....	0.0045	0.64	......	....
20	91	0.038	2.05	......	....	0.027	2.06	0.0339	2.68	......	....	0.038	3.24
22	64	0.103	2.31	0.0143	0.81	......	....	0.056	2.86	0.010	0.62	......	....
24	46	0.238	2.07	0.020	0.80	0.064	2.03	0.089	2.92	0.0145	0.63	0.080	3.21
26	35	......	....	0.0286	0.80	......	....	......	....	0.020	0.65	......	....
28	27	......	....	0.041	0.81	0.175	2.09	......	....	0.025	0.62	0.155	3.27
30	21	......	....	......	....	......	....	......	....	0.033	0.63	......	....
33	15	......	....	......	....	......	....	......	....	......	....	0.36	3.28

described.[11] Seven series of equal "make" stimuli were then taken at primary voltages ranging from 2 to 12 volts. The seven experiments agree in indicating that the value of C for this coil is 8. The experiments are given in Tables VI and VII. By way of corrobora-

TABLE V.

Representative Experiments showing the Application of the Formula $Z_m = \frac{\frac{M}{L}}{\frac{1}{I}+\frac{36}{E}}$ to the Measurement of "Make" Shocks, Coil B. In these Experiments the Position of the Secondary Coil was unaltered during Individual Experiments.

Val. of E.	Nov. 7, 1907. Secondary at 16 cm. $\frac{M}{L}=250$.		Nov. 19, 1907. Secondary at 16 cm. $\frac{M}{L}=250$.		Nov. 2, 1908. Secondary at 22 cm. $\frac{M}{L}=64$.		Nov. 20, 1908. Secondary at 12 cm. $\frac{M}{L}=1100$.	
	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.
1	....	....	0.07	4.97	....	....	....	....
2	0.0102	2.15	0.027	4.55	0.097	2.26	0.040	25.6
4	0.0096	2.21	0.0208	4.40	0.048	2.15	0.0328	27.9
6	....	....	....	....	0.042	2.15	0.0305	28.4
8	0.0092	2.21	0.0199	4.60	0.038	2.08	0.030	29.1
10	....	....	....	....	0.0365	2.06	0.29	28.9
20	0.0088	2.16	....	....	....	....	....	....
21	....	....	0.0188	4.55	....	....	....	....

tion of this value of C some experiments performed with coil A two years before the "make" shock formula was developed were examined and found to give constant values of Z when the formula indicated by the experiments cited above was applied to them. Representative experiments from this group are given in Table VIII.

Comparison of the General Formulæ for "Break" and "Make" Stimuli.

If the general equation for "break" shocks be written in the same form as the one for "make" shocks and the two placed side

[11] Martin: *Loc. cit.*, p. 119.

TABLE VI.

EXPERIMENTS WHICH ESTABLISHED IN COIL A THE VALUE OF C EQUAL TO 8 IN THE GENERAL "MAKE"-SHOCK FORMULA $Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + \frac{C}{E}}$. IN THESE EXPERIMENTS THE VALUE OF E WAS UNALTERED DURING INDIVIDUAL EXPERIMENTS.

Position of secondary in cm.	Value of $\frac{M}{L}$.	Jan. 15, 1909. $E=2$ volts.		Jan. 18, 1909. $E=2$ volts.		Jan. 19, 1909. $E=2$ volts.		Jan. 16, 1909. $E=4$ volts.		Jan. 16, 1909. $E=8$ volts.	
		Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.
0	2200	0.00175	3.82	0.00048	1.05	0.0011	2.40	......	....	0.00085	1.85
4	2000	0.002	3.97	......	....	......	....	......	....	......	....
8	1500	......	....	......	....	......	....	0.00105	1.57	0.0012	1.80
12	600	0.0069	4.03	0.00175	1.04	0.0034	2.00	0.00275	1.64	0.0031	1.85
16	120	0.036	3.78	......	....	......	....	......	....	......	....
18	69	......	....	0.0150	0.98	0.035	2.12	0.024	1.58	0.026	1.75
20	43.5	0.137	3.85	......	....	......	....	......	....	......	....
24	22	0.61	3.90	0.059	1.05	0.24	2.68	0.079	1.50	......	....
26	16.8	....	....	......	....	....	....	0.115	1.57	......	....
30	10	....	....	0.20	1.11	....	....	....	....	....	....

by side, the simple mathematical relationship existing between "make" and "break" stimuli becomes apparent. Written thus, the "break" shock formula is

$$Z_b = \frac{\frac{M}{L}}{\frac{1}{I}}.$$

TABLE VII.

EXPERIMENTS WHICH ESTABLISHED IN COIL A THE VALUE OF C EQUAL TO 8 IN THE GENERAL "MAKE"-SHOCK FORMULA $Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + \frac{C}{E}}$. IN THESE EXPERIMENTS THE POSITION OF THE SECONDARY COIL WAS UNALTERED DURING INDIVIDUAL EXPERIMENTS.

Value of E.	Jan. 16, 1909. Secondary at 20 cm. $\frac{M}{L}=43.5$.		Jan. 16, 1909. Secondary at 30 cm. $\frac{M}{L}=10$.	
	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.
2	0.18	4.57	0.325	1.41
4	0.125	4.35	0.19	1.38
6	0.122	4.57	0.175	1.42
8	0.11	4.30	0.162	1.39
10	0.11	4.40	0.160	1.42
12	....	....	0.158	1.43

Comparing this with the "make" shock formula

$$Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + \frac{C}{E}},$$

it is seen that the difference between them is wholly in the denominator, and consists of the addition of a simple expression to the denominator of the "break" shock formula to give the one for "make" shocks. Inasmuch as increasing the denominator of a fraction diminishes the value of the fraction, the formulæ express

the well-known fact that "make" shocks are weaker than "break" shocks produced under equivalent conditions.

TABLE VIII.

FURTHER CORROBORATION OF THE GENERAL "MAKE"-SHOCK FORMULA AS APPLIED TO COIL A.

Position of secondary in cm.	Value of $\frac{M}{L}$.	Nov. 1, 1906. E = 4.4 volts.		Nov. 2, 1906. E = 5.5 volts.		April 18, 1907. E = 8.8 volts.	
		Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.	Val. of I in amperes.	Z_m.
0	2200	......	......	0.00650	14.7	0.00265	5.70
2	2120	0.00285	6.02	0.00675	14.2	......	....
4	2000	0.00314	6.24	0.00725	14.3	0.00283	5.64
6	1800	0.00343	6.14	0.00800	14.2	......	....
8	1500	0.00397	5.92	0.00960	14.3	0.00366	5.48
10	1075	0.00565	6.02	0.0132	14.2	......	....
12	600	0.01013	5.96	0.0238	13.7	0.00927	5.50
14	250	0.0247	5.90	0.0595	13.7	0.0205	5.03
16	120	0.0530	5.80	0.1470	14.5	0.043	4.97
18	69	0.104	6.00	....	....	....	....
20	43.5	0.185	6.00	....	....	0.131	5.12
21	36	0.240	6.00	....	....	....	....
24	22	....	....	....	....	0.305	5.23
26	16.8	....	....	....	....	0.460	5.40

In the formulæ as here presented the numerators express the influence upon the value of Z of the position of the secondary coil with respect to the primary. The denominators express the influence upon Z of the intensity of the primary current, and for "make" stimuli of its voltage also. Since the numerator is the same in both formulæ, *i. e.*, $\frac{M}{L}$, it follows that however the "break" stimulus, Z_b, may compare with the "make" stimulus Z_m, changing the value of $\frac{M}{L}$ by moving the secondary coil does not affect the relationship between them. To illustrate, if we suppose the "break" stimulus to

be twice as intense as the "make" stimulus when the secondary coil is at zero, the "break" will continue to be twice as intense as the "make" wherever the secondary coil is placed, provided, of course, that all other conditions remain constant. I wish to emphasize this fact, because it is a direct contradiction of a statement made by me in the first paper of this series.[12] That statement was: "A . . . condition affecting the relative intensities of make and break shocks is the position of the secondary coil with respect to the primary." I presented evidence[13] which, in the light of the knowledge I had at that time, seemed to prove the truth of the statement. With the further study of "make" shocks it has become clear, however, that that evidence was misinterpreted. Its true significance will be discussed in a later paragraph.

Since the difference between the two formulæ is wholly in their denominators, we may expect careful analysis of these to yield a full understanding of the conditions upon which depend the relationships between "make" and "break" stimuli. It is clear, as stated above, that the denominator of the "make" shock formula will always be larger than that of the "break" formula, but the amount of difference between the two will vary greatly according to the relative values of $\frac{1}{I}$ and $\frac{C}{E}$. If we examine, for illustration, the experiments with coil *B* cited in Tables I to V of this paper, we find values of I ranging from 0.0005 ampere to 0.475 ampere, and values of E ranging from 1 to 21 volts. The value of C is constant throughout, being for coil *B* 36. Let us compare the values of Z_b and Z_m in a hypothetical experiment in which a primary current of 0.0005 ampere at 20 volts is employed. The expression for Z_b is

$$\frac{\frac{M}{L}}{2000}.$$

For Z_m the expression is

$$\frac{\frac{M}{L}}{2000 + \frac{36}{20}} = \frac{\frac{M}{L}}{2001.8}.$$

The difference between the stimulating intensities of the two sorts of shocks is in this case less than one tenth per cent. Compare now

[12] Martin: *Loc. cit.*, p. 69. [13] Martin: *Ibid.*, p. 70, Table V.

the values of Z_b and Z_m when a primary current of 0.4 ampere at 2 volts is used. The expression for Z_b is

$$\frac{\frac{M}{L}}{2.5}$$

and for Z_m is

$$\frac{\frac{M}{L}}{2.5 + \frac{36}{2}} = \frac{\frac{M}{L}}{20.5}.$$

In this case the "break" shock is more than eight times as intense as the "make."

The above illustrations present in concrete form the effects upon the relation between "break" and "make" stimuli of variations in intensity and voltage of the primary current. These effects may be stated in general thus: *The higher the voltage of the primary current and the less its intensity, the more nearly will "make" shocks equal "break" shocks; conversely, the lower the voltage of the primary current and the greater its intensity, the more will "break" shocks exceed "make" shocks.*

Relation of "Make" Stimuli to Primary Current Intensity.

The "make" shock formula shows that "make" stimuli do not vary directly with the intensity of the primary current, as Helmholtz [14] showed "break" stimuli to do. Although "make" shocks increase absolutely with every increase in primary intensity, other conditions remaining uniform, the increase is relatively slight when primary intensities of considerable magnitude are compared. For example, if with coil *B* a 2 volt primary current be increased from 0.5 ampere to 1.0 ampere, the "make" stimuli will be increased only 5 per cent, while "break" shocks under the same circumstances would be doubled.

This peculiarity of relation of "make" shocks to primary currents of high intensity has shown itself very strikingly in many of the experiments carried on in connection with this work. The use of minimal muscular contractions as indicators of stimulation

[14] Helmholtz: *Loc. cit.*

strengths requires that in the outer parts of the field of the inductorium, where the values of $\frac{M}{L}$ are small, primary currents of high intensity be employed to give shocks sufficient to elicit visible response. I have found it very often the case when studying "make" shocks, especially with primary currents of low voltage, that when the secondary coil was pushed out to a point where primary currents of 0.1 or 0.2 ampere failed to elicit response, no increase of primary intensity up to the limits of my apparatus would bring the stimulus up to the threshold. This frequent failure of relatively enormous primary currents to give detectible "make" stimuli was to me wholly inexplicable until the development of the "make" shock formula made its meaning clear.

The correct interpretation of the experiment cited by me in the first paper of this series in support of a wrong conclusion (see p. 282 above) is likewise simple in the light of the relation of "make" shocks to primary intensity brought out by the formula. The erroneous assumption which led me astray was that the strengths of "make" shocks as well as of "break" shocks vary directly with the intensities of the primary current. Until the present work no valid reason for doubting that assumption existed.

Summary.

1. The physiological intensities of "make" induction shocks may be expressed in terms of the calibration already proposed for "break" shocks by the use of the formula

$$Z_m = \frac{\frac{M}{L}}{\frac{1}{I} + \frac{C}{E}}.$$

In this formula Z_m represents the stimulating value of the "make" shock, $\frac{M}{L}$ the "calibration number" whose value depends on the position of the secondary coil, I the intensity of the primary current measured in amperes, E the E.M.D.P. of the primary current expressed in volts, and C a constant which is fixed for each inductorium.

2. Comparison of the formula for "make" shocks with the one previously established for "break" shocks shows that the relationship between the two sorts of stimuli depends in any given inductorium altogether upon the intensity and voltage of the current through the primary coil.

3. The effects upon the relation between "break" and "make" shocks of variations in the intensity and voltage of the primary current may be stated in general terms thus: The higher the voltage of the primary current and the less its intensity, the more nearly will "make" shocks equal "break" shocks; conversely, the lower the voltage of the primary current and the greater its intensity, the more will "break" shocks exceed "make" shocks.

4. With primary currents of high intensity and low voltage large variations in intensity produce relatively slight alterations in the stimulating values of "make" shocks.

A METHOD OF DETERMINING THE POSITION OF THE CENTRE OF GRAVITY IN ITS RELATION TO CERTAIN BONY LANDMARKS IN THE ERECT POSITION.[1]

By EDWARD REYNOLDS and ROBERT W. LOVETT.

SO far as can be learned from a study of the literature, there is at present no reliable method of estimating the position of the centre of gravity of the body in the upright position. Various loose statements as to its location are given in works dealing with the mechanics of the upright position, and there are a few carefully formulated attempts to determine it by a study of the masses of the body and their relation to each other,[2] but scarcely any two writers agree as to what the erect normal posture should be. The position of the centre of gravity would vary according to each one's idea of the normal posture, for the disposition of the various masses and their relation to each other would of course change as the posture changed.

In the horizontal position, however, the centre of gravity may be determined by the reliable method attributed to Borelli. The subject lies on the back on a long board balanced on the edge of a piece of wood triangular in section. When the subject lies in such a position that the board balances evenly, the centre of gravity lies in a plane vertical to the edge of the triangular support.

A study of this experiment led to the present method of determining the plane of the centre of gravity of the body in the standing position and its correlation with certain bony landmarks.

Upon the platform of a dial scale registering up to 100 pounds (Fig. 1, *A*) is placed a sharp edge which supports one end of a flat

[1] Presented before the Society of Medical Sciences on November 17, 1908.

[2] These methods are reviewed by NAGEL, Handbuch der Physiologie, iv, p. 564. The practical working out of the centre of gravity as determined by these methods is given in HORNER's monograph.

board (B), the other end of which is supported by a similar sharp edge placed upon a rigid block (C). The distance between the edges is 508 mm. (20 inches). A short distance behind the rigid block is placed an upright post (E) with a horizontal sliding arm (D, shown in section only), which furnishes a plane of reference from which the antero-posterior position of each of the important landmarks of the body can be determined by measuring their horizontal distance from this sliding arm. The determination of the antero-posterior position of the centre of gravity in the standing subject is then rapid and easy, the method being as follows:

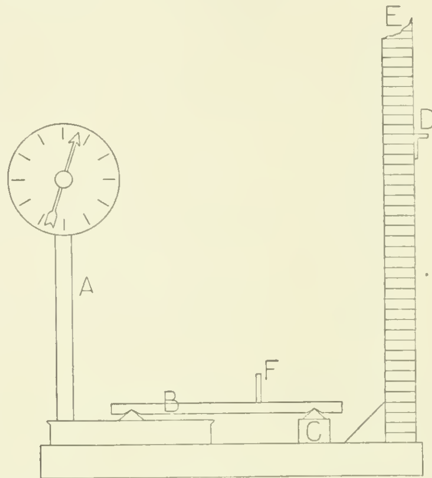


FIGURE 1. — Side view of the apparatus for estimating the centre of gravity. A, scale. B, balance plane on which patient stands facing A. C, block supporting triangular edge. D, movable horizontal arm on upright for obtaining horizontal distances. E, vertical upright for standard plane. F, ledge against which heels are placed.

The subject is weighed. He is then placed upon the balance plane (B) at a known point facing the scales. The exact point is unimportant, but after some experimentation we selected as most convenient that in which the heels are situated at one fourth the length of the plane from the posterior sharp edge. A movable ledge (F) against which the heels are placed is provided here. Since the balance plane upon which the subject stands acts as a lever, in which the weight is borne between the fulcrum and the supporting force (the posterior sharp edge and the spring which governs the scales), it is evident that the weight recorded on the dial (the balanced weight) will bear to the total weight the same proportion which obtains between the total length of the balance plane and the distance between the perpendicular dropped from the patient's centre of gravity and the posterior end of the plane. The formula is

$$\frac{\text{Wt.}}{\text{Bal. wt.}} = \frac{L}{X},$$

where L is the length of the balance plane.

In practice, and with this apparatus, the total weight is divided into twenty times the balance weight; the result is the disance in inches beween the perpendicular from the centre of gravity and the posterior edge.[3]

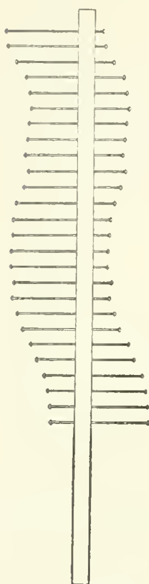


FIGURE 2. — Rod with metal arms for measuring back and abdominal contours.

After the determination of the position of the centre of gravity, which should occupy on an average one or two minutes, the following points having been marked on the skin are measured and recorded:

1. The length of the base of support, *i. e.*, the antero-posterior distance from the back of the heels to the most forward portion of the toes.

2. The position of the back edge of the malleolus, being its distance from the plane of measurement afforded by the horizontal arm and its height above the balance plane on which the subject stands.

In this and the following determinations the horizontal difference is obtained by a footrule, one end of which is placed against the malleolus, while the body of the rule is held by the thumb against the upper surface of the sliding arm. Since this arm (and therefore necessarily the ruler) is horizontal, the height of the point observed may be read at the same time, from a graduated scale which is marked upon the upright post.

3. The position of the back of the head of the fibula.

4. The position of the middle of the trochanter.

5. The position of the posterior part of the spine of the fifth lumbar vertebra.

6. The position of the posterior part of the spine of the seventh cervical vertebra.

All these points are taken under the usual conventions of somatological measurements on the living.

[3] To this in practice there must be added a constant of 4.25″, which represents the distance in this especial apparatus between the posterior sharp edge and the plane of measurement furnished by the movable posterior arm which is the point of departure throughout the graphic representation of results.

Each measurement is recorded on the subjoined blank.

Case. Date.

.... Observer.

Weight.

Balance weight.

	Distance from horizontal arm.	Height.
Malleolus		
Fibula		
Trochanter		
Fifth lumbar		
Seventh cervical. . . .		
Length of base . . .		
Fifth metatarsal . .		
Centre of gravity . (from calculation).		

The measurements are then easily transferred to graphic form by the reproduction of the observed measurements upon a sheet of paper, of which the bottom represents the balance plane, and the right edge the posterior plane of measurement (Fig. 3).

The above are all the exact measurements which we have taken, but since the value of their graphic representation is considerably enhanced by its combination with some sort of representation of the body profile of the individual, we have completed the examination by the use of a device which obtains this with fair accuracy and which is illustrated in Fig. 2. A series of horizontal metal arms, tipped with celluloid, slide easily through holes in the vertical wooden arm. These metal arms are shaken out to their full length, and their ends pushed rapidly and lightly against the subject's back in the median line, the point of the uppermost horizontal arm being applied to the seventh cervical vertebra. In the construction of the graphic record (Fig. 3), the position of this profile is known by its relation to the seventh cervical and fifth lumbar vertebræ; that is, these points are marked upon the paper from the measurements taken, and the end of the uppermost arm of the profile instrument is laid against the mark which represents the seventh cervical, while its lower portion is in contact with the mark representing the fifth lumbar vertebra. The curve is then traced from the ends of the pins throughout its length.

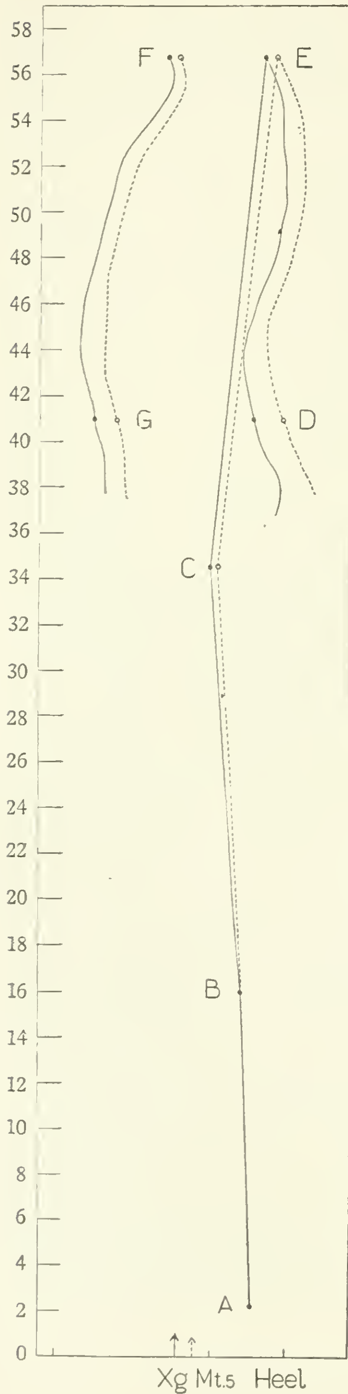


FIGURE 3. — Graphic representation of standing position under two conditions (shown in solid and broken line). *A*, external malleolus. *B*, head of fibula. *C*, trochanter. *D*, fifth lumbar vertebra. *E*, seventh cervical vertebra. *F*, *G*, horizontal points opposite *D* and *E*. *A C*, line of legs. *C E*, line connecting trochanter and seventh cervical.

The body curve of the ventral surface is obtained in the same way by the use of the same, or, to save time, preferably a second profile instrument, the uppermost arm of which is applied to the anterior surface of the neck at the level of the point accepted as the seventh cervical on the posterior surface. The position of this curve on the chart is ascertained by using as points of reference the horizontal distances between the posterior parts of the seventh cervical and fifth lumbar vertebræ and the points horizontally opposite on the ventral surface measured by a pelvimeter or other callipers.

It would be very desirable that this graphic record should be completed in every instance by a representation of the inclination of the brim of the pelvis, which would, of course, include its relation to the trochanter, but after much experimentation we have been unable to measure with accuracy the inclination of the pelvic brim in the living subject.

The use of the profile curves in the graphic representation involves the disadvantage that the chart must be drawn life size, but even though these profile curves are recognized as only approximately accurate, their use adds greatly to the value of the graphic represen-

tation. With two profile instruments the time occupied in obtaining the curves is unimportant.

The representation of attitude furnished by the completed record is practically accurate, and after a few records are taken becomes exceedingly satisfactory to the eye. The change effected in an individual by treatment or apparatus may be most convincingly displayed by the reproduction of its record on the same chart, as in Fig. 3.

The errors of the method have been carefully studied.

(*a*) **Swaying of the subject.** — When the subject first stands on the balanced board, there is a marked oscillation of the needle, which grows less and less as the subject sinks into an easy attitude. After a period usually from thirty seconds to one minute, the needle hovers around a definite point on the dial, making short excursions to one side and the other. The mean of the later oscillation is accepted at this time as the balanced weight. The cause of the oscillations of the needle was found to be the swaying of the subject. This fact was first roughly determined by measuring the distance of the various points from the fixed perpendicular. The sway was found to consist of a forward and backward movement of the body as a whole, least at the ankles and most of course at the head, and not in an undulating movement. These characters of the sway and the fact that it corresponded to the oscillations of the needle were proved by some forty observations, as follows:

Horizontal threads running backward were fastened to the subject's skin over the fifth cervical, the fifth lumbar, and the head of the fibula. At the plane of the upright the threads ran over pulleys and became vertical, carrying weights and cardboard indicators. By photographs it was shown that these indicators moved up when the subject swayed forward and down when the patient swayed back, that the excursions of the three were proportionate to the height of the points taken constant in their relation to each other and to the excursion of the dial needle recorded on the same plate with the movement of the cardboard.

(*b*) **Prevention of sway.** — The prevention of swaying without inducing distortion was then studied as a source of possible error. A small leather pad carried on a horizontal arm attached to an ordinary photographer's rest was used to support the back just above the fifth lumbar vertebra, and the subject was steadied in a position which was indicated by the needle to be the normal bal-

anced weight. It was found by measurements from the standard vertical plane of the various marked points that the most effective level for the fixation arm was near the waist and least efficient at the neck. That fixation by this method did not in any way distort the previous attitude was shown by composite photographs of the models, two exposures being made on the same plate, one before and one after fixation. The outlines were found to correspond exactly in the two positions.

(*c*) **Errors in measurement from the standard vertical plane.** — By pressing the rule hard against the skin an error of 3 or 4 mm. is easily made, but the observer soon learns to use the same amount of pressure. In every observation each measurement is taken twice. It is very unusual to find a variation of 4 mm. between the two sets, and we have accepted 6–5 mm. (¼ inch) as the limit of probable error in locating the various points. It is easy to see that, as this error is smaller than the deviations observed, it is of no practical importance in determining the erect position in life size.

(*d*) **Distortion of attitude after fixation.** — This may be prevented by observing the dial at various stages of the observation and seeing that no marked variation in its reading occurs.

(*e*) **Location of bony landmarks.** — The location of bony landmarks is obviously only approximate when made in the living by marks on the skin. For example, the position of the middle of the trochanter in any given case can be regarded only as a general indication of its position, nor would it be of great value with regard to locating the hip joints could it be accurately placed, for we have no exact knowledge of their relation. As the value of the method, in our opinion, lies in the comparison of tracings in the same individual under varying conditions, it is obvious that the same skin mark is reliable for comparison in this way in two or more consecutive observations, even if anatomically incorrect.

(*f*) **Errors from varying position of the feet.** — These are eliminated by having the inner borders of the feet in all observations placed on lines marked on the balanced board.

(*g*) **Rotation of pelvis.** — Some subjects tend to an unconscious rotation of the pelvis on a vertical axis which may occasionally necessitate the marking and observation of both trochanters.

(*h*) **Psychical influences.** — Care must be taken that the model is ignorant of the purpose of the observation, lest he should attempt to help or hinder it.

Aside from the purely scientific aspect of the work in its bearing on physiology and animal mechanics, it is our belief that it will prove of practical use, especially in the fields of orthopedic surgery and gynecology.

We acknowledge most gratefully our obligation to Professor Ira N. Hollis, of Harvard University, for his advice at various stages of our investigation.

A NOTE ON THE ABSORPTION OF FAT.

By R. H. WHITEHEAD.

[From the Anatomical Laboratory, University of Virginia.]

IT was generally believed for a long time that the fats in foods were absorbed in the form of a fine emulsion. As the result, however, of increasing knowledge concerning the bile and the pancreatic and intestinal secretions a rival theory soon appeared. It was shown repeatedly that much of the fat was split into the corresponding fatty acids and glycerol by the lipase of the pancreatic juice, and that these acids united with the alkalis of the bile and intestinal juice to form soluble soaps. Accordingly it was taught by many that ingested fat was absorbed both in the form of an emulsion and in the water-soluble form of soap, neither view excluding the other. The latter view, however, has prevailed in recent years, having been strengthened by various investigations, particularly those of Kastle and Loevenhart,[1] who demonstrated the almost universal presence of lipase in the tissues, and showed that this ferment was able by reversibility of action to synthesize fats as well as to split them; thus furnishing an explanation of the phenomena which had led many to believe that the fats after having been decomposed in the intestinal canal are built up again in the villi. Still, although practically all physiologists accept the view that fat is absorbed in the form of soluble soaps, many are reluctant to deny that it may also be absorbed, in part, as an emulsion; because there seem to be no *a priori* reasons why fine emulsions may not be absorbed, and because the intestinal contents are such as to favor the formation of emulsions. The histological evidence in favor of the absorption of emulsions has been obtained, for the most part, by staining sections of the small intestine with osmic acid a few hours after a meal of fat. In such cases globules of fat

[1] KASTLE and LOEVENHART: American chemical journal, 1900, xxiv; LOEVENHART: This journal, 1902, vi.

colored brown-black by the osmic acid are found in large numbers in the epithelium and lacteals of the villi. It is clear, however, in the light of the work of Kastle and Loevenhart that the finding of fat in these situations and under these circumstances does not prove that the fat was absorbed as such — it may have been absorbed in the soluble forms of its constituents and then synthesized in the villi.

It occurred to me to test the question by studying sections of the intestine after feeding fat which had been stained *before* feeding. For this purpose I chose Sudan III, the dye so much used in recent years for the staining of fat in histological examinations; it has been shown by Dr. S. H. Gage[2] that this dye can be fed to hens with impunity. Sudan III was dissolved in melted butter in sufficient quantity to give the butter a dark red color. After cooling the butter was fed to a half-grown cat which had been without food for fourteen hours. The animal was killed at the expiration of four and a half hours, and pieces of the small intestine were fixed in 10 per cent solution of commercial formaldehyde. It was noticed that, while the mucous membrane was stained quite red, the lacteals in the mesentery were white. Sections of the intestine were made with a freezing microtome and examined on the slide in glycerine, some of the sections having been immersed for a few seconds in 50 per cent alcohol according to the usual technique. It was found that, while the sections which had not been immersed in alcohol had a diffuse rosy tinge, in both sorts of sections there was entire absence of red globules in the villi, although masses of red butter still unsplit were evident in the lumen of the intestine. In the case of the sections which were dipped in the alcohol even the rosy hue was wanting.

These findings indicated that either (1) the fat had not been absorbed at all or (2) it had not been absorbed as such. To test this point the same sections which had just been examined were removed from the slides, stained by the usual technique in a saturated solution of Sudan III in 80 per cent alcohol, and re-examined. Fat was now demonstrated in great abundance both in the lining epithelium and in the lacteals of the villi, in the form of minute red globules. Undoubtedly, then, the fat was absorbed, and it is

[2] Gage: Proceedings of the Association of American Anatomists, Baltimore, 1908.

also evident that it had not been absorbed as such, but had been taken up by the villi in water-soluble forms of its constituents. The presumption is that the fatty acids entered the villi in soaps. For oleic acid dissolves Sudan III almost as readily as does fat itself, and if the red color of the mucous membrane had been due to the presence of this acid stained by the dye, it would not have been decolorized so readily by the slight exposure to the action of weak alcohol. On the other hand, a solution of a soap made with oleic acid and sodium carbonate dissolved Sudan III very feebly.

I would call attention, in conclusion, to the fact that the experiment is one that can easily be employed for class-work in the laboratory. No attempt has been made in this brief note to review the voluminous literature of the subject, but excellent reviews have been given by Oppel.[3]

[3] Oppel: Lehrbuch der vergleichenden mikroskopischen Anatomie, 1897, ii; and in Ergebnisse der Anatomie und der Entwicklung, 1902, xii.

THE EFFECTS OF BONE ASH IN THE DIET ON THE GASTRO-INTESTINAL CONDITIONS OF DOGS.

By ALFRED PEIRCE LOTHROP.

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

CONTENTS.

	Page
I. Introduction	297
II. Metabolism Experiments	301
1. General description of the metabolism experiments	301
2. First metabolism experiment	303
3. Second metabolism experiment	305
4. Third metabolism experiment	307
5. Fourth metabolism experiment	309
6. General comparison of the results of the four metabolism experiments	312
III. Artificial Digestion Experiments	317
1. Salivary	317
2. Peptic	318
3. Tryptic	321
4. Amylolytic	321
5. Lipolytic	322
6. General comparison of the results of the artificial digestion experiments	322
IV. Summary of General Conclusions	323

I. Introduction.

METABOLISM experiments on dogs, as usually conducted, are beset by many difficulties when the objects of the investigations require that the urine and feces be kept separate for analysis and that the feces be collected as quantitatively as possible. When an animal is fed a diet consisting of hashed meat, cracker meal, lard, and water in the proportions ordinarily given in this laboratory,[1] nearly all of the food is digested and assimilated so that the fecal discharges are comparatively infrequent (usually at intervals of three or four days), but the character of the discharges under such conditions is usually decidedly diarrheal. When such a condition exists, there is very apt to be a mingling of the urine and

[1] Fifteen gm. of meat, 4 gm. of cracker meal, 3 gm. of lard, and 35 c.c. of water per kilo of body weight.

feces if the animal is confined in a cage and the urine collected automatically in a receiver at the bottom. Such a mingling makes accurate analysis of the excreta impossible and may ruin the experiment.

Many observations have been made in this laboratory on the thorough assimilation of the food in dogs on the diet already referred to, and also on the fluidity, offensive odor, and troublesome nature of the fecal discharges from such dogs.[2] From every other standpoint, however, such a diet is satisfactory.

As the number of workers in metabolism experiments increased in this laboratory, the difficulties incident upon collecting and preparing the feces for analysis became more and more prominent, and some method of relief was sought. When dogs receive food containing bone, the feces eliminated by them are solid, gray in color, and practically free from odor. They dry quickly in the air and can be easily ground to a powder closely resembling calcium phosphate. The organic constituents of the bone are, of course, digested, and the insoluble bone salts, mostly calcium compounds, which remain unabsorbed, are eliminated almost wholly in the feces. Numerous investigators had previously employed indigestible products mixed with the diet to give solidity to the feces, but it did not seem desirable in carefully conducted experiments to continually administer material which is never contained in the natural diet of the animal and which may have undesirable mechanical effects during its passage through the digestive tract. This criticism cannot, however, apply to the use of bone, for it is a material which dogs naturally ingest, and which their digestive processes apparently dispose of without any deleterious effects. The administration of bone as such, however, presents many difficulties. It would be impossible to feed it in daily masses of uniform composition unless pulverized. In that condition it would be apt to contain much organic matter whose removal by digestion might not be uniform, but whose retention might cause irregular contributions to the feces and thus disturb the daily metabolic events.

The only possibility seemed to be the thoroughly roasted bone ash containing only the inorganic constituents of the bone with a slight increase in carbonate. Accordingly, a series of trials was made with bone ash, and its great advantages were clearly demon-

[2] MEAD and GIES: This journal, 1901, v, p. 104; also GIES and collaborators: Biochemical researches, 1903, i (Reprint No. 21).

strated. The ash seemed to have absolutely no deleterious effects; the animals made no objections to ingesting even large quantities mixed with their food; and the fecal matter was practically inodorous, white, and dry, and could be quantitatively collected for analysis. About five years ago the first mention of its systematic use was casually made in a report of an investigation,[3] and shortly afterward a more detailed account of the general advantages of its use in the diet of dogs, especially in metabolism experiments, was presented.[4]

Since that time bone ash has been added regularly to the food of practically all the animals used in experiments in this laboratory and has given nothing but satisfaction. It has even been given advantageously in milk to kittens to prevent diarrhea. Ordinarily 1 gm. per kilo of body weight has been added to the food mixture, but five times as much has often been given without causing any unfavorable effects. Besides preventing diarrhea, moderate amounts of bone ash in the diet considerably increase the bulk of the excrementitious matter and make its discharge more frequent and regular, defecation usually occurring daily. Thus intestinal putrefaction is minimized by regular evacuation of the intestinal tract. Occasionally dogs eat their own feces, especially if particularly odoriferous. Such an event in a metabolism experiment causes very disturbing conditions. The bone ash feces are so brittle and inodorous that they incite no inclination in the dog to ingest the material. Only two out of several hundred dogs have shown any tendency thus to dispose of "bone-ash feces."

Steel and Gies[5] have investigated the influence of bone ash on the elimination of calcium and phosphate in the urine. Their results seem to show that in spite of the large amounts of calcium and phosphate ingested, in the form of the bone ash calcium phosphate, there is no change in the urinary excretion of calcium and no increase in the elimination of phosphorus (presumably also of phosphate). As much as 50 gm. of the bone ash were given with one day's food to a dog weighing only 6 kilos, and, although this

[3] TALTAVALL and GIES: This journal, 1903, ix, p. xvi; also GIES and collaborators: Biochemical researches, 1903, i, p. 59.

[4] GIES: Proceedings of the American Physiological Society, This journal, 1904, x, p. xxii.

[5] STEEL and GIES: This journal, 1907, xx, p. 350.

is about eight times the amount usually given, the animal handled it readily and very little, if any, was absorbed.

It was also found that even freshly voided, strongly acid urine will not appreciably dissolve any of the bone ash from feces, fragments of which are sometimes flushed into the receiver by the urine if the fecal matter is not immediately removed from the cage. Bone ash (2 gm.) in contact with strongly acid urine (150 c.c.) for four hours yielded to the urine only 0.0003 gm. of CaO, an amount which is within the unavoidable experimental error of method.[6]

In the course of an investigation on the influence of magnesium sulphate on metabolism, Steel[7] found that bone ash will not appreciably offset the cathartic effect of magnesium sulphate, except in a purely mechanical way, by adding bulk to the excrementitious matter. Recovery from the cathartic effects of large doses of magnesium sulphate was almost immediate when the administration of the magnesium sulphate was discontinued and that of the bone ash continued.

In spite of the large amounts of phosphate administered in the bone ash added to the diet, Hawk and Gies[8] in hemorrhage experiments on dogs, and Berg and Welker[9] in radium researches on dogs, have found it possible to maintain true phosphorus balances as satisfactorily as those of nitrogen and sulphur. All that was required was special alertness and care in the collection of the excreta.

In view of the systematic use of bone ash in all the metabolism experiments in this laboratory, and the probability that its employment will become general if it is devoid of deleterious influences, it is desirable to inquire more fully into the effect of bone ash on gastro-intestinal conditions, and to investigate the chemical changes, if any, that it undergoes in passing through the digestive tract. What are the effects of bone ash on intestinal putrefaction? Is digestion modified unfavorably? Is absorption hindered? Are any of the bone ash constituents eliminated in the urine? Is the secretion of intestinal mucus increased? Is general metabolism affected? To answer these and other questions this investigation was undertaken.

[6] STEEL and GIES: *Loc. cit.*, p. 356.

[7] STEEL: Journal of biological chemistry, 1908, v, p. 98.

[8] HAWK and GIES: This journal, 1904, xi, p. 171.

[9] BERG and WELKER: Journal of biological chemistry, 1906, ii, p. 371.

II. Metabolism Experiments.

1. **General description of the metabolism experiments.** *Animals and environment.* — The metabolism experiments were performed on 4 healthy dogs, weighing from 7 to 12½ kilos. They were confined, while under observation, in cages devised in this laboratory and recently described.[10] The metabolism experiments were conducted along the lines usually employed in this laboratory.[11]

Food. — The daily food consisted of a mixture of hashed lean beef, cracker meal, lard, and water. The raw beef was taken from large supplies preserved in a frozen condition.[12] The cracker meal was obtained from large quantities of the commercial product kept in large glass bottles with ground glass stoppers. The lard used was Armour's best pure leaf lard, purchased in three-pound pails and kept in a refrigerator. Ordinary tap water was used. In the first two experiments the dogs received their daily rations in one portion at 12.45 P. M. and in the third and fourth experiments at 2.30 P. M.

Periods, weights. — In general each experiment was divided into four periods: a preparatory period during which the animal became accustomed to its surroundings and received its regular diet, but during which no analyses were made; a fore period and after period under normal conditions, and a middle dosage period during which bone ash was mixed with the food. These periods were of different lengths. In the first two experiments each day ended at 12.45 P. M. and in the second two at 2.30 P. M. The dogs were weighed just before being fed.

Collection of excreta. — The urine was collected daily just before feeding, and the feces were removed whenever noticed in the cage. As a rule, the cages were sprayed and well washed daily, and were cleaned especially thoroughly at the end of each period. The development of bacteria in the cage was thus kept as low as possible. The daily washings were used to dilute the urines to the uniform volume of 1 litre. The urine was preserved with powdered thymol. When necessary, the feces were desiccated over a water bath.

Analyses. — The total nitrogen of the meat and cracker meal was determined. In the *period* urines total ash, nitrogen, phosphorus, sulphur, and calcium were determined, and in the *daily* portions the ethereal sulphates. The

[10] Gies: This journal, 1905, xv, p. 403.

[11] Mead and Gies: This journal, 1901, v, p. 106; Gies and collaborators: Biochemical researches, 1903, i, p. 419 (Reprint No. 21).

[12] Gies: This journal, 1901, v. p. 235; also Gies: Proceedings of the Society for Experimental Biology and Medicine, 1908, v, p. 27.

period feces, after thorough grinding, sifting, and mixing, were analyzed for total nitrogen, ash, and fat (ether extraction). In two experiments the quantities of fecal lecithin were also determined.

Analytical methods. — The following methods of analysis were employed: *Nitrogen:* Kjeldahl process, oxidation being effected by concentrated sulphuric acid aided by a little copper sulphate. *Phosphorus:* Liebig alkali fusion method and final weighing as magnesium pyrophosphate. *Sulphur:* Liebig alkali fusion method; also the fuming nitric acid method of Gill and Grindley.[13] *Ethereal sulphates:* Folin method.[14] *Fat:* Complete ether extraction in the Soxhlet apparatus. *Lecithin:* Calculated from phosphorus content of ether extract (dry feces). *Calcium:*[15] To 150 c.c. of urine acetic acid was added. The solution was heated on a water bath for two hours. Any flocculent precipitate produced by this treatment was filtered off and washed with hot water. To the filtrate and washings 10 c.c. of ammonia were added and the mixture was then made slightly acid with acetic. To the acid liquid 15 c.c. of a saturated solution of ammonium oxalate were added and the mixture set aside in a warm place for at least four hours. The precipitate was then filtered off and washed free from oxalate with hot water. The calcium oxalate was dissolved from the paper with hot hydrochloric acid (sp. gr. 1.04), the paper was washed thoroughly with hot water, the solution and washings made up to 150 c.c., heated to 70° C. and titrated with $\frac{n}{20}$ potassium permanganate. The preliminary treatment with acetic acid seems to be necessary only after the urine has stood for a considerable length of time.

Bone ash. — The bone ash employed was a thoroughly incinerated carbon free commercial product. A large quantity was put through a fine sieve and preserved for the experiments in a glass stoppered bottle. In each of the metabolism experiments the amount of bone ash ordinarily given to animals under observation here, namely, 1 gm. per kilo of body weight, was employed. Analysis of the stock supply of bone ash gave the following data for calcium, magnesium, phosphate, sulphate, and residue insoluble in dilute HCl (1:5).

Ca	39.020 per cent	P_2O_5	40.650 per cent
Mg	0.486 per cent	SO_4	0.500 per cent
Insoluble residue	0.089 per cent		

[13] Gill and Grindley: Journal of the American Chemical Society, 1909, xxxi, p. 52.

[14] Folin: Journal of biological chemistry, 1905, i, p. 154, and This journal, 1905, xiii, p. 52.

[15] Steel and Gies: This journal, 1907, xx, p. 351.

2. **First metabolism experiment.** — A black and tan male dog weighing 9.56 kilos, was given daily 150 gm. of meat, 40 gm. of cracker meal, 30 gm. of lard, and 350 c.c. of water. The preparatory period was six days in length. Elimination of black, medium hard, foul-smelling stools occurred on the second and sixth days. On the eighth to fourteenth days inclusive, 10 gm.

TABLE I.

FIRST EXPERIMENT. DAILY RECORDS.

I. Fore period. Normal conditions.								
Number of the day . . .	1	2	3	4	5	6	7	Av.
Body weight (kilos) . . .	9.56	9.61	9.46	9.46	9.45	9.38	9.38	....
Urine: volume (c.c.) . . .	375	295	535	375	410	410	380	397
Sulphur of ethereal sulphates (gm.)	0.1608	0.0705	0.1039	0.1019	0.1030	0.0878	0.0822	0.1014
Feces: dry weight (gm.) .	0	22.5	0	0	0	19	0	5.93
II. Dosage period. 10 gm. of bone ash per diem.								
Number of the day . . .	8	9	10	11	12	13	14	Av.
Body weight (kilos) . . .	9.44	9.33	9.31	9.30	9.32	9.25	9.23	....
Urine: volume (c.c.) . . .	335	370	360	350	330	380	380	358
Sulphur of ethereal sulphates (gm.)	0.0809	0.0712	0.0688	0.0648	0.0580	0.0533	0.0681	0.0664
Feces: dry weight (gm.) .	0	41	17.5	11	23	23	21	19.5
III. After period. Normal conditions.								
Number of the day . . .	15	16	17	18	19	20	21	Av.
Body weight (kilos) . . .	9.24	9.25	9.21	9.25	9.28	9.27	9.28	....
Urine: volume (c.c.) . . .	360	375	330	325	345	420	425	368
Sulphur of ethereal sulphates (gm.)	0.0476	0.0549	0.0582	0.0570	0.0506	0.0612	0.0501	0.0542
Feces: dry weight (gm.) .	0	0	12	0	0	0	16.5	4.07

of bone ash were mixed with the daily food. On the ninth day there was a large amount of fecal matter, white and hard and free from odor. Defecation occurred daily throughout the rest of the dosage period. During the *after* period there was elimination, on the seventeenth and twenty-first days, of soft feces like those of the fore period. The dog showed absolutely no visible effects of the bone ash. The weight during the experiment fell to 9.28 kilos, doubtless because the prescribed diet was insufficient in amount. The reaction of the daily urine was uniformly acid to phenolthalin, amphoteric to litmus, and alkaline to rosolic acid. The specific gravity of the urine fluctuated between 1013 (twentieth day) and 1018 (seventeenth and eighteenth days).

The analytical data of this experiment are summarized in Tables I, II, and III.

TABLE II.

First Experiment. Urine. Analytical Totals and Daily Averages for each Period.

Period (7 d'ys).	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	Total.	D'ly av.	T'al.	D'y av.	Total.	Daily av.	T'al.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm	gm.	gm.	gm.	gm.	gm.
Fore .	2862	397	13.216	1.888	45.40	6.48	2.3835	0.3405	5.953	0.8505	0.1834	0.0262
Dosage	2505	358	11.564	1.652	43.09	6.16	2.2911	0.3273	3.824	0.5463	0.1099	0.0157
After .	2580	368	12.320	1.760	45.40	6.48	2.6418	0.3774	5.355	0.7651	0.1540	0.0220

TABLE III.

First Experiment. Feces. Analytical Totals and Daily Averages for each Period.

Period (7 days).	Weight.		Nitrogen.		Ash.		Fat.		Lecithin.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . . .	41.5	5.93	2.482	0.354	4.316	0.616	6.80	0.971	0.112	0.016
Dosage . .	136.5	19.50	4.518	0.647	74.530	10.650	7.07	1.010	0.105	0.015
After . . .	28.5	4.07	1.810	0.258	4.090	0.584	3.01	0.430	0.049	0.007

3. **Second metabolism experiment.** — A female Scotch terrier was used in the second experiment and received a daily diet of 135 gm. of meat, 36 gm. of cracker meal, 27 gm. of lard, and 315 c.c. of

TABLE IV.

SECOND EXPERIMENT. DAILY RECORDS.

I. Fore period. Normal conditions.							
Number of the day	1	2	3	4	5	6	Av.
Body weight (kilos)	8.95	8.97	8.97	8.98	8.96	8.93	....
Urine: volume (c.c.)	465	350	365	365	360	420	387
Sulphur of ethereal sulphates (gm.)	0.0415	0.0340	0.0334	0.0292	0.0401	0.0194	0.0329
Feces: dry weight (gm.)	0	0	9.0	0	3.5	4.5	2.83
II. Dosage period. 9 gm. of bone ash per diem.							
Number of the day	7	8	9	10	11	12	Av.
Body weight (kilos)	8.94	8.96	8.92	8.93	8.89	8.90	....
Urine: volume (c.c.)	335	335	375	357	350	355	351
Sulphur of ethereal sulphates (gm.)	0.0165	0.0165	0.0251	0.0134	0.0194	0.0141	0.0178
Feces: dry weight (gm.)	8	11	17.5	8.5	26	8.5	13.25
III. After period. Normal conditions.							
Number of the day	13	14	15	16	17	18	Av.
Body weight (kilos)	8.84	8.84	8.82	8.89	8.92	8.93	....
Urine: volume (c.c.)	320	398	305	260	355	325	327
Sulphur of ethereal sulphates (gm.)	0.0115	0.0232	0.0162	0.0063	0.0181	*	0.0151
Feces: dry weight (gm.)	18	0	10	3	0	11	7

* The urine was lost by accident after it had been made up to 1000 c.c., so that the after period in the quantitative records was shortened to five days.

water. Five days sufficed to accustom the animal to the cage. The weight remained fairly constant throughout the experiment, being 8.95 kilos at the start and 8.93 kilos at the finish. In the *fore* period of six days defecation of black, foul-smelling stools occurred on the third, fifth, and sixth days, and the effect of the

TABLE V.

SECOND EXPERIMENT. URINE. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	T'al.	D'ly av.	T'al.	D'l av.	Total.	Daily av.	T'al.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . .	2325	387	10.32	1.720	36.42	6.07	1.7958	0.2993	4.49	0.7485	0.0918	0.0153
Dosage .	2107	351	10.68	1.780	36.06	6.01	1.9704	0.3284	4.04	0.6732	0.0792	0.0132
After . .	1963	327	7.84	1.568	30.20	6.04	1.5350	0.3070	3.68	0.7370	0.0785	0.0157

TABLE VI.

SECOND EXPERIMENT. FECES. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period (6 days).	Weight.		Nitrogen.		Ash.		Fat.		Lecithin.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . . .	17.0	2.83	1.183	0.197	2.295	0.38	2.96	0.493	0.036	0.006
Dosage . .	79.5	13.25	2.258	0.376	48.020	8.00	2.86	0.477	0.024	0.004
After . . .	42.0	7.00	2.065	0.344	12.410	2.07	4.12	0.687	0.018	0.003

bone ash appeared immediately in the feces on the day after the first dose. During the *dosage* period there was daily defecation. The discontinuance of the bone ash seemed to have a disturbing effect, for on the first day of the *after* period there were two diarrheal movements, and on the third and fourth days the feces were still soft, and contained much mucous matter. The reaction of the daily urine was always acid to phenolthalin and alkaline to rosolic acid. It was acid to litmus on all but the last five days

of the after period, when it was amphoteric to that indicator. The specific gravity of the urine fluctuated between 1015 and 1018. During the experiment the animal was pregnant, and two sturdy pups were born a month after the close of the experiment. The bone ash had no discernible effects on the mother or the pups.

The daily records and analytical data of the second metabolism experiment are summarized in Tables IV–VI.

4. **Third metabolism experiment.** — The animal, a small shaggy black and tan male dog, weighing 6.90 kilos, was given a daily diet consisting of 105 gm. of meat, 28 gm. of cracker meal, 21 gm. of lard, and 245 c.c. of water. The dog was under preliminary observation in a cage for twelve days, during which there was apparently no normal defecation. It was suspected, however, that the dog had been defecating but that he was disposing of the fecal matter, for on the fifth day a very small amount of feces (½ gm.) was found on the pan. Accordingly, the animal was carefully watched. Meanwhile the daily records and excreta were kept. On the twelfth day a small amount of hard, black, and doughy material was passed. The dosage period was thereupon begun, and the urine samples of that and the five preceding days were taken to represent the fore period. Seven grams of bone ash were mixed with the food of the seventh day, and the characteristic bone ash feces appeared within twenty-four hours. The animal was kept under continuous observation during the daytime, and on the tenth day, about an hour after feeding, defecation occurred and it was noticed that the animal was licking his chops as if he had disposed of a portion of the feces. Although bone ash had been given on three successive days, the fecal matter was dark-colored and foul, and a small amount was purposely left on the wire to test the dog. It was soon ingested. On the following day fecal crumbs on the pan showed evidence of defecation, but there was no material on the wire. On the three succeeding days the excrements were dry and very hard, and were avoided by the animal. The dosage period was continued for eight days, and an after period of seven days followed, defecation occurring on the first, third, and last day of the period.

In spite of the fact that there was evident ingestion of some of the feces, the animal did not seem to exhibit any symptoms whatever. The output of ethereal sulphates showed an increasing amount of intestinal putrefaction up to the time when the fecal

matter became hard and dry and was avoided by the dog. However, the putrefactive products seemed to produce no toxic effect,

TABLE VII.

THIRD EXPERIMENT. DAILY RECORDS.

I. Fore period. Normal conditions.							
Number of the day	1	2	3	4	5	6	Av.
Body weight (kilos)	6.74	6.75	6.81	6.74	6.74	6.81	...
Urine: volume (c.c.)	260	230	170	310	215	180	227
Sulphur of ethereal sulphates (gm.)	0.0253	0.0253	0.0217	0.030	0.0263	0.0339	0.0271
Feces: dry weight (gm.)	0	0	0	0	0	9.0	1.5

II. Dosage period. 7 gm. of bone ash per diem.								
Number of the day	7	8	9	10	11	12	13	14	Av.
Body weight (kilos)	6.87	6.92	6.96	6.97	6.95	6.97	6.92	6.90	...
Urine: volume (c.c.)	150	190	170	215	200	190	225	240	201
Sulphur of ethereal sulphates (gm.)	0.0274	0.0369	0.0306	0.0438	0.0552	0.0416	0.046	0.0425	0.0405
Feces: dry wt. (gm.)	0	7	0	14.5	0	17.5	15.0	11.5	8.2

III. After period. Normal conditions.								
Number of the day	15	16	17	18	19	20	21	Av.
Body weight (kilos)	6.81	6.84	6.85	6.88	6.88	6.91	6.91	...
Urine: volume (c.c.)	235	215	220	200	240	215	235	223
Sulphur of ethereal sulphates (gm.)	0.0386	0.0288	0.0247	0.0328	0.0412	0.0325	0.0318	0.0329
Feces: dry weight (gm.)	14.0	0	3.5	0	0	0	3.5	3

for the animal was very lively and apparently normal throughout the experiment and never became at all lethargic.

The reaction of the daily urine was uniformly acid to phenolthalin, amphoteric to litmus, and alkaline to rosolic acid. The specific

gravity of the urine fluctuated between 1026 (seventh day) and 1015 (twenty-first day); it was usually above 1016 and below 1022.

The daily records and analytical data of the third metabolism experiment are summarized in Tables VII–IX.

TABLE VIII.

THIRD EXPERIMENT. URINE. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	T'al.	D'ly av.	T'al.	D'y av.	Total.	Daily av.	T'al.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore .	1365	227	7.584	1.264	26.72	4.45	1.5222	0.2537	3.190	0.5317	0.1152	0.0192
Dosage.	1610	201	9.888	1.236	33.18	4.14	2.4500	0.3064	4.495	0.5619	0.2512	0.0314
After .	1560	223	8.568	1.224	29.42	4.20	1.6569	0.2367	3.713	0.5305	0.1596	0.0228

TABLE IX.

THIRD EXPERIMENT. FECES. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Weight.		Nitrogen.		Ash.		Fat.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore	9.0	1.50	0.473	0.0788	9.85	0.164	1.70	0.283
Dosage	65.5	8.20	1.520	0.1900	37.27	4.660	3.12	0.390
After	21.0	3.00	0.619	0.0884	11.13	1.576	0.93	0.133

5. **Fourth metabolism experiment.** — The animal chosen for this experiment was a young dog of a white mongrel type. The daily diet was as follows: 180 gm. of meat, 48 gm. of cracker meal, 36 gm. of lard, and 420 c.c. of water. When the dog was first confined in the cage, his weight was 11.72 kilos, and during the first week it increased about 50 gm. per day, so that at the end of seven days it had risen to 12.12 kilos. During the subsequent week he continued to gain in weight, and it was evident that he was a growing dog. It was finally decided to start the experiment on this

ascending scale of weight. The dog continued to increase from 12.37 kilos at the beginning of the experiment to 12.91 kilos at the end.

During a *fore* period of four days defecation occurred daily, and the feces were black, diarrheal, and contained much mucous matter. On the fifth day 12 gm. of bone ash were mixed with the food. The feces on the two following days were still soft but yellow and

TABLE X.

FOURTH EXPERIMENT. DAILY RECORDS.

I. Fore period. Normal conditions.					
Number of the day	1	2	3	4	Average.
Body weight (kilos)	12.37	12.34	12.47	12.47	...
Urine: volume (c.c.)	300	320	250	280	287
Sulphur of ethereal sulphates (gm.)	0.0329	0.0277	0.0193	0.0346	0.0286
Feces: dry weight (gm.)	7	8.5	2	12	7.4

II. Dosage period. 12 gm. of bone ash per diem.							
Number of the day	5	6	7	8	9	10	Av.
Body weight (kilos)	12.50	12.52	12.57	12.62	12.66	12.71	...
Urine: volume (c.c.)	275	295	320	270	260	275	283
Sulphur of ethereal sulphates (gm.)	0.0255	0.0191	0.0339	0.0379	0.0307	0.0272	0.0280
Feces: dry weight (gm.)	18	19	9	17	16.5	20	16.6

III. After period. Normal conditions.						
Number of the day	11	12	13	14	15	Av.
Body weight (kilos)	12.77	12.82	12.84	12.87	12.91	...
Urine: volume (c.c.)	300	320	330	360	300	322
Sulphur of ethereal sulphates (gm.)	0.0263	0.0255	0.0211	0.0289	0.0293	0.0262
Feces: dry weight (gm.)	13.5	0	10	0	4.5	5.6

free from mucus. On the seventh day the feces were formed, and on the ninth day they were hard and dry. Bone ash was administered for six days. During the *after* period of five days defecation occurred on alternate days, and on the thirteenth day the feces were

TABLE XI.

FOURTH EXPERIMENT. URINE. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	T'l.	D'y av.	T'al.	D'y av.	Total.	Daily av.	T'l.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . . .	1150	287	5.68	1.42	22.94	5.73	1.2652	0.3163	3.09	0.7727	0.0680	0.0170
Dosage . .	1695	283	8.93	1.49	33.91	5.65	1.9074	0.3179	4.06	0.6771	0.1152	0.0192
After . . .	1610	322	7.16	1.44	30.06	6.01	1.7655	0.3531	3.47	0.6949	0.0720	0.0144

TABLE XII.

FOURTH EXPERIMENT. FECES. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Weight.		Nitrogen.		Ash.		Fat.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore	29.5	7.4	2.407	0.602	2.64	0.66	3.62	0.905
Dosage	99.5	16.6	3.283	0.547	58.01	9.66	5.15	0.858
After	28.0	5.6	1.540	0.308	8.87	1.77	3.05	0.610

again black and free from bone ash, but did not show the diarrheal characteristics of the feces of the *fore* period.

The great utility of bone ash in preventing diarrhea was strikingly shown in this animal. The discharges at first were very watery, and twice the experiment had to be begun anew on account of the mingling of urine and feces. The first dose of bone ash had a marked effect, and on the third day the diarrhea was completely stopped.

The reaction of the daily urine was uniformly acid to phenol-

thalin, amphoteric to litmus, and alkaline to rosolic acid. The specific gravity of the urine fluctuated between 1017 (twelfth and fourteenth days) and 1021 (first day).

The daily records and summaries of the fourth metabolism experiment are given in Tables X–XII.

6. **General comparison of the results of the four metabolism experiments.** — A study of the summaries in Tables XIII and XIV, together with the data in Tables I and XII inclusive, warrants the following deductions:

Nitrogen balance. — In the *first* experiment the slight negative balance is probably accounted for by the fact that the prescribed diet, as mentioned on page 304, was not sufficient for that particular animal. There was, therefore, a utilization of a small amount of body protein with a decrease in weight during the experiment. The weight was very constant during the after period of the first experiment, when nitrogenous equilibrium was practically established. In the *fourth* experiment there was a steady increase in weight throughout the work, and the excessive plus balances show that a relatively large amount of nitrogen was utilized in building up body tissue. The *third* animal gained somewhat in body weight; slightly more in the dosage than in the other periods. The positive balances in the different periods accord with that observation. With the *second* dog the weight was practically constant throughout the experiment (page 306), although there was a continuous negative balance.

It is obvious that the administration of bone ash had no particular effect in these experiments on the total nitrogenous metabolism.

Urine volume. — In all the experiments there was a *decrease* in urine volume during the periods of the administration of bone ash, probably because of greater elimination of water in the increased bulk of fecal matter. In all but one case the average daily volume in the after period was greater than that in the corresponding dosage period. In only one instance was the average volume in the after period greater than that in the corresponding fore period. In the second animal the discontinuance of the dosage with bone ash was coincident with a tendency to diarrheal movements and a consequent diminution in the daily average urine volume for the after period. The large increase exhibited by the fourth animal in the after period was probably due to the fact that he was a growing dog, and, as he continued to gain in weight, less water was utilized

TABLE XIII.

Summary of Comparative Daily Averages of the Analytical Data, per Period, of the Four Metabolism Experiments.

Urine (volume in c.c.).						Feces (weight in gm.).				
Period.	1	2	3	4	Gen. av.	1	2	3	4	Gen. av.
Fore . . .	397	387	227	287	324	5.93	2.83	1.50	7.38	4.41
Dosage . .	358	351	201	283	298	19.50	13.25	8.20	16.58	14.38
After . . .	368	327	223	322	310	4.07	7.00	3.00	5.60	4.92
Nitrogen.						Nitrogen.				
Fore . . .	6.48	6.07	4.45	5.74	5.69	0.354	0.197	0.079	0.602	0.308
Dosage . .	6.16	6.01	4.14	5.65	5.49	0.647	0.376	0.190	0.547	0.440
After . . .	6.48	6.04	4.20	6.01	5.68	0.258	0.344	0.088	0.308	0.249
Ash.						Ash.				
Fore . . .	1.88	1.72	1.26	1.42	1.57	0.616	0.382	0.164	0.660	0.455
Dosage . .	1.65	1.78	1.23	1.49	1.53	10.650	8.000	4.660	9.660	8.240
After . . .	1.76	1.57	1.22	1.44	1.49	0.584	2.070	1.580	1.770	1.500
Sulphur.						Fat (ether extract).				
Fore . . .	0.340	0.299	0.254	0.316	0.302	0.971	0.493	0.283	0.905	0.663
Dosage . .	0.327	0.328	0.306	0.318	0.319	1.010	0.477	0.390	0.858	0.684
After . . .	0.377	0.307	0.237	0.353	0.318	0.430	0.687	0.133	0.610	0.465
CaO.						Lecithin (ether extract).				
Fore . . .	0.026	0.015	0.019	0.017	0.019	0.016	0.006	...	...	...
Dosage . .	0.016	0.013	0.031	0.019	0.020	0.015	0.004	...	...	...
After . . .	0.022	0.016	0.023	0.014	0.019	0.007	0.003	...	..	...
P_2O_5.						Urine. Sulphur of ethereal sulphates.				
Fore . . .	0.850	0.743	0.532	0.773	0.726	0.101	0.033	0.027	0.029	0.047
Dosage	0.546	0.673	0.562	0.677	0.614	0.066	0.018	0.040	0.028	0.038
After . . .	0.765	0.738	0.530	0.695	0.682	0.054	0.015	0.033	0.026	0.032

TABLE XIV.

Nitrogen Balance in each Period of the Four Metabolism Experiments.

Experiment.	1			2			3			4		
Period.	Fore. (7 days.)	Dosage. (7 days.)	After. (7 days.)	Fore. (6 days.)	Dosage. (6 days.)	After. (5 days.)	Fore. (6 days.)	Dosage. (8 days.)	After. (7 days.)	Fore. (4 days).	Dosage. (6 days.)	After. (5 days.)
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Food	46.97	46.97	46.97	36.23	36.23	30.20	29.50	39.36	34.44	33.72	50.58	42.15
Excreta	47.84	47.63	47.17	37.60	38.32	32.26	27.17	34.64	30.00	25.34	37.18	31.60
Balance	−0.87	−0.66	−0.20	−1.37	−2.09	−2.06	+2.33	+4.72	+4.44	+8.38	+13.40	+10.55

than at the start. Although receiving 420 c.c. daily, the largest volume eliminated on any one day was 360 c.c., — on the day previous to the close of the experiment.

Ash. — The amount of ash that was excreted in the urine was practically constant; in the last two experiments it was almost identical for each period. This fact indicates that there was no appreciable absorption of the inorganic constituents of the administered bone ash, although such an absorption might occur without any corresponding increase in the amount of urinary ash. In two cases there was a decrease and in the two others a slight increase of urinary ash during the dosage period. The amount of fecal ash was, of course, very much greater for the periods during which bone ash was given. The large amount of fecal ash for the after period in three of the four experiments showed that considerable bone ash persisted in the intestinal tract and was not completely eliminated for several days. With the first animal practically complete elimination of ingested bone ash occurred within twenty-four hours after the interruption of the treatment with bone ash. The daily average amount of fecal ash in the corresponding *after* period was 0.58 gm., practically the same as that of the *fore* period, — 0.62 gm.

Nitrogen. — In each case there was a *slight* decrease in urinary

nitrogen, and in three cases corresponding increases in fecal nitrogen, during the bone ash periods. Possibly there was *slightly* less assimilation of nitrogenous material during the dosage period, and probably, also, at the same time an entanglement of a *slightly* larger amount of mucous matter by the increased bulk of excrementitious material. The urinary nitrogen in each after period was always greater than that of the corresponding dosage period. The fourth dog, which was not in weight equilibrium, but utilizing nitrogenous matter in building up body tissue, did not exhibit the increase of fecal nitrogen shown by the other animals during the dosage periods. The high content of fecal nitrogen for this dog in the fore period confirmed the observation of the presence of much mucous matter in the diarrheal stools mentioned on page 310, the same cause accounting for the high figure in the feces of the after period of the experiment on the second dog (page 306).

Sulphur. — (*a*) Ethereal sulphates. In the first two experiments there was a constant decrease in the output of ethereal sulphates. A minimum was reached during the latter part of the dosage period, showing that bone ash, probably by increasing the bulk of the feces and so causing daily evacuation, kept down intestinal putrefaction. In the third experiment, where there was evidence of the ingestion of fecal matter, the amounts of ethereal sulphates rose gradually until the middle of the dosage period, when the feces became hard and dry and were avoided by the animal as mentioned on p. 307. Then, as *daily* defecation was inaugurated and no feces were ingested, the quantitative output of ethereal sulphate gradually decreased again. In the fourth experiment the quantitative elimination was practically the same in each period. (*b*) Total sulphur. The amount of total sulphur showed no consistent regularity. In two experiments (II and III) it was highest during the dosage periods, and in the other two (I and IV) in the after periods. Probably the increase in these two periods was largely dependent on the elimination of the sulphur in the bone ash (page 302).

Calcium. — The variations in the output of urinary calcium were hardly more than the normal fluctuations. The quantity in dog's urine is small at best, and shows no noticeable increase during the ingestion of bone ash, notwithstanding the relatively large amount of calcium present in bone ash. In two cases there were slight decreases and in two slight increases of calcium output in the urine

during the dosage periods. In the third experiment there was a perceptible increase not only in urinary calcium excretion, but also in the amount of urinary phosphorus during the dosage period. Probably the ingestion of the fecal material already noted (page 307) brought about conditions which allowed a somewhat greater absorption of calcium phosphate. In that experiment (III) the amount of urinary sulphur was also much higher.

Phosphorus. — Except in the instance already mentioned in the discussion of the calcium content, there was in each experiment a marked decrease in urinary phosphorus during the dosage period with a subsequent increase during the after period, the latter amounts, however, in no case quite reaching the high mark of the fore period. The third experiment shows considerable deviation from the other three. The results prove without doubt that there was no appreciable absorption of the calcium phosphate of the bone ash. The decrease in phosphorus output in the urine may be accounted for, as suggested by Steel and Gies, by the probability of a diminished absorption of alkali phosphate from the food. "Such a result might ensue from interaction, in the intestine particularly, between calcium and phosphate with the production of less soluble or precipitated products. Possibly such interaction would occur especially between alkali phosphate and that portion of the available calcium that had been converted into chlorid from carbonate, and which chlorid would be prone, in the intestine, to combine with phosphate in increasing proportion as the mixture containing them became less acid or perhaps alkaline in reaction. . . . The *total* amount of phosphorus (phosphate) in the excreta would doubtless be unaffected, for the phosphate withheld from absorption would be passed, within a few hours, as calcium phosphate into the feces."[16]

Fat absorption. — The amounts of unabsorbed fat in the feces indicated only a normal variation. The differences were in no case great enough to warrant the statement that the bone ash exercised any influence one way or the other upon the absorption of fats. In each of two experiments there was a slight increase and in the other two slight decreases in the amounts excreted. In three cases there was a more perceptible decrease during the after periods, suggesting, perhaps, a somewhat more complete assimilation, but

[16] Steel and Gies: This journal, 1907, xx, p. 351.

not more than that of half a gram at the most out of 30 gm. given in the diet.

Fecal lecithin. — It seemed desirable to determine the approximate amount of lecithin in the fecal fatty matter in order to obtain, if possible, a suggestion regarding the effect of bone ash on the secretion of bile. Phosphorus in the residue was determined, and the amount of lecithin calculated therefrom with the aid of the figure for the phosphorus content of distearyl lecithin: 3.57 per cent. The results showed that there was no effect of the bone ash on the elimination of lecithin and probably none on the secretion of bile.

III. Artificial Digestion Experiments.

Although the experiments described on the foregoing pages made it seem quite improbable that bone ash affects unfavorably either the digestive or absorptive processes, the following experiments were performed in order to reveal any possible effects on the activity of the gastro-intestinal enzymes.

1. **Salivary digestion.** — Two cubic centimetres of saliva immediately digested the starch in 18 c.c. of 1 per cent paste containing as much as 0.4 gm. of bone ash. Preliminary tests with saliva greatly diluted with water to prevent rapid digestion of starch made it evident that ptyalin is markedly restrained by comparatively large proportions of bone ash. The following data are among the most significant that were obtained in this connection:

A. Conditions: Starch paste, 1 per cent — 18 c.c. Saliva, 1 in 5 — 2 c.c.

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.025	0.05	0.1	0.2	0.4
Achromic point attained in	4 min.	6 min.	10 min.	Red after 1½ hrs.		Purple after 1½ hrs.

B. Conditions: Starch paste, 1 per cent — 13 c.c. Saliva, 1 in 50 — 2 c.c.

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.025	0.05	0.1	0.2	0.3
Achromic point attained in	1 hr., 10 min.	11 hrs.	24 hrs.	Blue, 24 hrs. later		

C. Conditions: Starch paste, 1 per cent — 18 c.c. Saliva, 1 in 20 — 2 c.c.

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.005	0.01	0.02	0.05	0.1
Achromic point attained in	20 min.	20 min.	35 min.	35 min.	36 hrs.	42 hrs.

Slight quantities of soluble calcium salts, such as calcium chloride, seem to have beneficial effects in such experiments, but tribasic calcium phosphate was found to behave like bone ash. Calcium carbonate, while not so effective as tribasic calcium phosphate, retarded markedly the action of the ptyalin in similar experiments under the same conditions.

Analogous experiments with solutions of diastase gave similar results.

D. Conditions: Starch paste, 1 per cent — 18 c.c. Diastase sol., 0.1 per cent — 2 c.c (Digestion started at 5 P. M.)

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.01	0.02	0.5	0.1	0.5
Achromic point attained in	3 hrs.	Complete at 9 A. M.	Red at 9 A. M.	Purple at 9 A. M.	Still blue at 9 A. M.	

E. Conditions: Starch paste, 1 per cent — 15 c.c. Diastase sol., 0.1 per cent — 5 c.c.

No.	1	2	3	4	5
Bone ash (gm.)	0	0.01	0.02	0.05	0.1
Achromic point attained in	1½ hrs.	2 hrs.	2¾ hrs.	4 hrs.	Red 24 hrs. later.

Other insoluble substances, such as glass wool, cotton, sand, infusorial earth, and barium sulphate, were tried under the same conditions, but none of them hindered the action of either ptyalin or diastase. The effect of bone ash seemed to be due to the insoluble calcium salts contained in it.

The water soluble material from 0.5 gm. of bone ash was obtained by shaking repeatedly with 20 c.c. of water. After filtration, 10 c.c. of the extract were mixed with an equal volume of starch paste, and 2 c.c. of diluted saliva (1 : 5) were added. The digestion was not appreciably affected. This seems to show that the interference of the bone ash is mechanical rather than chemical.

2. **Peptic digestion.** — Experiments with pepsin-HCl were carried out, in general, according to the method described by Berg and Gies,[17] a brief outline of which follows:

Weighed amounts of bone ash were transferred to wide-mouthed glass-stoppered bottles having a capacity of about 150 c.c. One hundred cubic centimetres of a solution containing 0.2 per cent of hydrochloric acid and 0.1 per cent or 0.3 per cent of pepsin ("Merck, Ph. G. IV") were then added to each bottle, followed by 1 gm. of fibrin or elastin, which had been dried to constant weight at 110° C. The bottles containing the mixtures

[17] Berg and Gies: Journal of biological chemistry, 1907, ii, p. 497.

were then placed in a water bath maintained at 40° C. throughout the experiment. Controls were also run: protein alone, and bone ash alone, in comparable volumes of the pepsin-acid solution. Each mixture in a series was subjected, of course, to the same general conditions as all the others.

At the conclusion of the digestive period the mixtures were filtered on dry weighed papers which were subsequently desiccated and weighed, giving, by difference, the amount of undigested protein plus any undissolved bone ash. The amount of undissolved ash in the corresponding control subtracted from the total weight of undissolved protein and ash gave the amount of protein residue (undigested matter).

In the tests with fibrin aliquot portions of the filtrates were used for the quantitative determination of the metaprotein.[18] To stop digestion at a definite time after filtration was started, the liquid was made alkaline with dilute potassium hydroxide solution. Finally, while hot, the alkaline filtrates were neutralized and then made very faintly acid to lacmoid with dilute hydrochloric acid, in order to keep in solution the calcium phosphate from the bone ash and also to favor the complete precipitation of the metaprotein. After standing over night the solutions were filtered through weighed papers, the precipitates washed free from saline matters, and the papers dried and weighed. The combined weight of the neutralization precipitate (per 100 c.c.) plus that of the protein residue subtracted from 1 gm. gave the weight of combined fibrin proteoses and peptones. In the case of elastin no precipitable metaprotein is formed in peptic digestion, so that the weight of the protein residue subtracted from 1 gm. gave the weight of the corresponding elastin proteoses and peptones.

Observations of the digestive process in these experiments indicated that with increase in the amount of bone ash there were also larger amounts of protein residue, due, possibly, merely to the decrease in the amount of hydrochloric acid by the neutralizing effect of the bone ash. The quantitative data show that in 100 c.c. of the pepsin-acid mixtures, containing not more than 300 mg. of bone ash (which amounts were completely dissolved in the control), digestion of fibrin was not seriously interrupted and the amounts of combined proteoses and peptones that were formed did not vary appreciably. Larger amounts of bone ash, however, by greatly reducing the acidity, appreciably retarded the peptic digestive process in the case of fibrin, as was shown by the corre-

[18] Hawk and Gies: This journal, 1902, vii, p. 460; also Gies and collaborators: Biochemical researches, 1903, i, p. 615.

spondingly larger amounts of residue and by the production of much less material beyond the metaprotein stage. Even larger quantities of bone ash were required to appreciably affect the peptic digestion of elastin.

Typical data in this connection are summarized below:

A. Elastin. 1 gm. 100 c.c. of 0.2 per cent HCl — 0.3 per cent pepsin solution. Digestive period: 10 hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	300	500	700
Residue (mg.)	849	861	869	862	870	903

B. Fibrin I. 1 gm. 100 c.c. of 0.2 per cent HCl — 0.1 per cent pepsin solution. Digestive period: $1\frac{3}{4}$ hours.

No.	1	2	3	4	5
Bone ash (mg.)	0	100	200	300	500
Residue (mg.)	25	33	31	55	127
Neutralization precipitate (mg.)	264	255	206	243	303
Combined proteoses and peptones (mg.)	711	712	763	702	570

C. Fibrin II. 1 gm. 100 c.c. of 0.2 per cent HCl—0.1 per cent pepsin solution. Digestive period: $1\frac{3}{4}$ hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	300	400	700
Residue (mg.)	72	84	96	127	248	315
Neutralization precipitate (mg.)	149	186	158	206	229	301
Combined proteoses and peptones (mg.)	779	730	746	667	523	384

Twenty milligrams of pepsin, dissolved in about 2 c.c. of water, were added to 5 gm. of bone ash and the thin paste mixed at intervals for about four hours. The mixture was filtered and 1 c.c. added to 20 c.c. of 0.2 per cent HCl. A small piece of fibrin, kept in the mixture at 40° C. for an hour, was unaffected. Evidently the alkalinity of the bone ash was sufficient to destroy the pepsin in the comparatively concentrated solution employed. In the stomach, however, there is normally sufficient acid to immediately neutralize the slight alkalinity of the bone ash, in amounts even greater than those of the doses given in the metabolism experiments.

The effect of bone ash on specially *dilute* pepsin solution was determined under the following conditions: 20 c.c. of 0.2 per cent HCl containing 0.01 per cent of pepsin (Merck, "Ph. G. IV"); fibrin, 0.1 gm.; temperature, 40° C. The experiment was started at 9 P. M.

No.	1	2	3	4
Bone ash (mg.)	0	50	100	200

After three hours digestion was evident in 1 and 2; it was practically complete in 1 and 2 at nine o'clock on the following morning. In each of 3 and 4 there was an appreciable residue, even at the end of twenty-four hours, but after standing for several days digestion in 3 and 4 was also practically complete, although free acid was absent from 4.

3. **Tryptic digestion.** —The general method of procedure in the tryptic experiments was the same as in the peptic digestions. A solution containing 0.25 per cent of Na_2CO_3 and 0.1 per cent of trypsin (Merck) was used, with fibrin or elastin as the indicator. The weight of the soluble matter in 1 gm. of bone ash was determined, and from this the corresponding amounts in the various quantities used were calculated. Observations of the digestion as it proceeded suggested that the amounts of residue were practically the same in all the bottles. The quantitative data indicate that artificial tryptic digestion is not materially interfered with by bone ash under the conditions of these experiments. The amounts of elastin residue decreased as the amounts of bone ash were increased in a series, but, in the case of fibrin, there was a gradual rise in the corresponding amounts of metaprotein, and a consequent decrease in the quantities of combined proteoses and peptones. The increase in the amount of metaprotein was gradual and fairly constant, amounting to an increase of 100 mg. in the presence of 2 gm. of the ash. Typical data are subjoined:

A. Fibrin. 1 gm. 100 c.c. of 0.25 per cent Na_2CO_3—0.1 per cent trypsin solution. Digestive period: 2 hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	500	1000	2000
Residue (mg.)	53	66	46	57	56	72
Neutralization precipitate (mg.)	177	191	201	215	271	275
Proteoses and peptones (mg.)	770	743	753	728	673	653

B. Elastin. 1 gm. 100 c.c. of 0.25 per cent Na_2CO_3—0.1 per cent trypsin solution. Digestive period: 1¼ hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	500	1000	2000
Residue (mg.) Exp. 1	136	117	92	78	99	79
Residue (mg.) Exp. 2	116	51	45	44	62	71

4. **Pancreatic amylolytic digestion.** — For digestive experiments with amylopsin a glycerol extract of sheep's pancreas was employed. The acidity of the extract, due to acid phosphates, was

neutralized with 0.5 per cent Na_2CO_3, which was then added in excess until the reaction became faintly alkaline to litmus. A 1 per cent starch paste solution was employed, and the observations conducted under conditions similar to those of the salivary digestion experiments (page 317). Ten drops of the extract and 20 c.c. of starch paste were mixed with different amounts of bone ash. The results were very similar to those obtained in the salivary experiments. Small amounts of bone ash had a decided retarding effect, and larger amounts prevented digestion to the achromic point for many hours. The bone ash decidedly interferes with the action of the amylases, seemingly in a mechanical way. The effect on the amylopsin was less marked than that on the ptyalin, probably because of the greater concentration of the former under the prevailing conditions.

5. **Lipolytic digestion.** — A portion of the glycerol pancreatic extract that had been prepared for use in the amylolytic experiments was also employed for the lipolytic experiments. The acid reaction of fresh milk was neutralized with Na_2CO_3, and then the milk was colored strongly blue with litmus solution. Three cubic centimetres of the pancreatic extract were added to 25 c.c. of milk in several bottles containing different amounts of bone ash. The bottles were kept in water at 40° C. and the colors compared from time to time. If under these conditions an extract displays lipolytic power, there is a gradual change from blue to red, caused by the hydrolytic production of fatty acid from milk fats, the intensity of the red coloration indicating the relative speed of the reaction.

It was very difficult to distinguish between the shades of color in the control tubes and those containing 0.1, 0.2, or 0.4 gm. of bone ash. The tubes containing 0.8 and 1.6 gm showed a distinct bluish color. The tube containing 1.6 gm. remained as blue as the original milk for a long time. On long standing there was a slow change of color in all the samples. The action of the lipase was not appreciably interfered with by moderate amounts of bone ash. It is a question whether the larger amounts of bone ash interfered chemically by neutralizing the fatty acids as they were formed, or merely mechanically, as appeared to be the case with the amylases.

6. **General comparison of the results of the artificial digestion experiments.** — The action of ptyalin, diastase, and amylopsin in dilute solutions was seriously impaired even by small quantities of bone

ash. In comparatively large proportions bone ash retarded the action of each of these enzymes for many hours or inhibited it in each case altogether. Peptic digestion was retarded somewhat by large amounts of bone ash, apparently because of consequent decrease in the amount of acid. Small quantities of bone ash had very little influence on the peptic process. Tryptic digestion was not disturbed, but, on the other hand, seemed to be favored somewhat by the presence of bone ash, probably as a result of the increased alkalinity imparted by the bone ash. Lipase hydrated milk fats readily in the presence of bone ash. Even relatively large amounts of bone ash did not appreciably retard the lipolysis.

IV. Summary of General Conclusions.

Moderate quantities of bone ash mixed with the food of dogs failed to affect perceptibly the reaction or the specific gravity of the urine, but increased the bulk, and frequency of elimination, of the feces. Such doses of bone ash appeared to reduce the volume of the urine by about the volume of the extra amount of water that was eliminated at the same time in the correspondingly greater bulk of feces. Diarrhea was immediately greatly reduced or stopped entirely by dosage with bone ash.

Moderate doses of bone ash failed to affect appreciably the elimination of total urinary inorganic matter, but the ash from the feces was correspondingly increased in amount. Calcium elimination by the kidneys was unaffected by the bone ash treatment, but the excretion of phosphate in the urine was slightly diminished thereby.

General protein metabolism, as registered by the nitrogen balances for successive periods, was not materially affected by the amounts of bone ash administered in these experiments. The data for urinary sulphur and phosphorus were practically in accord with this conclusion. Urinary nitrogen and phosphorus were decreased somewhat during the dosage periods, whereas the amounts of these elements in the feces were increased in equivalent absolute amounts during the same periods.

Although protein assimilation was perhaps slightly diminished, the reduction, on the most radical assumption the data would permit, must have been trivial. Absorption of both fat and protein

seemed to be unaffected and the excretion of bile unmodified. The elimination of mucus appeared to be increased slightly by mechanical influences. Intestinal putrefaction was perceptibly diminished by the bone ash dosage, doubtless as a result of the more frequent evacuations of the bowels that were induced.

Although the action of various amylases was seriously affected by bone ash in the artificial digestion experiments, the two proteases that were studied *in vitro* were not unfavorably influenced when contained in solutions of normal reaction. Tryptic action seemed to be quickened by the bone ash. Lipase likewise was rendered more active *in vitro,* if it was at all affected by the ash.

The results of the artificial digestion experiments warrant the hypothesis that large quantities of bone ash do not unfavorably influence the intestinal enzymotic processes, and that such doses of bone ash have little or no effect on gastric digestion. Such a hypothesis regarding the normal gastro-intestinal digestive processes is further emphasized by the observation that none of the many dogs subjected to bone ash dosage in this laboratory ever indicated any symptoms of indigestion.

The observations recorded by Steel and Gies [19] in some of these connections have been confirmed in every instance.

The use of bone ash in the diet of dogs seems to offer no metabolic disadvantages whatever. The advantages in its use have been referred to in the introduction to this paper and were mentioned in some detail by Steel and Gies.

In conclusion I desire to acknowledge my gratitude to Dr. William J. Gies for his counsel and guidance throughout this work, and to whose interest and advice I owe its completion.

[19] Steel and Gies: *Loc. cit.*

AN IMPROVED METHOD OF DESICCATION, WITH SOME APPLICATIONS TO BIOLOGICAL PROBLEMS

By L. F. SHACKELL.

[From the Physiological Laboratory of the St. Louis University School of Medicine.][1]

THE present communication embodies the results of an attempt made more than a year ago to shorten the time of vacuum desiccation needed to obtain the percentage of water in various materials. The resultant method has proved much more widely applicable than was anticipated in the beginning. The numerical data and applications to analytical chemistry presented in the first part of this paper are therefore incidental to the use of the latest modification — the essential of which is the desiccation of materials in the frozen condition — in the fields of therapeutics and pure biology subsequently mentioned. No attempt has been made to detail the work done along the latter lines, since a series of extended researches involving the present method is in progress.

Vacuum Desiccation.

The use of a vacuum has been recommended for many years in the drying of substances which would be changed in composition by heating to the boiling-point of water. In general, no rule has been prescribed as to the degree of attenuation required. In a recent article on the determination of water in foods, etc., Benedict and Manning[2] have shown that the extent and rate of desiccation of such materials is largely dependent on the rarefaction of the atmosphere within the containing vessel. These authors have proposed a "chemical" method for obtaining a high vacuum, which consists in vaporizing a few cubic centimetres of ethyl ether

[1] The writer collected the numerical data given, and developed the present method, while Assistant Chemist to the Missouri Agricultural Experiment Station.

[2] Benedict and Manning: This journal, 1905, xiii, p. 309.

in a tubulated desiccator by evacuating the latter with an ordinary water suction pump. The ether vapor displaces the air and subsequently dissolves in sulphuric acid contained in the desiccator. By this means, if proper attention is paid to the lubrication of the vessel, an air pressure of less than 5 mm. of mercury can easily be secured and maintained for practically an indefinite period. Using the method as just outlined, Benedict and Manning have determined the water content in various air-dried materials, or in substances containing less than 20 per cent water; "the agreement of duplicates was striking in all cases. With animal materials (collagen, ossein, gelatin) in general the drying operation is complete at the end of two weeks."

With but slight modification the method was used for nearly a year in the chemical laboratories of the Missouri Agricultural Experiment Station with uniformly satisfactory results. Instead of air-dried substances, however, the material dried — fresh animal tissues — contained usually 60 per cent to 80 per cent of water.

The writer early noticed that drying was much facilitated by rotating the desiccator, since otherwise the surface of the sulphuric acid soon became saturated with water, and desiccation from that point was coincident with the diffusion of the water throughout the concentrated acid below. This fact suggested a series of experiments to determine the minimal time required to completely dry various materials. At this time the attention of the writer was brought to the "Geryk" vacuum pump,[3] with its possible application to the problem at hand. This pump will lower the air pressure in an ordinary desiccator (2–3 litres) to a small fraction of a millimetre of mercury within two minutes. Where water pressure is variable, this pump is a much more reliable source of high vacua than the ether-water-pump method, though it is believed that the analytical results which were obtained with the aid of such a pump, and are presented in the following tables, could have been duplicated under proper conditions with the method of Benedict and Manning. There are, however, several factors which tend to lessen the value of the latter method in water determinations. Where it is necessary to run hundreds of such determinations at the same

[3] I am indebted to Dr. Hermann Schlundt of the University of Missouri for suggesting the use of this pump, which does much to simplify the method to be described. A very efficient form suitable for hand or power can be obtained from the Cleveland Lamp Company, Cleveland, Ohio.

time and many desiccators must be evacuated, much valuable time is lost in waiting for the complete volatilization of the ether. Carbonization of the latter which is absorbed by the sulphuric acid gradually takes place. By reduction of the sulphuric acid sulphur dioxid is produced, and this is absorbed by many substances, especially such as contain much fat. For the same reason the use of commercial sulphuric acid is questionable because of the sulphur dioxid dissolved therein. Without going into detail, the following modifications of ordinary vacuum desiccation were used to obtain the analytical data published herewith, namely: (1) The rapid production and maintenance, by means of the pump mentioned, of a very high vacuum, — less than 1 mm. of mercury column; (2) thorough mixing of the absorbing sulphuric acid by semi-occasional rotation, especially during the early period of drying.

Discussion of Analytical Data.

In Table I are given the results obtained on a sample each of soil, corn chop, air-dried feces, milk, and honey. All the substances were weighed out into crucibles previously ignited and tared, using a tared cover to prevent absorption of moisture by the dry sample during the period of weighing.

The honey used was of the consistency of putty, and an attempt was made to dry it without dilution; this failed from the fact that whenever a high vacuum was used the honey foamed excessively. Water determinations were, however, run upon the fresh sample of honey by careful manipulation to compare with the percentages obtained from the diluted material. A weighed amount (14.1102 gm.) of the fresh honey was dissolved in water, and the volume made up to 50 c.c.; 10 c.c. portions — equivalent to 2.8220 gm. of the original sample — were taken in duplicate for the determination of water. This diluted honey, and the samples of milk, of which the same volume was taken, were absorbed in cotton previously tared with the respective crucibles.

In each table, in the first line opposite the description of the sample are given the percentages of water obtained; below these in the case of each sample are given the dry weights. These indicate simply the weight variations during drying. In every instance the results of duplicate determinations are given. In this series the first weights were taken after a desiccation of twenty-four hours,

and every twelve hours thereafter until the samples became constant. It will be noticed in Table I that ninety-six hours sufficed to completely dry the materials enumerated. The practically absolute desiccation in this length of time of an air-dried feces (steer), as well as of a corn chop, indicates the value of the modifications previously mentioned in the rapid drying of materials containing much cellulose. Benedict and Manning admit that desiccation of such substances by their method requires generally a drying period of more than two weeks.

Because of the time required to completely dry the liquids in this series it was thought that the cotton used as an absorbent might not be thoroughly dry, and it was found to be moreover very hygroscopic. A new series (II) was therefore started, in which materials were used that would perhaps be most affected by drying at the temperature of boiling water. These included samples of levulose, butter, cheese, milk, vinegar, and soap. 10.4267 gm. of levulose (crystalline) were dissolved in water and diluted to 50 c.c.; 5 c.c. portions of this solution — 1.0427 gm. of fresh sample — and the same volumes of the milk and vinegar were used for the determinations of water. In this series the same containers (crucibles) and method of drying were used, except that clean, ignited sea sand was tried instead of cotton as an absorbent for the liquids. From the results, sand has much to recommend itself in this connection; it does not spatter during the removal of the air from the desiccator; like cotton, it spreads the liquid, thus facilitating desiccation; it does not absorb a weighable amount of water, and hygroscopicity is limited to the amount of dry substance in the original sample.

As a check on the vacuum method, control determinations of water were made on the samples of Series II in strict accordance with the methods prescribed for the several materials by the Association of Official Agricultural Chemists.[4]

The results of Series II by the vacuum method are given in Table II, and the results by official methods are given in Table III. Each weighing in Table II was made after a drying period of twelve hours. Inspection of Tables I and II shows that the materials investigated, which it is believed are quite representative, could be dried within five days, and in the case of some — soil, butter,

[4] See Bulletin 107 (Revised) of the United States Department of Agriculture.

TABLE I.

First Series. — Vacuum Desiccation. — All Liquids Absorbed on Cotton.

Description of sample.		Weight of sample.	Period of drying. — Hours.						
			24	36	48	60	72	84	96
		gm.							
Soil I.	Per cent . . .	9.3856	2.38	2.39	....	....	....	....	....
	Weights . . .		34.6054	34.6046	....	....	....	....	....
Soil II.	Per cent . . .	10.8513	2.40	....	....	....	....	....	....
	Weights . . .		36.8289	....	....	....	....	....	....
Corn Chop I.	Per cent	4.7345	12.35	12.75	12.90	13.12	13.54	13.57	13.52
	Weights		29.2571	29.2382	29.2310	29.2208	29.2010	29.1993	29.2016
Corn Chop II.	Per cent	5.2997	12.37	12.75	12.91	13.08	13.57	13.48	13.49
	Weights		29.8729	29.8529	29.8443	29.8355	29.8095	29.8143	29.8136
Feces I. (air dry)	Per cent	3.0525	6.75	6.73	6.77	6.80	6.84	6.87	....
	Weights		27.8651	27.8656	27.8643	27.8634	27.8623	27.8613	....
Feces II.	Per cent . .	2.7224	6.60	6.80	6.83	6.86	6.92	6.92	....
	Weights . .		28.0211	28.0155	28.0147	28.0139	28.0123	28.0122	....
Milk I.	Per cent . .	10.290	84.11	84.30	84.36	84.38	84.42	84.45	84.46
	Weights . .		26.4555	26.4356	26.4294	26.4278	26.4236	26.4199	26.4192
Milk II.	Per cent . .	10.290	84.02	84.24	84.28	84.32	84.36	84.36	84.38
	Weights . .		29.1857	29.1630	29.1590	29.1548	29.1505	29.1507	29.1488
Honey I.	Per cent . .	2.8364	7.10	8.25	9.25	9.56	10.74	11.01	11.18
	Weights . .		27.8866	27.8540	27.8258	27.8170	27.7836	27.7758	27.7710
Honey II.	Per cent . .	3.6366	6.73	8.08	8.98	9.28	10.77	11.01	11.19
	Weights . .		27.7726	27.7235	27.6910	27.6799	27.6257	27.6170	27.6105
Honey I. (diluted)	Per cent	2.8220	11.20	14.58	14.79	14.99	....	....	....
	Weights		29.9702	29.8749	29.8689	29.8633	....	....	....
Honey II.	Per cent .	2.8220	10.77	14.57	14.80	15.04	....	....	....
	Weights .		29.6539	29.5464	29.5400	29.5333	....	....	....

TABLE II.

SECOND SERIES. — VACUUM DESICCATION. — ALL LIQUIDS ABSORBED ON SAND.

Description of sample.		Weight of sample.	Period of drying. — Hours.									
			12	24	36	48	60	72	84	96	108	120
		gm.										
Levulose I. (solution)	Per cent	1.0427	.23	.46	1.14	1.22	1.42	1.72	1.86	1.92	....	....
	Weights		53.9230	53.9206	53.9146	53.9127	53.9106	53.9075	53.9060	53.9054	....	....
Levulose II. (solution)	Per cent	1.0427	.59	.99	1.41	1.54	1.67	1.97	2.14	2.09 [1]	....	....
	Weights		55.9591	55.9550	55.9506	55.9492	55.9479	55.9448	55.9430	55.9435	....	....
Butter I.	Per cent	6.3048	16.34	16.34	....	....	....	....	....	....	....	....
	Weights		30.6363	30.6365	....	....	....	....	....	....	....	....
Butter II.	Per cent	7.5582	16.32	16.32	....	....	....	....	....	....	....	....
	Weights		31.7942	31.7943	....	....	....	....	....	....	....	....
Cheese I.	Per cent	8.3582	31.45	32 29	32.55	32.64	32.72	32.79	32.85	32.90	32.94	32.96
	Weights		30.3304	30.2599	30.2387	30.2306	30.2243	30.2180	30.2136	30.2095	30.2059	30.2045
Cheese II.	Per cent	8.4656	31.43	32.32	32.56	32.66	32.74	32.81	32.86	32.91	32.96	32.97
	Weights		32.4931	32.4180	32.3980	32.3891	32.3825	32.3765	32.3722	32.3682	32.3640	32.3632
Milk I.	Per cent	5.1450	86.66	86.68	86.68	....	....	....	....	....	....	....
	Weights		54.5434	54.5420	54.5421	....	....	....	....	....	....	....
Milk II.	Per cent	5.1450	86.59	86.62	86.62	....	....	....	....	....	....	....
	Weights		53.7691	53.7674	53.7673	....	....	....	....	....	....	....
Vinegar I. (distilled)	Per cent	5.0500	99.59	99.61	....	....	....	....	....	....	....	....
	Weights		55.2924	55.2916	....	....	....	....	....	....	....	....
Vinegar II. (distilled)	Per cent	5.0500	99.58	99.59	....	....	....	....	....	....	....	....
	Weights		52.2313	52.2306	....	....	....	....	....	....	....	....
Soap I.	Per cent	5.3986	13.97	15.83	17.31	18.40	19.10	19.53	19.94	20.29	20.42	20.56
	Weights		31.3843	31.2836	31.2038	31.1450	31.1072	31.0841	31.0617	31.0427	31.0357	31.0284
Soap II.	Per cent	5.3388	11.30	13.77	15.96	17.73	18.95	19.66	20.46	21.01	21.23	21.40
	Weights		29.6894	29.5580	29.4408	29.3464	29.2810	29.2434	29.2004	29.1711	29.1594	29.1505

[1] Result high, due to loss of a little sand from the crucible.

TABLE III.

SECOND SERIES. — CHECK ON VACUUM DESICCATION BY "OFFICIAL METHODS."

Description of sample.		Weight of sample.									
		gm.									
Levulose I. (solution)	Per cent	1.0427	6.44	6.43	6.50	....	....	....	....	....	....
	Weights		25.6861	25.6863	25.6854	....	....	....	....	....	....
Levulose II. (solution)	Per cent	1.0427	6.50	6.81	6.74	....	....	....	....	....	....
	Weights		26.6221	26.6188	26.6195	....	....	....	....	....	....
Butter I.	Per cent	3.4460	16.57	16.61	....	....	....	....	....	....	....
	Weights		18.3383	18.3369	....	....	....	....	....	....	....
Butter II.	Per cent	3.4179	16.46	16.48	....	....	....	....	....	....	....
	Weights		18.2112	18.2106	....	....	....	....	....	....	....
Cheese I.	Per cent	5.7654	33.44	....	....	....	....	....	....	....	....
	Weights		31.7410	....	....	....	....	....	....	....	....
Cheese II.	Per cent	5.2118	33.25	....	....	....	....	....	....	....	....
	Weights		31.5903	....	....	....	....	....	....	....	....
Milk I.	Per cent	10.290	86.12	86.32	86.41	86.51	86.56	86.61	86.65	86.69	86.71
	Weights		15.1038	15.0827	15.0738	15.0639	15.0581	15.0532	15.0489	15.0450	15.0434
Milk II.	Per cent	10.290	86.32	86.52	86.59	86.64	86.66	86.69	86.72	86.76	86.76
	Weights		15.9071	15.8873	15.8800	15.8750	15.8722	15.8693	15.8660	15.8628	15.8630
Vinegar I. (distilled)	Per cent	10.100	99.64	....	....	....	....	....	....	....	....
	Weights		14.6526	....	....	....	....	....	....	....	....
Vinegar II. (distilled)	Per cent	10.100	99.64	....	....	....	....	....	....	....	....
	Weights		14.9502	....	....	....	....	....	....	....	....
Soap I.	Per cent	3.3515	21.72	21.72	....	....	....	....	....	....	....
	Weights		51.1414	51.1414	....	....	....	....	....	....	....
Soap II.	Per cent	4.6816	21.91	21.95	....	....	....	....	....	....	....
	Weights		49.5440	49.5420	....	....	....	....	....	....	....

milk, vinegar, and the like — within a comparatively few hours. In the case of the cheese and soap — the latter giving the only discordant results — dried in the vacuum, the difficulty in abstraction of the last traces of water is explained by the fact that these substances dried to hard masses, and it is probable that traces of water were held quite firmly within the hard, outer crust. Later experiments have shown that if clean, ignited sand is mixed with fresh meat much of the shrinkage of the sample is prevented, and complete desiccation is proportionately facilitated. This is easy to do in the case of meats, cheese, and the like if the container is tared with sufficient sand and a suitable stirrer for thoroughly mixing the former with the sample. Where it is possible, it is suggested that weighed portions of soaps be dissolved in and made up with water to a definite volume, and aliquots absorbed on sand as described previously for honey, milk, etc.

Though the data given in the tables are meagre, they still present several points for consideration. An inspection of Tables I and II in which vacuum desiccation was employed shows that in the large majority of cases given exceedingly accurate results, as shown by duplicates, have been obtained.

It is well here to call attention to the possibility of obtaining good duplicates where the samples are not thoroughly dry. In this connection the reader is asked to compare the first and last percentages given for the corn chop in Table I and for the cheese in Table II. In each case striking duplication was obtained, though there was still more than 1 per cent of water in each sample. The obvious conclusion is that drying should always be continued until constancy in weight is obtained; furthermore, to prevent the same conditions from prevailing during drying, duplicates should not be allowed to dry in the same desiccator.

In comparing the results obtained by the vacuum with those by "official methods," it will be seen that in a majority of instances there was a greater loss of volatile material by heating the samples to drive off the water than by volatilizing the latter in a high vacuum. This is simply corroborative of conclusions that many other workers have reached, and shows the necessity of using the vacuum method where anything like absolute values are desired in water determinations.

Benedict and Manning have shown the value of a very high vacuum in obtaining completeness of desiccation and accuracy of

result. To this the writer desires to add his observation that, *given a high vacuum, whether obtained "chemically" or mechanically, the time of desiccation will still be unduly prolonged unless precautions are taken to keep the absorbent sulphuric acid at the same concentration throughout by rotation and thorough mixing in the desiccators.* The frequency of the rotation will, of course, depend entirely upon the amount of water being absorbed, a little experience enabling the operator to judge for himself. During the first few hours of drying, however, in which the largest part of the water is lost by the samples, the writer finds it necessary to rotate each desiccator for a few seconds every fifteen minutes to half an hour.[5]

As stated in the prefatory remarks, the object of the work just recorded was a reduction in the time required for complete vacuum desiccation. The results show that this has been accomplished, the time element in several cases being shortened to but a small fraction of that required for the same or similar materials by the method of Benedict and Manning. This fact, it is hoped, emphasizes the value of the simple expedient employed by the writer of keeping the sulphuric acid homogeneous during the drying period, and should make the vacuum method much more generally used, especially in work of research calibre; even in technical work, where time is an all-important factor, it seems quite possible that this method can be used to advantage for the determination of water in materials like soaps, low-grade sugars, molasses, etc., where the existent methods are entirely empirical and the results often grossly inaccurate.

Vacuum Desiccation of Frozen Materials.

Previous to the work just detailed, which was completed in July, 1908, the writer had undertaken at the University of Missouri, under the direction of Dr. P. F. Trowbridge, a study of the glycogen content in liver and muscle of steers under various conditions of feeding. Results were so discordant, however, due to the very rapid post-mortem hydrolysis of the glycogen, especially in the liver, that no definite conclusions could be reached. The rapid

[5] It is obvious that various types of mechanical rotators can be devised, where the routine determination of water by this method is carried on to any extent.

drying of materials in the high vacuum now reverted the attention of the author to a problem related to that just mentioned, namely, the cause of post-mortem hydrolysis of glycogen. This question was energetically debated for several years by Noel Paton [6] and Pavy.[7] According to the former's view, the post-mortem change is due in the main to exaggerated life action of the liver cells, though the formation of an amylolytic enzyme which causes subsequent slow hydrolysis is possible. Pavy maintains, on the other hand, that conversion of glycogen to dextrose under these circumstances is entirely the result of unorganized ferment action. The latter idea is generally regarded as correct; nevertheless it seemed that by the use of the vacuum method of drying a definite solution of the problem could be effected. The line of reasoning follows:

1. The extensive vasculation of the liver, and the fact that by crushing the freshly excised gland unusually rapid hydrolysis takes place, seems to argue that the latter may be due in part to a diastatic ferment in the blood, the presence of which has been demonstrated by Bial and others. Rapid irrigation of the liver with physiological saline should obviate this factor, if any such importance attaches to it.

2. By drying such bloodless liver in a vacuum, any diastatic enzyme in the gland tissue should be preserved; the practical absence of fat makes it possible, as experiments have shown, to reduce the dried material to an impalpable powder, thus insuring the destruction of any living cells.

3. The glycogen or sugar could be determined in weighed portions of the powdered liver after incubation in water for varying periods of time. Any decrease in the glycogen or increase in the sugar percentage would indicate the presence of a hydrolytic agent.

There was still left unanswered the question of preventing hydrolysis during the period of drying. This could seemingly be done only by keeping the substance frozen. Bearing in mind the fact that ice may be volatilized in a very high vacuum without passing through the liquid phase, an attempt was made to dry frozen materials without allowing the ice in them at any moment to liquefy. Very gratifying results have been obtained; in fact *all experiments*

[6] D. Noel Paton: Proceedings of the Royal Society, liv, p. 313; Journal of physiology, 1897, xxii, p. 121; *Ibid.*, 1899, xxiv, p. 36.

[7] F. W. Pavy: Journal of physiology, 1898, xxii, p. 391; Pavy and Siau, *Ibid.* 1902, xxvii, p. 452.

to be cited as well as those at present under way are fundamentally dependent upon the rapid and complete desiccation of materials in the frozen condition.

Practical Applications of the Freezing Method.

Though the conception of the present method was due to the glycogenic problem just mentioned, the latter has given way to a study of questions of a more practical nature. For the present paper, a simple statement of results thus far obtained, more extended investigations of which are in progress or projected, will indicate the significance and scope of this latest modification.

1. The mixing of sand with samples to prevent shrinkage and hardening and consequent occlusion of water need now be used only with those that contain too little water to be solidly frozen. Materials containing sufficient water so that they can be frozen to solid masses — meats, fruits, vegetables — can be brought to complete dryness in less time than by any other modification of the vacuum method of desiccation. The reason for this is that the size and physical structure of the substance being dried are preserved practically intact and shrinkage is prevented by its solidity while frozen. It is sufficient to mention in this connection that the writer has samples of beef round that have been dried for nearly four months. No attempt has been made to keep this material from contact with the air or ordinary atmospheric moisture, yet not the lightest deterioration can be detected. The dried meat is perfectly porous throughout, a fact that accounts for the ease with which accurate moisture determinations can be made. This same porosity very greatly facilitates the extraction of the material with ether for the ordinary determination of fat or extraction by other organic solvents.

It may not be amiss to suggest at this point the drying of large samples (100–500 gm.) — this has been done with as much ease as 5 gm. samples — in the extensive problems of animal nutrition that are being undertaken at the present time, and in which analyses of the fresh substance as a rule prevail; the fact that there is an extremely small chemical change, if any, during drying and none whatever afterward if the simplest precautions are taken, makes it always possible to refer to the equivalent of fresh material in case of question as to previous results.

It is at times practically impossible to obtain thorough mixture of samples for analysis, as for example the white and gray matter of the central nervous system; with the use of this method such materials can be completely dried, triturated, and mixed in the powdered form to complete homogeneity.

2. The application of this method to immunity work early suggested itself. An investigation along this line was begun in November last. Diphtheritic or tetanus antitoxin was not then available, but, through the kindness of Dr. J. W. Connaway of the Missouri Agricultural Experiment Station, the writer was supplied with the toxic blood as well as the immune serum of hog cholera. This was for the most part dried while frozen. Unavoidable delays and the difficulty of obtaining proper animals for the tests to be made render it impossible to report at present the results in this case. The following experiments make it probable, however, that the present method can be used for the absolute preservation of immune sera as well as the concentration of low-potency toxins: (*a*) The serum of guinea pigs was desiccated while frozen. Though the dried material has been exposed to sunlight and the ordinary temperature for many weeks, its complement content remains unchanged, as shown by its successful use in the Wassermann sero-diagnostic reaction for syphilis. (*b*) Dog's blood caught directly from the femoral artery into a receiver surrounded by a freezing mixture was congealed to a solid mass and completely dried without coagulation. On addition of water to a small portion of the dry residue, a typical fibrin clot invariably forms in a few moments, showing that none of the factors involved in the formation of thrombin have been affected by this method of desiccation. (*c*) A rabbit's brain containing the fixed virus of rabies, obtained through the kindness of Dr. D. L. Harris, City Bacteriologist of St. Louis, was dried in the frozen condition. An emulsion of a small amount of the dried brain injected subdurally into a rabbit causes typical symptoms of rabic paralysis, with subsequent death of the animal.

A matter of considerable importance is the solubility of products of this method. A serious objection to the use of desiccated antitoxins as now put on the market is that such products do not give clear solutions when diluted. The present method is not open to this objection, for every experiment so far has shown that, if perfectly clear solutions are frozen and dried, the same clearness and perfect solubility will again obtain on dilution. The turbidity re-

sulting on solution of serum residues when desiccated by ordinary vacuum methods may be attributed to concentration of the original solutions with probable accompaniment of chemical changes, even though comparatively low temperatures have been employed during desiccation. A striking illustration of this point is the work on rabies just cited. As is well known, the Pasteur treatment of hydrophobia depends on the attenuation of virulence in the spinal cords of inoculated rabbits when dried for several days over caustic potash. In this latter case concentration is undoubtedly destructive to the virus, as shown by the fact that a cord well dried by Pasteur's method loses its virulence completely. On the other hand, the same material dried by the present method retains its virulence, because the disturbing factor, concentration, has been removed. *The present method employs the only possible means by which concentration — that is, increase in percentage of solids in solution — can be prevented.* From a physical standpoint it is interesting to record that drying proceeds from the surface toward the centre, and that when say 50 per cent of the water is removed from a certain material, one half is practically absolutely dry and the other half contains its original percentage of water, still in the frozen condition.

3. A very interesting phenomenon noted while drying blood was that the blood gases were retained quantitatively or nearly so in the dry residue; at least no change in the mercury levels of a differential manometer could be noted during the drying of 40 c.c. of brightly scarlet defibrinated blood. The residue was perfectly soluble in water or physiological saline, the corpuscles having been destroyed at some time during the process. Such a solution subjected to a vacuum without previous freezing gave off the gases in the same manner as fresh blood. It has not yet been determined whether elemental nitrogen is present in the dry residue, but the oxyhemoglobin is undoubtedly preserved.

Technic of the Improved Method.

To obtain results such as have been given, the following fundamentals of the technic must be rigidly adhered to: (1) Well-made desiccators; (2) thorough solidification of the material, if freezing is used at all; (3) the production and maintenance of a very high vacuum; (4) the use of a proper lubricant for the stopcock and joints; (5) proper closure of desiccator stopcocks after exhaus-

tion; (6) thorough mixing at intervals of the sulphuric acid in the desiccators.

1. A desiccator whose inner diameter is more than 6 inches cannot safely be used with the very high vacuum now employed. Larger desiccators are almost invariably crushed by the external pressure. It is very important to have the parts of the desiccator fit well. All ground-glass surfaces, especially those of the stopcock, should be as smooth as possible.

2. Freezing of the material before desiccation needs no discussion. The writer has always used an ordinary ice-and-salt mixture. It is obvious that unless thorough freezing is effected, that part of the material still semi-solid will shrink and harden in the same manner as unchilled samples.

3. In work on frozen substances, an efficient pump of the type previously mentioned is indispensable for the rapid production of a vacuum sufficiently high to prevent thawing.

4. In all this work the author has used as a lubricant a mixture of 5 parts by weight of commercial vaseline (petrolatum) and 3 parts of ordinary paraffin. The ingredients are melted together and heated to the boiling-point of the mixture for several minutes. Unless this precaution is taken to insure reciprocal solution of the components, small particles of paraffin are apt to be found throughout the lubricant, rendering it worthless for the purpose intended. The proportion of paraffin may be increased or decreased, dependent upon any considerable rise or fall in temperature of the laboratory.

5. Simple turning of the stopcock after exhaustion of the desiccator has been found insufficient to entirely prevent entrance of air into the vessel; minute pathways in the lubricant are formed in the direction of revolution and allow small quantities of air to enter the desiccator despite additional closure by solid rubber stoppers, etc. To prevent this the writer proceeds as follows: the outer surface of the core and inner surface of the shell of the stopcock are lubricated, the first at its larger and the second at its smaller end. The core is then pushed into the shell until the two films of lubricant meet. This procedure tends to prevent stoppage of the exit tube with the lubricating mixture. After the stopcock is finally closed, the core is simply pressed tightly into the outer shell, thus destroying the pathways formed on closing. By this means it has been possible to maintain the highest obtainable vacuum indefinitely

when necessary. A small manometer is always used within the desiccator to indicate any chance diminution in the height of the vacuum.

6. As detailed in the first part of this paper, thorough mixing of the sulphuric acid in the desiccators is absolutely necessary to prevent saturation of the surface of the acid with water, the latter causing increased tension of water vapor in the vessel sufficient to allow frozen material to thaw, and consequent failure of the experiment. Since the vapor tension of water is 4.6 mm. of mercury at 0° C., it is obvious that the levels of a differential manometer must never show a difference of more than 3 mm., if it is desired to prevent melting of frozen material. Even after sulphuric acid has absorbed an equal volume of water it is very hygroscopic, and if kept well mixed will absorb water vapor as rapidly as the ice in any frozen material is volatilized. The poor thermal conductivity of a high vacuum, as well as the fact that frozen substances are cooled to several degrees below 0° C. by the rapid volatilization of the ice in them, has made it possible to obtain eminently satisfactory results even in a very warm laboratory.

Summary.

1. The improvements in the present method over ordinary methods of vacuum desiccation are (*a*) the very rapid production of extreme vacua with the so-called "Geryk" type pump; (*b*) the freezing of the material prior to desiccation to obviate primarily any concentration of substances, and to a lesser degree shrinkage and hardening; (*c*) mixing of the sulphuric acid absorbent to prevent saturation of its exposed surface with its consequently greatly lessened efficiency.

2. The method affords a comparatively rapid and exceedingly accurate means for determining water in various materials, especially such as can be frozen solidly. Because of the friability and porosity of such dried materials, the extract obtained by organic solvents can be secured with greater ease than by the usual method of extracting oven-dried materials or those that have been shrivelled and hardened by ordinary methods of vacuum desiccation.

Liquids need not be frozen for determinations of water, but can be absorbed on dry sand in proper containers.

3. Blood dried in the frozen condition retains the largest part if not all of the gases originally in it. To what extent these gases remain in chemical combination or physical occlusion has not been as yet determined. Fresh blood quickly frozen and dried retains its power of coagulation on later addition of water.

4. Experiments so far indicate that all materials, especially those unstable substances associated with immunity work, can be desiccated as outlined and can be indefinitely preserved.

It is generally recognized that no chemical changes take place in perfectly dry substances. The products of this method are for all purposes entirely moisture-free. Deterioration in them is therefore absolutely precluded, providing the ordinary precautions of stoppered containers are taken to prevent contact with atmospheric moisture. If necessary, substances after drying can be hermetically sealed *in vacuo*.

The special value of the method in medicine lies undoubtedly in the field of serum-therapy. One of the great practical problems has been the prevention of auto-degeneration in serums and toxins. It has been found possible, however, by the use of this method to obviate autolysis of such typically unstable substances as the complement of guinea-pig serum and the virus of hydrophobia.

Though but a few of the most important results of the method have been mentioned in the present paper, the writer and his colleagues appreciate the probability of its widely extended application to many current problems, several of which are being attacked at the present time.

The writer wishes, in conclusion, to acknowledge his indebtedness to Dr. E. P. Lyon, whose appreciation of the method and provision of experimental facilities have furnished much of the incentive for the more recent work here recorded, as well as for the series of researches at present in progress.

THE INFLUENCE OF THE TEMPERATURE OF THE HEART ON THE ACTIVITY OF THE VAGUS IN THE TORTOISE.

By G. N. STEWART.

[*From the Laboratory of Experimental Medicine, Western Reserve University.*]

J. M. LUDWIG and B. Luchsinger[1] long ago concluded that in the tortoise, as in the frog, "not only does the vagus remain completely active at the highest temperatures which the heart can still endure, but it appears rather to have an increased activity at these lethal temperatures."

In an investigation of the influence of temperature and other factors on the heart and particularly on the action of the vagus and cardiac sympathetic nerves[2] in the frog, I incidentally made some observations on the inhibitory nerves of the ordinary European land tortoise, and concluded that "in general the inhibitory action of the right vagus of that animal is affected by the temperature of the heart in the same sense as that of the vagus in the frog; although the effect seems to be less marked than in the frog, and a much greater change of temperature is necessary to cause a sensible alteration in the inhibitory activity of the nerve. At very low temperatures it is unquestionably more difficult to obtain complete standstill of the heart than at the ordinary or at a higher temperature. But for a considerable range above and below the ordinary temperature it may be difficult to demonstrate any marked difference. It is by no means easy to show in the tortoise what is seen in the frog, that the minimum strength of stimulus needed to produce a given inhibitory effect increases as the temperature falls and decreases as the temperature rises. It needs a considerable fall of temperature to appreciably increase the minimum stimulus."

[1] LUDWIG and LUCHSINGER: Archiv für die gesammte Physiologie, 1881, xxv, p. 211.

[2] STEWART: Journal of physiology, 1892, xiii, p. 59.

These conclusions have been called in question by E. G. Martin,[3] working with the terrapin, but confirmed by Bassin,[4] working in Kronecker's laboratory with the European land tortoise. Bassin, who does not seem to know of any work published in English, states that the strength of stimulation of the vagus which causes inhibition at the ordinary temperature causes as great an inhibition when the heart is heated to about 40°. Inspection of his curves and protocols shows indeed that complete inhibition may be obtained at the higher temperature with a strength of stimulation distinctly smaller than at the lower.

I have not been able to spare time to repeat the work, but, particularly since the appearance of Bassin's paper, hold this to be unnecessary. With the single exception of Lépine and Tridon,[5] all observers who have worked with European tortoises are agreed that the inhibitory activity of the vagus is, at any rate, as great at temperatures around 40° as at ordinary air temperatures. Why, then, has Martin reached the opposite result? (1) Chiefly, I think, because he has adopted a different criterion of vagus activity from the other observers cited. I am merely noting this difference, not criticising it. Ludwig and Luchsinger, Bassin and myself, determined a strength of stimulation sufficient to cause complete stoppage of the heart at the higher temperature, and then observed whether stoppage was caused by the same strength of stimulus at the lower temperature; or, *vice versa*, a strength of stimulation just insufficient to produce complete inhibition at the lower temperature was sought and its effect determined at the higher. The stimulation was kept up only long enough to produce a perfectly definite effect. Martin, on the other hand, in his first set of observations, "selected a strength of stimulus which would hold the heart at a practical standstill for several minutes at ordinary temperature, and then the effect of raising and lowering the temperatures was observed for this strength of stimulus." In the second set "advantage was taken of the fact that the heart of the terrapin can be maintained in standstill by stimulation of the vagus for a number of minutes without the effect of the stimulus becoming appreciably weaker. The heart was inhibited at ordinary tem-

[3] Martin: This journal, 1904, xi, p. 387.

[4] Bassin: Archiv für Anatomie und Physiologie, 1907, p. 429.

[5] Lépine and Tridon: Mémoires de la Société de Biologie, March 4, 1876.

perature, and then, while the stimulus was still on, the temperature of the organ was raised ten degrees or more." [6]

Now Martin found in general that at the higher temperature the heart during vagus stimulation gave a larger number of beats than at the lower temperature, or could not be so long prevented from beating. What was observed here was really the capacity of the heart to escape eventually from vagus control at the higher and lower temperatures, not the capacity of the vagus to cause complete, even if transient, inhibition, as in the experiments of the other writers.

The two tests could hardly be expected to give the same result. For if the vagus, acting upon an intracardial inhibitory mechanism whose excitability is increased by increase of temperature, is able to cause inhibition even more easily at high than at lower temperatures, it does not follow that the inhibition will be as long maintained. One would rather expect that the automatic "contractile energy," accumulating more rapidly at the high temperature in the automatic ganglia or the muscular fibres or in both, would sooner reach the threshold at which it overflows in spite of the continued stimulation of the inhibitory nerve. As I pointed out in my previous paper,[7] "although a high temperature is favorable to the initiation of inhibition, it is not necessarily favorable to its continuance. So far is this from being the case that the standstill obtained by chemical stimulation of the medulla oblongata at the ordinary temperature can be removed by gradually raising the temperature of the heart. And with electrical stimulation of the mixed vagus nerve the standstill, which is more easily obtained at a high temperature than at a low, often passes off sooner."

[6] Martin also made a few experiments in which he endeavored to see whether the minimal strength of stimulation needed to produce inhibition was altered by altering the temperature of the heart. The results, however, are such as are not easily interpreted. For example, in one experiment (he cites only two) he got no inhibitory effect at all with the strongest stimulation which could safely be employed when the temperature of the heart was 31.5°, although complete stoppage was caused with a much weaker stimulus at 22° before the heart was heated and at 23° after it was cooled again. If this is a typical result, the terrapin's heart must differ essentially in this respect from that of the tortoise, for I have never seen anything to suggest that a temperature of 31.5° will abolish the inhibitory power of the vagus, nor is there anything in the work of Ludwig and Luchsinger or of Bassin which even remotely indicates such a possibility.

[7] STEWART: *Op. cit.*, p. 99.

Even to this test, however, the vagus action at the higher temperature in Martin's experiments really was much more slightly impaired than would appear on a hasty comparison of the number of beats at the two temperatures during the period of vagus stimulation. For to get the true measure of the extent of the inhibition we must consider the number (and strength) of the beats the heated heart would have executed at the given temperature had the nerve not been stimulated. When, for example, we read that at 29° the heart beat 9 times in three minutes during stimulation of the vagus, whereas at 20° it contracted only thrice in five minutes, we must remember that, had it been left to itself, it would have beat probably not less than 90 or 100 times at the higher temperature and perhaps not half as frequently at the lower. The absolute amount of inhibitory effect, measured by the amount of work suppressed, is therefore very considerable at the higher temperature.

(2) Martin did not use such high temperatures (only up to 31° or 32°) as the other observers (up to 40° or a little more). Now, as already mentioned, I found that for a considerable range above and below the ordinary temperature it is not easy to demonstrate any marked difference in the minimal strength of stimulus which will cause inhibition. It is, of course, quite conceivable that the excitability of the intracardiac inhibitory mechanism or the capacity of the muscle fibres to respond to inhibitory stimuli may be a different function of the temperature at different temperatures in the same heart, and that the curves may not be the same for the hearts of different animals. Further, the relation between the effect produced on the contractile power of the heart and on the inhibitory mechanism by a given change of temperature need not be the same for all temperatures in one and the same or in different hearts. So that, if it is much easier, as I have shown, to demonstrate the progressive change in strength of the minimal stimulus required to produce inhibition for moderate increase or diminution of temperature from the ordinary temperature in the frog than in the land tortoise, there may also be a difference between the land tortoise of Europe and the terrapin in this regard.

AN APPARATUS FOR STUDYING THE RESPIRATORY EXCHANGE.

By FRANCIS G. BENEDICT.

[*From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.*]

Introduction.

A CAREFUL examination of the qualitative and quantitative changes in the air passing through the lungs furnishes most valuable data for interpreting the nature and extent of the oxidation processes in the body. The exhalation of carbon dioxide and the absorption of oxygen have been called the respiratory exchange. When the body is at rest and without food, the exchange is continuous, proceeds with considerable regularity, and, if rhythmical, the variations are usually fairly constant from day to day. The respiratory exchange is, however, markedly influenced by the ingestion of food and muscular exercise and to a less extent by many other factors.

Of the respiratory products, carbon dioxide has been longest and most accurately studied. The numerous delicate methods for determining this constituent in the air, the small amount present in normal air, the rapid excretion of carbon dioxide following muscular exercise, all contributed to the general feeling on the part of the earlier physiologists that a knowledge of the carbon metabolism was of utmost importance. When we consider that all three of the main organic materials in the body — the proteins, the fats, and the carbohydrates — yield carbon dioxide as the result of their partial or complete oxidation, it is seen that while the determination of the total carbon dioxide output may indicate in a general way the amount of the total katabolism, it of itself cannot indicate in any way the nature of the material burned.

To aid in apportioning the katabolism between the nitrogenous and the non-nitrogenous material of the body, it was found that the nitrogen excretion of the urine represented very closely the

amount of protein disintegrated. The proportion of nitrogen in the protein molecule is relatively constant, as is indeed the carbon content. From these factors the total carbon resulting from disintegrated protein can be computed with considerable accuracy. In thus including the carbon of protein in the calculation it became necessary to determine the unoxidized carbon in the urine. This determination even at this date is far from exact, and only too frequently investigators rely upon the relatively constant ratio between nitrogen and carbon in the urine found in healthy men to compute the carbon from the amount of nitrogen determined by means of the rapid and exact method of Kjeldahl.

Deducting from the total carbon excretion that computed as belonging to the disintegration of the protein, we have left the carbon derived from fat or carbohydrate. The apportionment of this remaining carbon between the katabolism of fat and carbohydrate has long been a source of much difficulty in metabolism experiments. For many years for want of direct determination of oxygen it was necessary to assume that during inanition the carbon other than carbon of protein was wholly derived from fat, and the calculations were based on this assumption. The recent researches in inanition,[1] however, have demonstrated that there may be a very considerable draft upon glycogen in the body, at least on the first day of fasting, and hence it is wholly erroneous to consider the carbon excretion other than that of protein as carbon of fat. In experiments where food is ingested, relying upon the well-known rapid absorption of carbohydrates in the diet, physiologists have usually assumed that the total carbohydrate in the diet was absorbed and oxidized inside of twenty-four hours, and hence the carbon unassigned to protein has been considered as made up in part from the carbon resulting from the combustion of carbohydrates in the diet. Making due allowance for the amount of carbon that can be derived from the carbohydrates in the diet, the remaining carbon has been ascribed to the fat of the diet, or, in case the diet was inadequate, to the fat of the diet plus a certain amount of body fat.

If to the determination of carbon dioxide exhaled is added a determination of the amount of oxygen absorbed, the interpretation of the results is very much more satisfactory, for the ratio between the volume of carbon dioxide given off and the oxygen

[1] Benedict: Carnegie Institution of Washington, Publication No. 77, 1907.

absorbed, the so-called respiratory quotient, gives a reasonably accurate indication of the nature of the substance burned.

While with the combustion tube or the calorimetric bomb the oxidation of organic material proceeds with perfect regularity and completeness to carbon dioxide and water, inside the human body we have to deal with other conditions. The carbohydrates are, it is assumed, completely oxidized; the fat with normal man is likewise assumed to be completely oxidized; but, on the other hand, the complex nitrogenous molecule of the protein is but partially disintegrated, and we have therefore of the original protein carbon part disintegrated in the form of carbon in the urine and part as carbon dioxide in the expired air.

In disease, where there may be abnormal metabolism, we have also the possibilities of a partial excretion of organic material in the urine other than that derived from protein. This is noticeably so in the case of B-oxybutyric acid and sugar found in the urine of diabetics. In calculating, therefore, the carbon dioxide resulting from the disintegration of organic material in the body and particularly in calculating the oxygen required for this disintegration, it is commonly assumed that in health no compounds resulting from the partial disintegration of either fat or carbohydrate are excreted in the urine. As a matter of fact, this is not, strictly speaking, true.

The chemical composition of the various ingredients of the body as well as the food stuffs has been determined. There is not the uniform agreement that could be expected to be found when analyzing definite, well-crystallized, organic materials of simple molecular structure; but as the result of a large number of analyses, the percentage composition of body material has been assumed to be that shown in the following table:

Body material.	N.	C.	H.	O.	Mineral matters (including S.)
	per cent.	per cent.	per cent.	per cent.	per cent.
Proteins	16.67	52.80	7.00	22.00	1.53
Fat	. . .	76.10	11.80	12.10	. . .
Carbohydrate (Glycogen) . . .	. . .	44.40	6.20	49.40	. . .

For materials in the food, the composition of starch, cane sugar, and glucose can be computed from the chemical formulas directly. For the composition of normal fat, average values given by Koenig [2] have been chosen, namely, C, 76.65 per cent, H, 11.92 per cent, and O, 11.43 per cent.

The calculation of the respiratory quotient for substances of simple molecular structure — namely, the starches, carbohydrates, and the fats — presents very little difficulty. Thus, for example, starch has the chemical formula $(C_6H_{10}O_5)_x$. For purposes of calculation the molecule represented by the formula $C_6H_{10}O_5$ and not the multiple can be taken without affecting the mathematics in any way. The molecular weight, therefore, may be considered as 162. The chemical reaction may be expressed as follows: $C_6H_{10}O_5 + 6\ O_2 = 6\ CO_2 + 5\ H_2O$.

In order to oxidize, therefore, 6 atoms of carbon or rather the 72 gm. of carbon existing in, say, 162 gm. of starch, 12 atoms coresponding to 192 gm. of oxygen are necessary. As a result of this oxidation, $6 \times 44 = 264$ gm. of carbon dioxide are produced.

The respiratory quotient deals with volumes rather than with weights, however, and on reducing these values to volumes, assuming that 1 litre of O weighs 1.43 gm and 1 litre of CO_2, 1.966, we have, as a result of the combustion of this amount of starch, 134.26 litres of CO_2 produced and the same volume of oxygen absorbed. Therefore:

$$\frac{CO_2}{O_2} = \frac{134.26}{134.26} = 1.00.$$

In order to calculate the respiratory quotient for fat, we can find, from the molecular composition given in the table above, that 1 gm. of human fat requires 2.844 gm. of oxygen in its combustion and 2.790 gm. of carbon dioxide are produced. There is, therefore, an absorption of 1990.8 c.c. of oxygen to form 1240.4 c.c. of carbon dioxide, and hence the respiratory quotient would be

$$\frac{CO_2}{O_2} = \frac{1240.4}{1990.8} = 0.713.$$

The calculation for protein is somewhat more elaborate, owing to the fact that it is only incompletely burned, as has been pointed

[2] Koenig: Chemie der menschlichen Nahrungs-und Genussmittel, third edition, i, p. 198.

out above. The calculations have been made in a number of ways by different writers on this subject, each assuming a somewhat different molecular composition for the protein, and each ascribing in turn various values to the unoxidized portion of the protein excreted in feces as well as in the urine. Furthermore, there is considerable latitude among various observers as to what degree the sulphur of protein is oxidized, for there is unoxidized as well as completely oxidized sulphur in the urine. The following calculation is, however, taken directly from Loewy,[3] in which it is assumed that 100 gm. of fat-free, dry substance of flesh contain 52.38 gm. of C, 7.27 gm. of H, 22.68 gm. of O, 16.65 gm. of N, and 1.02 gm. of S. Of these it is assumed that there are found

	C.	H.	O.	N.	S.
In the urine	9.406	2.663	14.099	16.28	1.02
In the feces	1.471	0.212	0.889	0.37	0.0
Remainder	41.50	4.40	7.690	0.00	0.0

By the combustion of 41.5 gm. of carbon and 4.4 gm. of hydrogen, there were used 145.87 gm. of oxygen. Deducting from this the 7.69 gm. originally in the protein and not excreted in the urine or feces, there were required from the air 138.18 gm. During the process of oxidation there were formed 152.17 gm. of CO_2. Reducing these values to volumes, we then have the ratio:

$$\frac{CO_2}{O_2} = \frac{77.39}{96.63} = 0.801.$$

The oxygen required for combustion, the products of combustion, and the respiratory quotient for several typical materials have been calculated and placed in the accompanying table (see page 350).

It will be seen that the values as used by Loewy differ slightly from those given in the table above, in that the amount of oxygen required to oxidize 100 gm. of protein was 138.18 gm, while in the above table the amount is 1.367 gm. per gram of protein. There is likewise a slight change in the carbon dioxide production. It should be stated, however, that apparently the substance upon which the greatest error falls in calculating the respiratory quotient contributes the least to the total katabolism, and hence the error is wholly negligible when the total katabolism is measured.

[3] Loewy: Oppenheimer's Handbuch der Biochemie, 1908, iv, p. 156.

The carbohydrates have a respiratory quotient of 1.00; fats, in general, of 0.71, and protein of 0.81. From these factors, therefore, we can see that during inanition, when the subject is subsisting for the greater part upon body fat and protein, the respiratory quotient would tend to approach 0.71, and, as a matter of fact, in a long series of observations it has been found that in the later days of a three- to six-day fast, the respiratory quotient is fairly constant at about 0.74.[4] On the other hand, if a diet is taken consisting in large part of carbohydrates, the respiratory quotient tends to approach unity.

RESPIRATORY QUOTIENTS FOR PROTEIN, FATS, AND CARBOHYDRATES.

Materials.	Oxygen required to oxidize 1 gm.		Products of the oxidation of 1 gm.				Respiratory quotient $\frac{CO_2\ cc.}{O_2\ cc.}$
			Carbon dioxide.		Water.	Heat.	
	Weight.	Volume.	Weight.	Volume.			
	gm.	c.cm.	gm.	c.cm.	gm.	Cal.	
Starch . . .	1.185	829.3	1.629	829.3	0.556	4.20	1.000
Cane sugar	1.122	785.5	1.543	785.5	0.579	3.96	1.000
Glucose . .	1.066	756.2	1.466	746.2	0.600	3.75	1.000
Animal fat .	2.876	2013.2	2.811	1431.1	1.065	9.50	0.711
Human fat .	2.844	1990.8	2.790	1420.4	1.055	9.54	0.713
Protein . .	1.367	956.9	1.520	773.8	0.340	4.40	0.809

Since the metabolism of the protein remains relatively constant from day to day and from hour to hour and is but a small proportion of the whole, the errors involved in its calculation are not of sufficient magnitude to influence seriously any deductions drawn from the results in which these calculations occur. Usually the disintegration of the protein is about 15 per cent of the total katabolism, and Magnus-Levy[5] has calculated that if the remaining 85 per cent is wholly from carbohydrates, the respiratory quotient would be 0.971, and if, on the other hand, the remainder of the

[4] BENEDICT: *Loc. cit.*, p. 451.

[5] MAGNUS-LEVY: VON NOORDEN'S Handbuch der Pathologie des Stoffwechsels, p. 217.

energy is derived solely from fat, the respiratory quotient would be 0.772. Under ordinary conditions the respiratory quotient would lie between these two figures, and values above or below these points might reasonably be considered as due to faulty technique or to distinctly abnormal metabolism or to possible formation of fat from the carbohydrate or carbohydrate from fat. The consideration of these points, however, would lead us too far afield in this discussion.[6]

A carefully determined respiratory quotient is therefore of direct value in indicating the nature of the material oxidized.

The absolute values for the amounts of carbon dioxide exhaled and oxygen absorbed in a given amount of time, usually one minute or one hour or twenty-four hours, are also of great importance in indicating the quantitative relations of the total katabolism. From the twenty-four hours' amount especially is it possible to strike a daily balance, and by determining or computing the carbon of the diet the adequacy of the ration for maintenance may be proved.

Of still more importance has been the use made by a number of physiologists of the respiratory exchange to compute the total calorimetry by the so-called method of indirect calorimetry.

From the determination of nitrogen in the urine (protein katabolism) and the respiratory exchange, it is possible to apportion the total katabolism between protein, fat, and carbohydrate.

It is generally assumed that all of the food materials are first transformed into similar substances found in the body, and the calculations may then with propriety be based upon the values for glycogen, body protein, and human fat.

As each of these materials gives rise, when completely burned, to definite amounts of energy, it is customary to multiply the number of grams of katabolized protein by the factor 4.1, fat by 9.54, and carbohydrates by 4.19, and then obtain the total energy resulting from the oxidation.

That this method gives a reasonably accurate indication of the calorimetric transformations in the body is commonly assumed. It is absolutely proved that the method of indirect calorimetry, when applied to experiments of not less than twenty-four hours' duration, does give accurate results for the total heat production. This has been shown not only with fasting men but likewise with those

[6] For an exhaustive treatment of this subject, see MAGNUS-LEVY: *Loc. cit.*, p. 218.

consuming food. As a result, then, of the information given by the study of the respiratory exchange, it is clear that a method for this most important study should be perfected which in so far as possible shall be free from errors, be as little complicated as possible, and permit of reasonably accurate and satisfactory results in the hands of others than skilled chemists especially trained to use the apparatus.

Recognizing at a very early date the noticeable variations in composition of inspired and expired air, and foreseeing with remarkable clearness the great advantage of the knowledge of the quantitative relations existing in these transformations, chemists and physiologists began to study methods for separating the inspired and expired air, for determining the changes in composition, and finally for noting the actual amount of carbon dioxide produced and oxygen absorbed in a definite period of time.

The methods of studying the respiratory exchange are based upon two distinct principles: First,[7] the animal or subject is placed in a chamber, large enough to have him remain with comfort, and the air is there allowed to become vitiated by products of oxidation, and the changes in the composition of the air from period to period studied; or there is a small movement of air throughout the chamber during the whole time, thus checking to a certain extent the rapid rise of the carbon dioxide content of the air inside the chamber; or the air is rapidly circulated through the chamber so as to maintain the atmospheric conditions approximately normal.

The other method depends upon the separation of the expired and inspired air by means of suitable valves, the respiration being maintained through tubes in the nose, a mouthpiece held between the teeth or lips, or a mask, or, in the case of animals, a cannula in a tracheal fistula. The expired air is measured either in a spirometer or gas meter.

During the past two decades Zuntz[8] and his co-workers have made admirable use of an apparatus employing a mouthpiece, valves

[7] In connection with a description of an apparatus of this type a review of the earlier respiration chambers was given in Publication No. 42 of the Carnegie Institution of Washington, 1906.

[8] For a complete discussion of the development of this remarkable apparatus, see Loewy: Oppenheimer's Handbuch der Biochemie, 1908, iv, p. 134.

similar to those of Speck[9] or of special fish bladders,[10] and an Elster wet gas meter.[11]

Chauveau and Tissot[12] make use of rigid glass nosepieces, metallic valves, and collect the expired air in an ingeniously devised spirometer.[13] This French apparatus has not, however, been accorded the general use given the apparatus of Zuntz.

Description of New Apparatus.

This apparatus is the logical outcome of an attempt to apply the principle used in the large respiration chambers in use in this laboratory to a movable type of apparatus. The precursor of these chambers has been described in great detail.[14] In the large respiration chamber a man sits in an armchair or lies on a bed in a chamber of sufficient size to allow him to move about with comfort. A current of air is maintained by a rotary blower. As the air leaves the chamber, it contains, in addition to the nitrogen normally present, carbon dioxide, water vapor, and is somewhat deficient in oxygen, inasmuch as the oxygen has been used for the support of combustion in the body. The outgoing air is caused to pass through a purifying system consisting, first, of a sulphuric acid vessel which removes the water, second, a soda lime vessel which removes the carbon dioxide, and, third, a sulphuric acid vessel for absorbing the water yielded to the current by the moist soda lime; and the air is then returned to the chamber, the deficiency in the oxygen being made up by admitting oxygen from a steel cylinder of highly compressed gas. By noting the increment in weights of the different purifying systems and the loss in weight of the oxygen cylinder, a rough approximation of the total carbon dioxide production and the oxygen consumption is obtained. By making

[9] Speck: Schriften der Gesellschaft zur Beförderung der gesammte Naturwissenschaften zu Marburg, x, 1871. See also Physiologie des menschlichen Athmens, Leipzig, 1892, p. 9.

[10] Durig: Biochemische Zeitschrift, 1907, iv, p. 68.

[11] Loewy: *Loc. cit.*, p. 136. For details of construction, see description by Zuntz: Landwirthschaftliche Jahrbücher, 1889, xviii, p. 1; also Flügge: Hygienische Untersuchungsmethoden, p. 531.

[12] Chauveau and Tissot: Comptes rendus, cxxxii, p. 1532.

[13] Tissot: Journal de physiologie et de pathologie générale, 1904, p. 692.

[14] Atwater and Benedict: Carnegie Institution of Washington, Publication No. 42, 1905.

due allowance for variations in pressure and temperature of the air in the chamber, variations in the carbon dioxide and water and oxygen contents of the air in the chamber, exact information regarding the carbon dioxide production and oxygen absorption of the subject during periods as short as one hour can be obtained. This method obviously involves the use of an elaborate respiration chamber, which, as a matter of fact, is fitted with calorimetric appliances and hence cannot be used as a portable or, indeed, as a semi-portable apparatus.

The large respiration calorimeter, although giving exact data regarding the respiratory exchange and the heat production, is unfortunately not adapted, either by reason of the expense of installation or the technique of manipulation, for general use in most hospitals, clinics, or laboratories. Hence an apparatus which, while not giving necessarily the calorimetric data, will give with great exactness the gaseous exchange and thus permit an approximation of the energy transformations, can be of great service to many laboratories and hospitals. It was planned, therefore, to attempt to so adjust this apparatus that, by taking the expired air from the mouth or nose, the carbon dioxide exhaled and the oxygen actually absorbed could be determined with great exactness. With this end in view, a small apparatus was constructed, using a rotary blower, sulphuric acid and soda lime purifying vessels, and supplying the oxygen either from a cylinder of the compressed gas or from the decomposition of sodium peroxide. The whole apparatus is placed upon one small portable table, with an electric motor directly belted to the blower. Thus the whole apparatus can be moved at will around the laboratory. In a hospital it can easily be taken to different wards and, indeed, to the bedside of a patient. This method, as outlined above, involves no gas analysis, and simply requires that the vessels absorbing the carbon dioxide and the oxygen cylinder should be weighed accurately.

Such an apparatus permits the use of almost any form of nasal tube, mouthpiece, mask, or, indeed, a hood covering the entire head. In practice, it has been found advisable in the large majority of cases to use special forms of nosepieces described beyond. Occasionally the mouthpiece and nose clip of Zuntz have been used.

The subject lying or sitting is fitted with the proper nose tubes, which are in turn connected to a three-way valve attached to the ventilating air pipe of the respiration apparatus.

The course of the ventilating air current is shown diagrammatically in Fig. 1. As the air leaves the lungs and passes into the constantly moving current of air, it is carried along by a rotary blower and forced through a suitable vessel containing strong sulphuric acid for removing the water vapor imparted to the air by the lungs. The air then leaves this absorber freed from water vapor but containing all the carbon dioxide. For the removal of this gas the air is caused to pass through finely divided soda lime, which absorbs the carbon dioxide with great rapidity. As has been found from a large number of experiments, it is desirable to have the soda lime somewhat moist,[15] and hence the water vapor taken up by the dry air as it passes through the soda lime is removed from the air by passing the air through another vessel containing sulphuric acid. The air is now dry and free from carbon dioxide, and if passed directly to the lungs would dry out the nasal and throat passages so quickly as to make respiration uncomfortable for the subject; hence it has been found advisable to moisten the air and thus render it respirable.

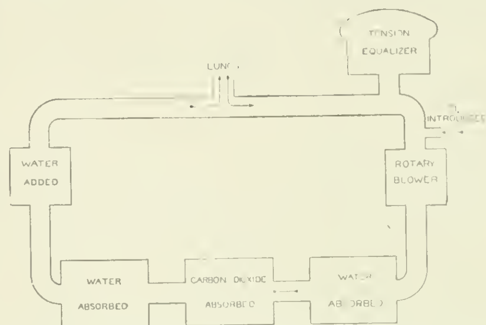


FIGURE 1.— Diagrammatic scheme of air circuit, and purifying arrangements.

Attached to the ventilating pipe near the point of entrance of the air into the lungs is a pan with a rubber diaphragm called a tension equalizer. Inasmuch as the whole circuit is closed, obviously the inspiration of air into the lungs would cause a decreased tension in the system which would seriously interfere with the respiration were not some proper provision made for keeping the tension always that of the atmosphere. By means of the rubber diaphragm, there is practically no difference between the external and internal tension; as the air is drawn into the lungs the rubber diaphragm sinks, and as the air is expelled from the lungs the diaphragm rises. The carbon dioxide formed by the process of oxidation in the body is absorbed by the soda lime, and conse-

[15] BENEDICT and TOWER: Journal of the American Chemical Society, 1899, xxi, p. 396.

quently, on the assumption that the same quantity of air is inspired as is expired, the total volume of air becomes diminished by the amount of oxygen used in the process of the combustion of both organic hydrogen and carbon. It is necessary, therefore, to admit oxygen to maintain the percentage of oxygen in the air approximately normal, and likewise to avoid a diminished tension on the system in case the oxygen used out of the system was greater in amount than the volume of the tension equalizer. With this apparatus, therefore, it is seen from the diagram that the man simply breathes into and out of a pipe through which a current of air, freed from carbonic acid but moistened to a comfortable degree of humidity, is constantly passing. Valves are not used.

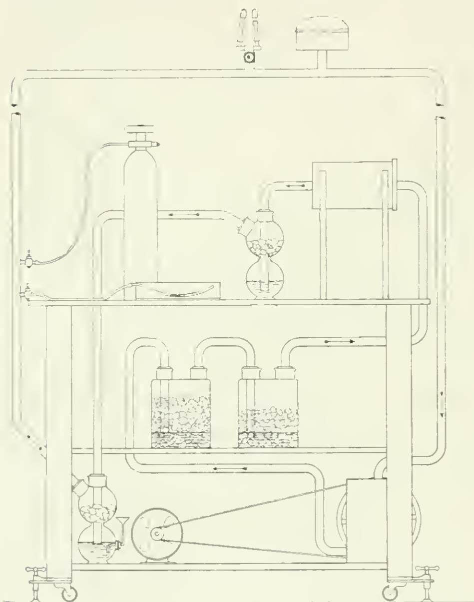


FIGURE 2. — Diagrammatic arrangement of respiration apparatus, showing nose tubes for breathing, tension equalizer, air-purifying apparatus, and oxygen cylinder.

The whole apparatus is mounted on a table. A somewhat diagrammatic representation is given in Fig. 2. The rotary blower driven by an electric motor is on the lower shelf of the table, and the air, leaving the lungs by means of the two nosepieces shown in the diagram, is sucked down by the blower and carried in a pipe beneath the lower shelf and introduced into two Wolff bottles containing pumice stone and sulphuric acid. The first of these absorbs a very large proportion of the moisture and the second is used for precaution. The dry air is then passed through the top of the table by a pipe and conducted to the can containing soda lime. It then enters into a glass vessel (as a matter of fact, the lower part of an ordinary Kipp generator is used) in the bottom of which is placed sulphuric acid and in the upper section some large pieces of pumice stone to prevent spattered acid from leaving

the vessel. The dry air freed from carbon dioxide is then conducted through a pipe into a second Kipp generator, which is, however, supplied with water and with pumice stone moistened with water. A small funnel attached to the side opening in the bottom of the generator permits the addition of water as fast as it evaporates. The moistened air then ascends through a tube to the point where it is respired by the subject. Two pet cocks in this pipe permit, the one the introduction of oxygen from the cylinder of compressed gas and the other the connection with a very delicate petroleum manometer.

Tension equalizer. — The tension equalizer consists of a rubber diaphragm fitted to a copper can 16 cm. in diameter and 9 cm. high. On opposite sides near the bottom of the can are soldered two ordinary hose couplings such as are used on garden hose with an internal diameter of 16 mm. The rubber diaphragm used in these experiments is of thin sheet rubber; we have found a ladies' pure rubber bathing cap to be especially serviceable and inexpensive for this purpose.

The large volume of air in this tension equalizer and the fact that the entrance and exit are opposite each other would make it possible for a direct passage of carbon-dioxide-laden air directly across the can with a minimum amount of circulation, and hence the after-ventilation to sweep out the carbon dioxide might be incomplete. Accordingly a semi-cylindrical piece of sheet copper is soldered to the can near the entrance coupling in such a way that the air striking this copper is deflected upward against the rubber diaphragm. This insures a thorough circulatory movement of the air inside the tension equalizer. The ordinary size of ladies' bathing cap permits considerable fluctuation of the volume of respiration, and consequently oxygen may be admitted at rather irregular periods. It is only necessary that the operator so adjust the supply of oxygen as to keep the bag from becoming either too much distended or too much flattened. To aid in minimizing the resistance of the movement of this rubber diaphragm, it is so placed that the open end of the tension equalizer is vertical and the diaphragm tends to hang down on the side. Thus the weight of the diaphragm is in large part taken up by the edge of the metal can. Furthermore, the tension equalizer is always pointed away from the subject, so that he cannot himself note the rise and fall of the diaphragm with each respiration.

Piping, hose, and couplings. — In the apparatus as here used, standard ½-inch pipe is used. This has an actual internal diameter of 15 mm. The rubber hose has an internal diameter of 19 mm., and the total length necessary for an apparatus of this type is approximately 2 meters. Since all of the work of maintaining the ventilating current of the air falls not upon the lungs of the subject but upon the rotary blower, it is obvious that if the distance between the three-way valve and the tension equalizer is not too great, the remaining piping may be of almost any length. Thus it is not out of the range of possibilities that the apparatus could be placed in one room of a hospital or clinic and pipes carried to another room. Since, however, the whole apparatus is very easily transported on the table, such a procedure would not be necessary except in rare instances.

Rotary blower. — The blower used in this apparatus is of the so-called positive type, that is, there is a movable piston on an eccentric shaft which forces the air around a circular chamber and out through an opening in the bottom. It has been described in detail elsewhere.[16] Pressures up to some 40 to 50 cm. of mercury are readily obtained with this blower, pressures far in excess of that demanded in work of this kind. A large wheel on the shaft of the blower is belted directly to the small electric motor, and by varying the resistances in the circuit the speed of the motor can be adjusted at will. The speed of the motor and the revolutions of the blower are so adjusted that the total amount of air passing through the ventilating pipe is not far from 35 litres per minute. To insure absence of leaks, the blower is immersed in oil; if a leak occurs at any time, it is made manifest instantly by the bubbling of air through the oil.

Drying apparatus. — The air current brings with it the water absorbed from the air moistener and a certain amount of water vapor from the lungs. Since, in the ordinary experiments on the respiratory exchange, no particular use is made of the determination of water, and as the carbon dioxide is determined by weight rather than by volume, it is necessary to dry the air before it enters the carbon-dioxide absorbers. The air leaving the blower passes through the two ordinary Wolff bottles. For the sake of convenience in cleaning, 3-neck Wolff bottles are commonly used. They

[16] Atwater and Benedict: Carnegie Institution of Washington, Publication No. 42, 1905, p. 18.

are filled with acid to a certain level marked on the bottle, and as the water is absorbed and the acid becomes diluted, experience has shown that a certain increase in volume indicates the time for the renewal of acid. The acid in the second Wolff bottle rarely has to be renewed. Two Wolff bottles, fitted with glass tubing 16 mm. internal diameter, permit the passage of 35 litres of air per minute, completely depriving it of moisture without difficulty.

Carbon dioxide absorber. — These absorbers are of very much the same type as those described for the large respiration chamber and are made of brass, silver-plated to resist the action of the alkali. They are 26 cm. long, 12 cm. in diameter, and at each end is a hose coupling of standard size, so that there is perfect interchangeability of all parts. Such a can, filled with soda lime, will absorb about 60 gm. of carbon dioxide without allowing any to pass.

Water absorber. — The dry air entering the soda lime can takes up in its passage through the can some of the moisture from the reagent, and since the amount of carbon dioxide absorbed is determined by weight, the amount of water removed from the can must be known accurately. In order to know how much water has been removed, the air passes through a Kipp generator containing strong sulphuric acid; the drying is accomplished by the bubbling of the gas once through the acid, but the pumice stone in the chamber above which is also drenched with sulphuric acid, aids materially in the removal of the last traces. It has been found that one of these Kipp generators will dry the air to the same degree of moisture content that it has when it leaves the Wolff bottles below. This apparatus is likewise provided with ordinary hose couplings.

Air moistener. — The dry air leaving the Kipp generator is moistened before being breathed again by passing it through a second Kipp generator with water. To this water is added a small amount of sodium bicarbonate to neutralize any possible acid fumes that may leave the Kipp generator. The passage of 35 litres of air through this water results in the saturation of the air to about 65 per cent, a degree of humidity that makes respiration very comfortable.

Manometer. — At the beginning of the experiment with the motor not in motion, the whole system is filled with air under a definite pressure. At the end of the experiment sufficient oxygen should be admitted to bring the pressure to what it was at first.

The most delicate form of manometer for this purpose that we have found is that used so successfully by Pettersson[17] on his gas

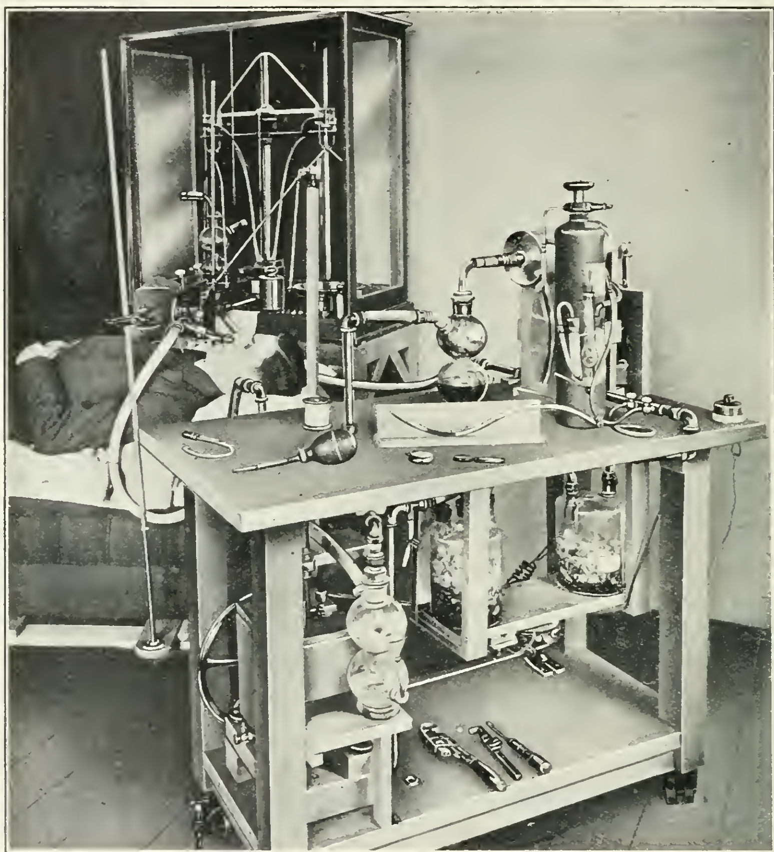


FIGURE 3. — General view of respiration apparatus in use. Rotary blower, electric motor, and two Wolff bottles on lower shelves. Air moistener in lower foreground. Carbon dioxide absorbing apparatus, oxygen cylinder, petroleum manometer, on top shelf. Nosepieces in position on subject. Tension equalizer, and three-way valve supported on cross rod attached to upright standards. Balance for weighing absorbers in rear. Specimen nosepiece and bulb for dilating on top of table.

analysis apparatus. It consists of a glass tube bent in the arc of a large circle and containing a short column of petroleum. The movement of this petroleum column along the arc of the circle for a few degrees is a very delicate measure of pressure and leaves little to be desired for an apparatus to be used as a manometer.

[17] PETTERSSON: Zeitschrift für analytische Chemie, 1886, xxv, p. 467.

Supply of oxygen. — Although steel cylinders of compressed oxygen weigh more than is desirable, the gas can be obtained readily and of a high degree of purity, and by use of the balances described beyond, it is possible to weigh the cylinders to a centigram. The gas as it leaves the cylinder contains a small amount of carbon dioxide, water vapor, and nitrogen. The apparatus has been described in detail elsewhere.[18] The general appearance of the cylinder can be seen in Fig. 3. The carbon dioxide is removed by a soda lime tube and the gas dried by sulphuric acid in a special form of glass tube. Both purifiers are attached by rubber bands to the oxygen cylinder and weighed with it. In order to prevent any sudden escape of gas through the tubes, a rubber bag is attached to the valve. This rubber bag should be deflated completely each time the cylinder is weighed, and care should be taken in admitting the gas to deflate the bag before the valve is finally closed. More recently we have obtained small cylinders weighing but 3 kilos and containing 143 litres of 97 per cent oxygen from the Linde Air Products Company of Buffalo. The gas is practically free from carbon dioxide and water, and purifying attachments are unnecessary. By means of a somewhat expensive reduction valve, the rubber bag may likewise be rejected. The oxygen ordinarily contains about 3 to 5 per cent of nitrogen, and a small correction on the weight of the gas is made, as is pointed out later.

While the increasing use of cylinders of compressed oxygen in medical practice makes this gas available in most places, it was thought advisable to attempt to substitute some form of portable oxygen generator that could be used with equal success. A great many experiments were made with a generator[19] supplying oxygen from sodium peroxide. The oxygen made by the interaction of sodium peroxide and water is remarkably pure, and the simplicity of the operation is such as to commend it for use.

The form of generator modified for use in these experiments is shown in Fig. 4. A metal bell, *A*, is submerged in a metal vessel containing water. A tin can containing fused sodium peroxide, sold under the trade name of oxone, is held in the bottom of the bell by two springs. Holes are punched through the top and

[18] Atwater and Benedict: *Loc. cit.*, p. 32.

[19] The generator is furnished by the Roessler & Hasslacher Chemical Co. of New York.

bottom of the can to allow the addition of water. On opening the valve *C*, which connects with a pipe screwed into the top of the bell, water rises inside the bell, comes in contact with the sodium peroxide, and generates oxygen. If the valve is closed, the oxygen generated forces the water down until it is beyond the reach of the sodium peroxide, and the generation of gas ceases. The gas thus formed is remarkably pure, containing only moisture. The whole apparatus can be weighed on the balances used for weighing the soda lime absorbers, and hence the amount of oxygen generated can best be noted by differences in weight.

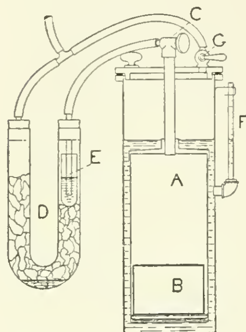


FIGURE 4.—Generator supplying oxygen from sodium peroxide. *A*, inner gas chamber; *B*, cartridge of sodium peroxide; *C*, needle valve; *D*, drying tube with pumice stone and sulphuric acid; *E*, tube with sulphuric acid for drying; *G*, valve communicating to interior of generator; *F*, water level tube.

In order to dry the issuing gas, a U-tube filled with pumice stone drenched with sulphuric acid is attached to the side of the cylinder by rubber bands. That the major portion of the drying may be accomplished and at the same time the rate of flow of gas indicated, the glass tube in one arm of the U-tube is caused to dip into concentrated sulphuric acid in a short piece of test tube. The gas bubbles through the acid, passes through the pumice stone, and escapes through a T-tube at the top. Occasionally, when the rate of flow is very rapid, the oxygen will be generated so fast as to force the water away from *B* so rapidly that some gas may even escape under the edge of the bell, consequently the valve *G* in the top of the apparatus is opened and connected directly with the T-tube. The top of the apparatus is, as a matter of fact, securely fastened by two thumb screws to a rubber gasket indicated in heavy black on the diagram. To assist in regulating the flow of gas, the level of the water in the metal cylinder is made visible by means of a small, arbitrarily graduated water gauge, *F*, attached to the outside of the cylinder. By means of this gauge one can tell exactly the level of the water at any time, and after a few preliminary trials the point to which water may be allowed to rise without danger of loss of oxygen is readily found.

In practice the U-tube is attached by rubber bands to the upper part of the cylinder.

One objection to this apparatus is the fact that during the action there is intense heat and the cylinder and liquid become very much heated. This interferes considerably with accurate weighing. It has been found practical to place the generator in a pan of cold water, allowing water to rise until it is just below the U-tube. Under these conditions the excessive heat is easily controlled, and by wiping off the cylinder carefully, the weight is accurately and quickly obtained. In the generation of the hot gas steam is apt to condense in the pipe at the top of the bell. This will occasionally collect in the fine needle valve, *C*, and make the flow of gas intermittent, and difficulties have been experienced in the use of the apparatus at just this point.

Wherever cylinders of compressed oxygen of suitable size and weight cannot be obtained, the above modified form of generator can be used with success. The cylinders of compressed gas are invariably to be recommended.

Balances. — The oxygen cylinders with the purifying attachments weigh some 8 to 10 kilos. Fortunately balances can easily be obtained in the market capable of weighing these cylinders to within 1 centigram. The balance has been described elsewhere.[20] They are furnished by most of the large supply houses. The balance here in use (shown in the background in Fig. 3) is of the size sold as having a carrying capacity of 10 kilograms in each pan. Usually, in placing the oxygen cylinders on the balance, it is necessary to have some form of hook to hold the cylinder in place. Since, however, differences rather than absolute weights are of value, this hook system can be left on the balance permanently.

Appliances for Breathing.

The successful use of this apparatus necessitates that the air should be breathed into and out of the ventilating air pipe without any escape of air into the room or without any entrance of room air to the lungs or the apparatus. This implies, then, that there should be a very close seal between the mouth or nose and

[20] Atwater and Benedict: Carnegie Institution of Washington, Publication No. 42, 1905, p. 57; Benedict and Milner: U. S. Dept. of Agriculture, Office of Experiment Stations, Bulletin 175, 1907, p. 20.

the ventilating air pipe. A very large number of experiments has been made in connection with this apparatus in which the Zuntz mouthpiece, the Tissot glass nosepieces, and several forms of masks have been used, and, as a result of all this experimenting, a form of nosepiece has been developed which gives by far the best results.

Nosepiece. — The difficulties experienced with the Tissot nosepiece have been serious, namely, the rigid glass tubes entering the nose must be forced into the nose with such great pressure that their use becomes very painful. Instead of relying upon the flexibility of the nose to adapt itself to the rigid glass surface, the attempt is made here to insert a nosepiece with an inflatable rubber cover in such a manner that the rubber could fit into the inequalities in the nasal orifice and thus produce tight closure. This result was obtained in a very simple way by preparing a nosepiece as shown in Fig. 5. A glass tube, 7 mm. internal diameter, serves to conduct the air into and out of the nose. A thin rubber finger cot, *B*, has a hole cut in the end, and the end is then slipped over the glass tube, *A*. This is well tied to the glass with white silk and shellacked. The rubber finger cot is then turned inside out and turned back over the glass tube, *A*, and tied about a rubber stopper, *C*. This rubber stopper has a small glass tube passing through it leading to the space between the rubber finger cot and the large glass tube. A rubber tube *D*, provided with a pinchcock, permits the introduction of air in the annular space between the glass and the rubber, thus allowing *B* to be inflated. After the inflation has proceeded to the proper point, the pinchcock is closed and the apparatus holds indefinitely. In use, the deflated apparatus is first inserted in the nose in the proper position; then, by using a syringe bulb, air is forced into the annular space until the desired tension is secured. As the rubber expands, it fills in perfectly the inequalities in the surface between the glass tube, *A*, and the nose. The second nasal tube is then inserted and inflated, and finally, if desired, the edge of the nose can be smeared with soapsuds and the subject can put pressure upon the tubes and test for any leak. When properly inserted, the tubes

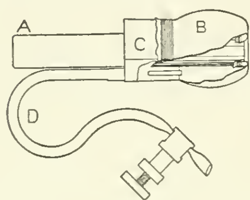


FIGURE 5.—Nose tube for respiration. *A*, glass tube connecting with respiration apparatus; *B*, rubber finger cot; *C*, rubber stopper; *D*, small rubber tube for dilating rubber cot.

cannot leak, and they may be worn an hour or more with perfect comfort. It has frequently happened with one of our subjects that he has slept through three or four experiments entirely unconscious of the presence of these tubes, thus showing that they cannot be very annoying or uncomfortable. The glass tube, *A*, is in turn connected by a short piece of rubber tubing with a metal tube attached to the three-way valve on the ventilating air pipe. In only those cases where nose breathing is impossible or difficult is the mouth apparatus recommended.

Occasionally it has been found advantageous to use the Zuntz mouthpiece and the noseclip to insure closure of the nose. Under these conditions it is necessary to secure tight closure around the mouth. The subject must not be allowed to fall asleep, as otherwise the muscles around the mouth will relax and a leak occur. The nose must be thoroughly tested for tightness.

When the nose tubes are used, it is necessary that the mouth be kept absolutely closed. It frequently happens that the noise of the blower produces a sense of drowsiness in the subject and he falls asleep. The lips may open slightly and leakage occur. It is occasionally necessary to insure that the mouth is kept closed by attaching a small piece of surgeon's plaster to the upper and lower lip, first having the subject draw the lips tightly together. With these precautions the subject can go to sleep and one can be sure that both mouth and nosepieces are perfectly tight.

Method of Use.

The subject, covered, if necessary, with a blanket, lies with the head in a comfortable position on a couch, and he should remain in this position for some minutes before the experiment begins. Too great stress cannot be laid upon the fact that the subject should be resting quietly, with the respirations normal and the pulse normal. After a slight physical exertion or any psychic excitement abnormal results are almost invariably found in the first of a series of experiments, due usually to the increased carbon dioxide exhaled unaccompanied by a corresponding intake of oxygen. During this preliminary period the nosepieces are properly adjusted and inflated.

The pulse can be taken as usual, but in most of the experiments it has been found of advantage to use a Bowles stethoscope with long air transmission through a rubber tube, so that the pulse can

be counted without the knowledge of the subject. While in most of these experiments a pneumograph placed about the trunk midway between the nipples and the umbilicus has been used, the respiration can be counted perfectly satisfactorily from the rise and fall of the chest wall.

Prior to the experiment proper, the subject lies quietly on the couch and breathes through the nose or mouth pieces into the three-way valve, one opening of which connects with the ventilating air pipe, the other open to the air. During the preliminary period he breathes through the side outlet and consequently is respiring ordinary air. Meanwhile the motor is started, and the air in the whole system is thoroughly mixed. The motor is then stopped and oxygen admitted from the cylinder until the rubber diaphragm is distended to such an extent that there is a slight positive, definitely known pressure on the system, as indicated by the petroleum manometer. At that point the oxygen supply is shut off, the manometer shut off, and the motor again started. The subject is carefully watched, and at the end of a normal expiration the three-way valve is suddenly turned by the operator, and the next inspiration consists of air removed from the ventilating air pipe.

In some of the earlier experiments attempts were made to have the subject personally throw the valve at the end of a normal expiration, but it was soon found that this could not be carried out satisfactorily, and hence the valve was so arranged as to be turned by the observer unknown to the subject. On the top of the valve is attached a socket wrench connected by brass rods with two universal joints to a hand wheel attached to the table (see Fig. 3). By turning this hand wheel the valve is thrown. It has been found that even subjects used to the apparatus are inclined to anticipate the moment of throwing the valve, and occasionally the respiration becomes abnormal.

In order to diminish the resistance as much as possible, the tension equalizer is placed as near the three-way valve as is convenient, *i. e.*, about 10 cm. from it. Under these conditions there is no noticeable variation in tension, whether the three-way valve is open to the air or open to the ventilating pipe.

The rate of ventilation is so adjusted that at no time is there any danger of the subject's breathing into the lungs air that has just been expired. For this purpose a number of experiments have

shown that a rate of 35 litres per minute is amply sufficient to take care of this point. In the first place, at the end of each expiration there is an instant's pause, during which time the last expired air is rapidly pushed along the tube, its place being taken by fresh, pure air. When the inspiration begins, this fresh air is carried to the lungs, and unless the rate of inhalation is more rapid than the flow of 35 litres per minute, obviously none of the vitiated air can be drawn back from the tension equalizer into the lungs.

During an experiment, as the oxygen is consumed out of the air and the rubber bag or the tension equalizer sinks more and more into the can, oxygen is supplied from the steel cylinder which has previously been weighed. This cylinder is not the one that has been used for filling the system to a constant tension before the experiment begins, as the preliminary introduction of oxygen need not be quantitatively known. Toward the end of the experiment (ten to twenty minutes), it is necessary to see that the rubber bag is not too much distended to permit the last expiration to take place without producing any tension on the rubber. The patient is then carefully watched, and at the end of a normal exhalation the valve again thrown, this time so that the opening to the air pipe is closed and the subject is breathing through the side outlet into the open air.

There is, then, in the system between the valve piece and the carbon dioxide absorber air which contains a large percentage of carbon dioxide. The current of ventilating air is maintained for some three or four minutes, during which time the system is thoroughly swept out, and at the end of which time there is no appreciable amount of carbon dioxide remaining. At the end of three minutes the motor is stopped and oxygen again admitted, this time from the weighed cylinder used during the experiment, until the petroleum manometer indicates the same tension on the system that was there at the beginning of the experiment.

In this apparatus, since for ordinary experiments the water expired from the lungs is of no particular value, it is unnecessary to weigh the two Wolff bottles, and only the soda-lime can and the Kipp generator containing sulphuric acid are weighed. The loss in weight of the oxygen cylinder is also carefully recorded.

In an experiment of but ten minutes' duration the carbon dioxide excretion is rarely below 3 gm; consequently, if the carbon dioxide absorbing vessel is weighed to within 0.03 gm., the error is but 1 per cent. Similarly the amount of oxygen absorbed is rarely

less than 3 gm., and an error of weighing of 0.03 gm. again involves an error of but 1 per cent. As a matter of fact, with balances which can be easily obtained and at small cost, it is possible to weigh these absorbing vessels rapidly to 0.01 gm., and hence the errors in weighing may practically be neglected.

Calculation of results. — While the amount of carbon dioxide absorbed by the soda lime is usually greater than the amount of water given up to the dry air as it passes through this can, it may happen that an actual loss in the weight of the soda lime can occurs simultaneously with a large gain in the acid vessel. Obviously the algebraic sum of these variations in weight represents directly the amount of carbon dioxide exhaled during the experiment.

The volume is found by multiplying the weight in grams by the factor 0.509.

If it were possible to admit absolutely pure oxygen, the calculations for oxygen would be equally simple. Where the sodium peroxide generator is used, this corresponds to the case, although marked variations in the level of the water inside of the generator should be taken into consideration.

With cylinders of compressed oxygen, however, the oxygen is by no means pure. It may at times contain but 90 per cent of oxygen, and hence apparently a large correction should be made. A close examination of the figures, however, shows that the correction is by no means as great as one would at first sight think. What is measured in this apparatus is the amount of oxygen required to replace an equal volume absorbed by the subject. The amount of oxygen admitted does not represent pure oxygen, but oxygen plus some nitrogen. For each litre of nitrogen admitted there would be a loss in weight of the cylinder amounting to but 1.26 gm. instead of 1.43 had the gas been pure oxygen. As a matter of fact, it has been found by testing that the correction to be applied with a cylinder containing, for example, 97 per cent of oxygen is about 0.4 per cent. Thus the loss in weight of the cylinder should be increased by 0.4 per cent to give the true loss in weight had the cylinder contained pure oxygen. In many experiments this slight correction may be neglected.

To find the volume of oxygen absorbed by the man during an experiment, the weight is multiplied by the factor 0.7. The respiratory quotient is the volume of carbon dioxide divided by the volume of oxygen.

Criticism of New Method.

In manipulating the whole system in the new method, it is assumed that the temperature of the system is absolutely the same at the beginning and the end of each experiment. Several possible sources of error may creep in here. As carbon dioxide is absorbed by the soda lime, and as water is absorbed by the sulphuric acid, there is considerable liberation of heat due to the chemical reaction. This results in the warming of the air in the spaces between the soda lime or the air above the acid, and consequently tends to increase somewhat the total volume inside of the system. This would decrease the oxygen introduced. The experimenter in moving about the apparatus, the subject in breathing into the tubes, would, especially during the first experiment of a series, tend to warm the system slightly. On the other hand, as the dry air leaving the Kipp generator passes through the moistening chamber, there is an evaporation of water, cooling the water in the generator and consequently the air above it. Thus the rise in temperature developed by the heat of reaction of the carbon dioxide and soda-lime and the water in the sulphuric acid is partly compensated by the contraction of the air due to the cooling of the air above the water. This is particularly the case in the first experiment of the series, where the water in the moistener may be at room temperature and is subsequently considerably lower.

Another possible source of error is the assumption that there is a constant degree of humidity in the air after it leaves the moistener. At the beginning of the experiment, before the valve is thrown, air is circulated through the system, and all the air leaving the moistener has a water content which by experiment has been found to be not far from 65 per cent. The amount of water evaporated into the air current as it passes through the Kipp generator will depend upon two factors, — first, the speed of the ventilating circuit, and, second, the temperature of the water in the generator. Consequently, if there are fluctuations in either of these, there may be variations in the amount of moisture in the air current. As a matter of fact, the fluctuations in the rapidity of ventilation are for the most part, rather small, and, in the second place, after the first experiment of the series, the temperature of the water in the moistener remains relatively constant. These errors are in large

part avoided by running the apparatus for several minutes before an actual experiment begins.

Another source of error lies in the fact that it is assumed that the barometric conditions remain constant throughout the experiment. As most of the experiments do not last over twenty minutes, it is to be doubted, however, whether there will be any material variation in barometric pressure during this time. The total volume of air, including that in the distended tension equalizer, is not far from 8000 c.c. A change in the barometer of 1 mm. would be equivalent to $1/760 \times 8000$, or about 10 c.c., corresponding to about 0.014 gm. of oxygen. This is, therefore, the possible maximum error on this apparatus. Obviously the error would be plus or minus, depending upon whether the barometer rose or fell. For the strictest accuracy, therefore, one should note the barometer at the beginning and end of each period.

Influence of variations in the residual air. — This method involves the assumption that the same quantity of air remains in the lungs at the end of each experiment that was present at the beginning. A large number of tests have seemed to indicate that with normal subjects lying quietly on a sofa with quiet respiration, this is the case.

Obviously with this method of studying the respiratory exchange, any errors involved in the change of residual air would affect noticeably the oxygen determination, and it is here that one finds the weakest point of the whole system. While a few preliminary experiments have been made on the use of the forced expiration as the moment to throw the valves, as a rule it has been found that the normal quiet respiration with the subjects lying on a bed can be so readily judged by the assistant that the throwing of the valves is quite a simple matter, and the inequalities in the volume of the residual air in the lungs are apparently so small as not to affect the determination of the respiratory quotient.

Influence of a leak on total metabolism. — In any of the methods now in use, including that here suggested, if there is a leak around the mouth or nose, the results are affected. Thus, in Zuntz's apparatus, while a leak would not influence perceptibly the composition of the expired air and consequently the respiratory quotient, obviously the total volume of expired air might be considerably affected. With the apparatus here described, the slightest leak influences enormously the determination of oxygen. On the other

hand, a small leak is without appreciable effect in the determination of the carbon dioxide production.

Disadvantages of the New Apparatus.

The type of apparatus described here has certain disadvantages that must obviously be taken into consideration. In the first place, it is impossible to make duplicate analyses. With the Zuntz method and the spirometer method, analyses can be made almost *ad libitum.* The new method, therefore, like the method of Hanriot and Richet,[21] must be classed with those that do not permit duplicate analyses. On the contrary, the rapidity with which these determinations can be made leaves very little necessity for duplicate analyses. The duplicate analyses have value only for insuring the accuracy of the composition of the air, and there is no method of securing duplicate determinations of the total volume of air expired in any given experiments. Hence this method fundamentally has no greater disadvantage in studying the total metabolism than has any other method involving a measure of the total products of respiration.

Second, the method likewise does not permit any measurement of the total ventilation of the lungs per minute or of volume of each expiration. As yet these values have not been of great physiological significance, especially when determined in connection with respiratory exchange. They are of value in the Zuntz method, and would be of value in this method in indicating the normal respiration at the beginning of a series of experiments where the subject had not previously been used. It is seriously to be questioned, however, whether similar data of equal value cannot be obtained by means of the pneumograph and tambour. This method has been used in this laboratory with considerable success to indicate normal respirations at the beginning of the experimenting.

Third, it is obvious that this apparatus cannot be adapted for a portable type of apparatus, thus making it impossible for studying many problems which have been investigated by means of the Zuntz apparatus.

[21] Hanriot and Richet: Comptes rendus, 1881, civ, p. 435.

Control Experiments with Burning Ether.

Experience with the large respiration chambers has shown that it is absolutely necessary in studying the respiratory exchange to control the apparatus from time to time by some delicate chemical tests proving the correctness of the determinations of carbon dioxide and oxygen. It seemed desirable, in connection with the development of this apparatus, likewise to have a method of checking it exactly. In order to test the apparatus it is only necessary to insert a combustion chamber of special construction in the ventilating air pipe at about the point where the three-way valve is ordinarily attached. After many experiments with various forms of combustion chambers and various types of alcohol burners, it was found impossible to secure satisfactory results by burning alcohol. The final type of combustion chamber was devised to permit the burning of ether vapor. The apparatus is shown in Fig. 6 herewith.

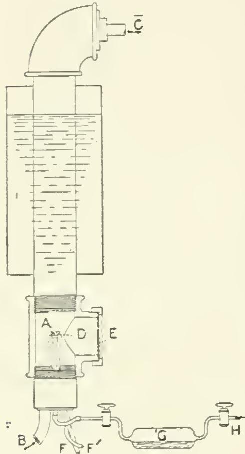


Figure 6.—Combustion chamber for control experiments with burning ether. *A*, combustion chamber; *B*, ingoing ventilating air current; *C*, outgoing air current; *D*, burner; *E*, glass window; *F*, *F'*, high tension sparking current lead wires; *G*, container for ether; *H*, supply of air under pressure; *I*, water cooler.

It consists of a large metal tee, *A* (standard 2-inch). Into this is screwed an upright piece of pipe which is surrounded by a tin water jacket, *I*. On the top an elbow is attached, into which a pipe, *C*, is screwed. In the bottom of the tee, *A*, is screwed a short piece of pipe having a rubber stopper in it, through which are passed, first, a rubber tube, *B*, through which the ventilating current of air passes, then a small brass pipe to which is attached a burner, and finally two electric wires, *F* and *F'*. A glass plate, *E*, permits a careful inspection of the flame. Ether is supplied from a glass vessel, *G*, which is, as a matter of fact, an ordinary so-called sulphur dioxide condensing tube. A current of air, entering the ether tube at *H*, passes over the ether, becomes saturated with ether vapor, enters the combustion chamber, and issues from the jet on the burner, *D*. By passing a high tension current through the wires

F and F', a spark is caused to jump across the gap just above the burner D, thus igniting the ether vapor. The excessive heat developed by the burning of the ether is absorbed readily by the water in I, and the gas issues at C, practically at room temperature.

In order to maintain a constant flame, a steady pressure of air must be obtained, and this is secured by inserting a T-tube between the blower and the first sulphuric acid Wolff bottle. A small supply of air taken from this point suffices to carry the ether vapor into the combustion chamber. In the test the vessel G is weighed before and after the experiment, and the amount of ether vaporized accurately known. At the end of the experiment the supply of ether vapor is shut off and the ventilating air current allowed to run for several minutes to sweep out the carbon dioxide already formed and to allow the whole system to acquire room temperature. At the end of that time the oxygen supply is added until the manometer indicates the same tension as at the start. The apparatus is then disconnected, and the soda lime vessel, the sulphuric acid water absorber, and the oxygen cylinder weighed. From these data it is possible to compute, first, the amount of carbon dioxide produced in the combustion of a given amount of ether; second, the amount of oxygen required to oxidize a given weight of ether; and, third, the ratio of carbon dioxide formed to oxygen absorbed in burning the ether. In certain experiments it was somewhat difficult to regulate the flame so as to secure perfect combustion, and occasionally unburned ether passed through the system. Under such circumstances, therefore, the amount of carbon dioxide actually absorbed was not so great as would be expected from the loss in weight of the ether vessel, but since the ether vapor thus unburned was immediately absorbed by the sulphuric acid in the absorbing vessels, it did not influence in any way the ratio of the carbon dioxide formed, and the oxygen absorbed of such ether as was burned, and consequently the respiratory quotient, so to speak, of ether was almost always found to correspond with the theoretical, irrespective of the absolute amount of ether burned. It was possible in most experiments to adjust the flame of the burner so as to have perfect combustion.

A typical test is given herewith. The experiment lasted fifteen minutes.

Ether Evaporated.

	Found.	Required.
Carbon dioxide	11.62 gm.	11.71 gm.
Oxygen	12.78 gm.	12.78 gm.
Respiratory quotient $\frac{CO_2}{O_2}$.	0.662	0.666

Physiological control. — While there may be reasonable questioning as to the true value of the respiratory quotient determined under conditions necessary with this or any other form of apparatus involving artificial breathing, it was possible to control the experiments with this particular form of apparatus by determining the respiratory quotient in a respiration chamber in this laboratory. Consequently it was possible to compare directly the respiratory quotient as determined by the new apparatus and that determined in a respiratory chamber where the subject was lying quietly and breathing normally. Several such comparisons have been made, and but one need be here given.

Thus, three consecutive ten-minute experiments on the new apparatus two hours after breakfast indicated a respiratory quotient of 0.87, and immediately following this, two one-hour periods in the large chamber gave values 0.89 and 0.90. Several hours later the values found with the new apparatus were 0.78, 0.82, and 0.79, and immediately following this, two consecutive one-hour periods in the respiration chamber, 0.77 and 0.76. While variations were found in the total metabolism during these periods, since in one case the subject was breathing through nosepieces and was in the laboratory, surrounded by a number of observers, and in the other case he was lying quietly in the chamber, the results show that there is nothing abnormal in the measurements as obtained with this type of apparatus.

In the development of this apparatus I have been much indebted to Mr. F. P. Fletcher, who was especially concerned with the apparatus for control with burning ether; Mr. J. A. Riche, who has conducted the greater number of experiments with men, and to Mr. T. M. Carpenter, who has personally supervised many of the details of construction and testing.

CAN FUNCTIONAL UNION BE RE-ESTABLISHED BETWEEN THE MAMMALIAN AURICLES AND VENTRICLES AFTER DESTRUCTION OF A SEGMENT OF THE AURICULO-VENTRICULAR BUNDLE?

By JOSEPH ERLANGER

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

(With a Histological Study by W. S. MILLER.)

THE sole functional connection between the auricles and ventricles of the mammalian heart, the auriculo-ventricular bundle, is composed of a tissue which in many ways resembles, although it is not identical with, heart tissue in general. When the continuity of this structure is completely interrupted in any way whatever, complete auriculo-ventricular heart block is the result, and this is permanent.[1] In other words, regeneration of the severed auriculo-ventricular bundle to the extent that it may become capable of again functioning does not occur. May we then be justified in concluding from this result that heart tissue in general does not regenerate?[2] This question is of the greatest practical signifi-

[1] Some new evidence bearing upon these subjects, as well as references to the literature, will be found in a paper by the author soon to appear in the Journal of Experimental Medicine.

[2] So far as the author is aware, the question of regeneration of heart muscle has been studied by histological methods only. The prevailing opinion seems to be (Thorel, in Lubarsch and Ostertag's Ergebnisse, 1903, ix, p. 861), that wounds of the heart muscle heal by the formation of scar tissue. The muscle cells show little if any tendency to regenerate. No effort seemingly has been made to determine if there is any restoration of conductivity across a healed wound. Bernstein's experiment (functional isolation of the tip of the frog's ventricle by means of a crush) throws little or no light upon this question because the period of survival probably does not suffice for the completion of regenerative processes. According to Tigerstedt (Physiologie des Kreislaufes, 1893, p. 157), the longest survival is recorded by Aubert, who has succeeded in keeping a frog alive for six weeks after performing Bernstein's operation. Another serious objection to the use of Bernstein's experiment for the elucidation of this question is mentioned on p. 378.

cance, since, if it can be shown that functional union of heart tissue never occurs, then must we admit the impossibility of re-establishing by operative interference, in cases of heart block due to destruction of the auriculo-ventricular bundle, a connection between the auricles and ventricles which would restore the normal sequence of heart beat.

The experiments which demonstrate that the auriculo-ventricular bundle, or a segment thereof, once destroyed does not regenerate, cannot be considered as conclusively proving the impossibility of establishing experimentally a functional union between the auricles and ventricles at some other place. The auriculo-ventricular bundle, it should be borne in mind in this connection, is a long and slender structure encased in connective tissue which in some places is particularly dense. Might not these relations of the auriculo-ventricular bundle interfere in some way with regenerative processes which otherwise might possibly proceed to complete restoration of function? Might it not be possible, it may be further argued, to unite auricles to ventricles by an operative procedure in such a way that regenerative processes can occur?

The Union of Auricles to Ventricles.

Stimulated by this line of thought, the making of a functional connection was attempted on several of the animals in which at the same time the effort was made to produce auriculo-ventricular heart block by damaging the auriculo-ventricular bundle. Auriculo-ventricular heart block was produced at the same time because it was thought that functional union of the coapted surfaces of the auricles and ventricles might be facilitated by the absence of any functioning connection between these chambers.

Methods. — The method of procedure was about as follows: After having exposed the heart and after having apparently successfully damaged the auriculo-ventricular bundle, a part of the contiguous surfaces of the right auricle and right ventricle were denuded of epicardium and fat, and, after all hæmorrhage had ceased, the denuded areas were carefully approximated with fine silk sutures. The operation is a difficult one, particularly the denudation of the auricle. This structure is so thin that there is great danger of opening it while stripping it of its epicardium. So difficult was the

operation that it was rarely successful, and in the most favorable of our cases could only a few square millimetres of the auricle be thus laid bare. Dogs alone were used. They were anæsthetized with morphine and ether.

Results. — The experiment was attempted four times. Two of the animals, Nos. 1 and 2,[3] died a few hours after the operation. Another, No. 4, survived the operation, but recovered of the block in the course of a day or two. Dog No. 3, however, survived the operation with a relatively complete heart block, from which it recovered completely in the course of twenty-six days. Evidently recovery in this case could have been due either to the successful operative union of the auricle to the ventricle or to a restoration of conductivity in the auriculo-ventricular bundle. For the purpose of deciding this question the heart was exposed on the sixty-first day, and, while recording the movements of the auricles and ventricles, the sutured area was crushed in a mass ligature. The heart beat remained normal. Complete heart block was then re-established by crushing the auriculo-ventricular bundle. The recovery was therefore due to a complete restoration of conductivity in the auriculo-ventricular bundle, which, as is discussed in another place, had escaped destruction at the time of the first operation.

The Regenerative Capabilities of Auricular Tissue.

Owing to the difficulties in the way of a successful experiment of the kind above described, we thought it might be well to test the question of functional regeneration of cardiac tissue under even more favorable conditions. This was done as follows:

Methods. — The auricular appendage was drawn through a clamp so made that with it the appendage could be crushed along a line parallel to, and near its base and extending from one edge of the appendage, either anterior or posterior, to points more than half-way across the ventral and dorsal surfaces. Immediately thereafter ligatures were laid to mark the apex of the appendage and the line of crush. For the latter purpose three ligatures were tied, namely, one at the edge of the auricle crushed by the clamp, and one each on the ventral and dorsal surfaces to mark the other ends of the line of crush. The wounds were then closed and the animals allowed

[3] See paper soon to appear in the Journal of Experimental Medicine.

to live. The general technique of the operation was similar to that employed in producing chronic auriculo-ventricular heart block.

By crushing only part way across the base of the appendage the appendicular muscle was left in a position to respond to cardiac impulses. In this way any danger of atrophy from disuse was obviated, a danger which otherwise would have defeated the object of the experiment. The failure to take this precaution renders valueless in this connection Bernstein's experiment, which otherwise might be considered as conclusively proving the impossibility of functional regeneration in the case of the frog's ventricle.

Results. — Two kittens thus operated upon died within a few hours. Three dogs experimented upon in the same way survived the operation. One of them, however, died some months later of causes not related to the subject in hand. Two of the animals, Nos. 3 and 5, lived for the final test, which was made on the one hundred and thirty-second and two hundred and sixty-eighth days, respectively.

At the final test the heart was exposed and, after having located the ligatures in the auricle, arrangements were made for stimulating the apex by the unipolar method and for recording the contractions of the body of the auricle by the method of air transmission.

It was found that stimuli applied at any point on the surface of the auricular appendage resulted in irregularities in the beat of the body of the auricle. Then the auricular tissue was crushed in a clamp along a line perpendicular to, and intersecting the base of, the appendage at about the middle points of the ventral and dorsal surfaces. This line met the edge of the auricles a little to one side or the other of the apex of the appendage and presumably intersected the old line of crush on the surfaces of the appendage, the ligatures laid at the time of the first operation to mark the latter line serving as guides in this connection. By this operation the auricular appendage was divided for our purposes into two areas, namely, one presumably completely isolated from the rest of the heart by the old and new lines of crush, which area, for the sake of convenience, we shall designate area *Y*; the other, termed area *X*, lying to the opposite side of the new line of crush and presumably still connected functionally with the rest of the heart at the base of the auricular appendage where the tissue had not been crushed at the first operation. Then each area in turn was stimulated tetanically, the stimulating electrode being applied to one or the other at different points but as far as possible from the old line of crush.

The results of the tests were as follows: In the case of Experiment III the auricle became irregular wherever in either area the stimulus was applied. Therefore neither area was completely isolated. For the purpose of demonstrating that the new line of crush was thorough and that the impulse was probably passing through the line as marked by the ligatures, the auricular tissue was crushed along a line parallel and immediately distal to the latter. Now the stimulus applied to area *Y* no longer elicited irregularities of the parts of the heart lying without it.

In the case of Experiment V a stimulus of considerably more than minimal density when applied to area *X* invariably caused the auricle to become irregular, whereas no irregularities whatever were recorded when the stimulus was applied to area *Y*. Area *Y*, evidently, was completely isolated.[4]

Discussion. — The results of these two experiments are therefore apparently contradictory. It would seem at first sight that in Experiment III functional regeneration had occurred over the old line of crush, whereas in Experiment V functional union had failed to take place. A decision as to the results of which experiment are to be considered as conclusive can be made only by taking into consideration the circumstances attendant upon each of the experiments. It should first be made clear that the difficulties in the way of a successful operation were very great. In the first place, we could not be sure as to the exact limits of the original line of crush. Although the marking ligatures were placed as carefully as possible at the ends of the line of crush where seen immediately after removing the clamp, it is possible that the auricular tissue may not have been thoroughly crushed quite up to that point; indeed, that some of the tissue included in the grasp of the clamp may have escaped thorough disintegration. Any one who has attempted to destroy the continuity of the auriculo-ventricular bundle by compression is in a position to appreciate the difficulties in the way of thoroughly and permanently destroying the functional continuity of two regions of the heart. We therefore cannot be certain that area *Y* was completely bounded by tissue that had been thoroughly destroyed. An experiment which shows that there is no functional

[4] The histological study by Dr. W. S. Miller of the material obtained from Experiment V is appended to this paper. It is to be regretted that the material from Experiment III could not be utilized for this study. The series of sections obtained from Experiment V, however, leaves nothing to be desired.

union over a line of crush is consequently of far greater significance than one that shows the existence of functional union. In the latter case functional union may never have been interrupted, although the possibility must be admitted that it may have been formed anew; whereas in the case of the former there is no such alternative.

When, in connection with these considerations, it is recalled that Experiment V was the last of our series and was performed under the very best of conditions, and that at the time we were perfectly certain of our procedures, whereas this was not so in the case of Experiment III, which was the first made upon the dog as subject, we have not the slightest hesitancy in accepting the results of Experiment V as conclusive.

Furthermore the histological study of Experiment V substantiates this conclusion in a most satisfactory manner. It shows that all muscular connection of area *Y* with the surrounding parts of the heart had been completely severed, whereas area *X* was still in connection with the body of the auricle through a band of uninjured muscular tissue which in the natural state was probably more than 0.7 mm. wide.

It is of the greatest interest that, although there was no muscular connection between area *Y* and the body of the auricle, these parts were none the less connected by several nerve trunks which either had escaped destruction at the time of the first operation or had grown across the old scar. Despite this nervous connection, impulses started in area *Y* were not conducted to the parts of the heart below. Evidently the cardiac excitation wave passes through myocardium and not through nerve trunks. The myocardium may for our purposes be considered as consisting of muscle and of the nerve plexus intimately associated therewith. There consequently remained open to the cardiac excitation wave, as in the normal heart, two possible paths of exit from area *X*. Assuming that the excitation wave normally takes but one of these paths, it evidently still remains to be determined which is the one. Our experiments throw but little light upon the question. The heart tissue was so prepared for histological examination that the course of the intrinsic nerve plexus could not be traced. If we may however assume that the nerve plexus, just as nerve trunks, can grow across a scar, then must area *Y* have been in connection with the heart through the plexus. Admitting this, we are led to the conclusion that the cardiac excitation wave is conducted through muscle fibres only.

We conclude therefore:

1. That functional union cannot be re-established between two parts of the heart whose functional continuity has been completely severed by thorough destruction of the connecting heart tissue. There is not the slightest hope of relieving heart block thus caused either by surgical interference or by the operation of natural regenerative processes.[5]

2. That the cardiac excitation wave is not conducted through the auricle in nerve trunks located in its tissue.

3. That the cardiac impulse is conducted through muscle fibres rather than through nerve fibres. Since heart tissue, as we understand it, is composed of muscle and nerve plexus, and since nerve fibres ordinarily regenerate and thus re-establish their former connections, these experiments would seem to support the myogenic theory of the heart beat. This conclusion is stated with some reserve, however, since our knowledge of regenerative processes in nerve plexuses is at present in a very unsatisfactory state.

HISTOLOGICAL STUDY.

By W. S. MILLER.

Method. — Fixed in Tellyesnicky's fluid; imbedded in celloidin; cut serially by a modification of Obregia's method in 478 sections μ 40 thick; stained with hæmatoxylin and eosin. The entire auricle was not sectioned; only a small portion, however, was left out of the series. That this was a negligible quantity may be seen by reference to Fig. 1, in which the line of the first crush, that of the second crush, and the plane of section are indicated.

First crush. — The first evidence of destruction of heart muscle is seen in section 102 and is situated at the outer rounded edge of the section. The destruction of the muscle is complete (Fig. 2). In section 124 the line of crush has moved around to the ventral

[5] We do not by this mean to imply that a bundle made functionally insufficient by some process that does not completely destroy its anatomical continuity, such, for example, as compression, infiltration, or recoverable degenerations, cannot again resume its function with the return of conditions to the normal. Indeed there are already on record instances of recovery from complete auriculo-ventricular heart block due probably to the relief from compression exerted upon the bundle from without.

side of the auricle while the opposite side is intact (Fig. 3). In section 138 a double line of crush is first noticed; that is, scar tissue

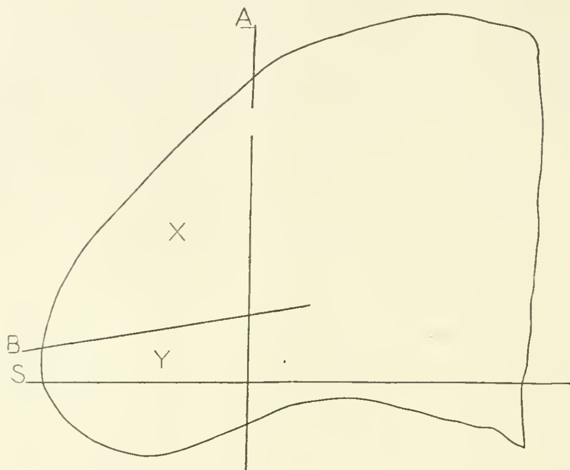


FIGURE 1. — Outline of portion of auricle sectioned. *A*, line of first crush; *B*, line of second crush; *S*, plane of section; the sections were cut as far as the crest of the convex base line. The break in line *A* indicates approximately the band of intact heart muscle. *X*, *Y*, areas mentioned in text.

is found on both sides of the section with intact muscle between (Fig. 4). From this point the two lines of scar tissue move

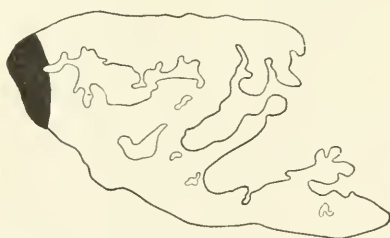


FIGURE 2. — In this and the following figures the crushed heart muscle is indicated in solid black. Figures 2, 3, 4, and 5 are camera lucida tracings of the sections named in the text.

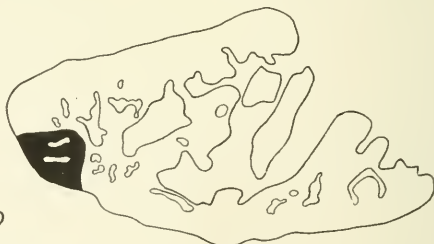


FIGURE 3.

farther apart, and through the remainder of the series each side of the section shows scar tissue 0.9 mm. in width extending through the entire wall of the auricle. No heart muscle crosses these two lines of crush which come to lie nearly opposite each other, that of

the ventral side being always situated slightly in advance of the dorsal side (Fig. 5, section 259).

Second crush. — It is somewhat difficult to locate the exact line of the second crush owing to the amount of the extravasated blood, but if the line of greatest injury be taken it can be stated that the line extends obliquely across the auricle from section 333 to section 276, where the line of the second crush crosses the old scar of the first crush.

FIGURE 4.

There is therefore an irregularly triangular area (area X) which is completely isolated from the rest of the auricle except for a narrow band, 0.56 mm. in width, situated on the dorsal side of the auricle.

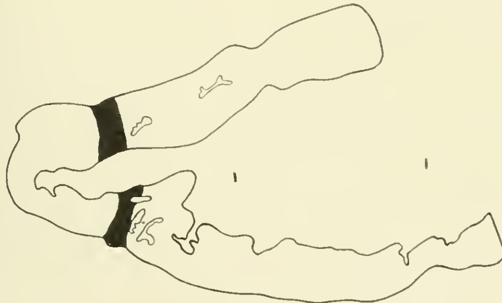


FIGURE 5.

In some places nerves can be traced across the line of the old crush; they are however found in area Y, that is, outside of the triangular area described above. In some sections the heart muscle appears to be regenerating; but whether this appearance is due to an actual new growth or recovery of some individual muscle elements which were injured at the time of the operation I am unable to determine. Wherever they are found they always lie along the old scar, but never penetrate into it.

PSEUDO-FATIGUE OF THE SPINAL CORD.

By FREDERIC S. LEE and SUMNER EVERINGHAM.

[*From the Department of Physiology of Columbia University, at the College of Physicians and Surgeons, New York.*]

IT is to be regretted that comparatively little is known of the phenomenon of fatigue in the central nervous system. Verworn's[1] demonstration of the significance of oxygen and carbon dioxide in the activity of the spinal cord is valuable, although the employment of strychnin therein might not be universally approved. The belief seems to be still prevalent that the brain and spinal cord are more susceptible to fatigue than are the parts of the organism lying outside of them. Mosso, Lombard, and Waller have contributed experimental evidence in advocacy of this idea. Against it we find results obtained by Kraepelin, G. E. Müller, Henri, R. Müller, Hough, Woodworth, Storey, and Joteyko. In his "Introduction to Human Physiology" Waller[2] makes the following statement: "If a series of induction shocks is applied to a frog's brain and bulb until the gastrocnemius has ceased to respond, a second series of contractions may be elicited by switching the current to a pair of electrodes applied to the sciatic nerve; if, when the muscle has ceased to respond to this excitation, the current is switched to electrodes applied to the muscle, a third series of contractions is obtained; from which we learn that maximum action of the superior organ does not elicit maximum action of the subordinate organ — in other words, that central fatigue is limitative of peripheral fatigue — and we may formulate, as a probable conclusion, that the incidence of normal voluntary fatigue is in diminishing gravity from centre to periphery, — relatively greatest at the former, relatively least at the latter." The present paper presents certain results of an attempt to study the fatigue of the central nervous system by direct stimulation of it.

[1] VERWORN: Archiv für Physiologie, Supplementband, 1900.

[2] WALLER: An introduction to human physiology, London, 1891, p. 551.

We have experimented with both frogs and turtles during the months of October to March inclusive. Although we have worked with great care, we have found direct stimulation of parts of the brain, such as the crura cerebri or the medulla, to result in only meagre contractions of the muscles of the fore legs, and no contractions of those of the hind legs. This has been the case, whatever the strength of stimulus and even when the circulation of the blood posterior to the cerebrum has not in any way been interfered with.

With the spinal cord it is different, and most of our work has been on this structure. In both frogs and turtles it is easy to stimulate directly the spinal cord and obtain reflex contractions of a single muscle, the nerves to all other muscles being cut. We have used for a stimulus in some cases a rapidly interrupted induction current, but in most experiments a series of single induction shocks. Sometimes we have used platinum electrodes, which were placed in close contact with and across the ventral surface of the cord, so as to enable the current to reach the motor tracts readily. At other times non-polarizable brush electrodes have been employed, the brushes being sufficiently long to enable them to be tied around the cord. All contractions have been recorded upon a slowly moving drum by means of an isotonic lever.

With the frog our custom has been, after destroying the brain, to prepare both gastrocnemius muscles for the graphic record of their contractions, to cut one sciatic nerve near the spinal cord, and carefully to expose the cord. The same stimuli were then applied simultaneously to the dorsal cord and to the cut sciatic nerve, exciting one gastrocnemius through descending tracts, its motor centre and nerve, and the other through its nerve only. Simultaneous records of the contractions of the two muscles were made. Sometimes we found that the muscle stimulated through the cord ceased to respond before its opposite ceased, but sometimes the perplexing reverse occurred. Contractions resulting from cord stimulation were usually not so great as those resulting from sciatic stimulation, which also was perplexing. Sometimes our object was defeated by an escape of the stimulating current from the cord into the nerve. Attempts to discover how far polarization occurred were frustrated by the short length of the cord. It was difficult to maintain, as fully as we wished, the circulation of the blood through the cord. We found it impossible also to obtain a systematic and continued series of reflex contractions from stimulation of the skin, which we very

much desired for comparison. In brief, we found the frog's spinal cord an unsatisfactory object for our purposes. We therefore turned to the turtle.

It was when we undertook experiments on this species that strong suspicions were aroused of the impossibility of fatiguing the spinal

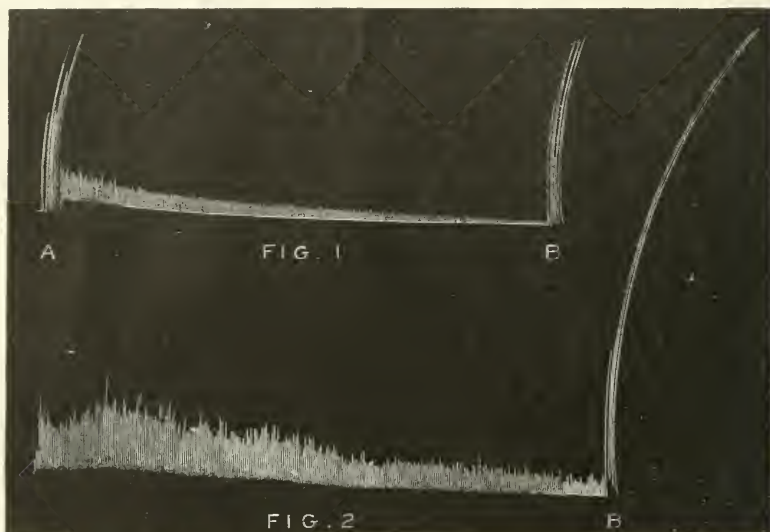


FIGURE 1.— Record of contractions of the ischio-caudali-tibialis (semimembranosus) of the turtle. To avoid shock the brain was destroyed twenty-four hours previously. *A* and *B* are reflex contractions of the muscle resulting from a mechanical stimulation, by pinching, of the skin in the region of the ischium. Between *A* and *B* is a series of reflex contractions resulting from electrical stimulation of the dorsal spinal cord. Break shocks, 16 per minute; secondary coil at 11.5 cm. with one Grove cell.

FIGURE 2. — Record of contractions of the ischio-caudali-tibialis of the turtle. To avoid shock the brain was destroyed two hours previously. The blood supply of the spinal cord was left intact. The record begins with a series of reflex contractions resulting from electrical stimulation of the dorsal spinal cord. Break shocks, 12 per minute; secondary coil at 12 cm. with one Grove cell. After "fatigue" appears to be well marked, at *B*, the skin was pinched near the ischium, and the resulting reflex contractions of the muscle were obtained.

cord by direct stimulation. It is possible in this species, as in the frog, to stimulate the cord above the sciatic centre and obtain contractions of an isolated muscle supplied by that nerve. The amplitude of the contractions is not great, however, and they cease comparatively early. Hence the total amount of work performed by the muscle is little. This is the more striking when the record of such an experiment is compared with that of the same muscle when acting

under the reflex influence of a stimulation of the skin. Records of typical experiments of this nature are shown in Figs. 1 and 2.

In both of these records there is observed an enormous disproportion between the reflex contractions arising from direct stimulation of the cord and those resulting from stimulation of the end organs of the afferent nerve, yet the two centripetal impulses undoubtedly make use of the same centrifugal nerve mechanism involving the same motor neurone. The disproportion is not a matter of strength of stimulus, since, by even the most intense stimuli applied directly to the cord, only moderate contractions are elicited, while a slight pinch of the skin suffices for a most intense contraction. It is not a matter of shock caused by destruction of the brain. We have tested this in a variety of ways, and have found neither the early nor the late destruction of the brain materially to affect the result. It is not a matter of anemia of the cord; we have taken great care to avoid anemia. It is not primarily a matter of polarization of the cord by the electric current. Moving the electrodes along the cord posteriorly and thus bringing new regions into activity has either no pronounced effect or produces only a slight increase in intensity. Moreover, the disproportion is present with the very first contraction. The disproportion is always present, no matter what portion of the cord is stimulated, whether the cervical, dorsal, or lumbar.

The most reasonable explanation of the disproportion which has occurred to us is that stimulation of the cord excites both motor and inhibitory impulses, and that the subsequent contraction is the algebraic resultant of the effects of the two. Stimulation of the cord as a whole is a stimulation of a variety of tracts. Presumably there is sent to the motor centres that give origin to the nerves of the muscle in question, other than simple motor impulses, and it is not improbable that they may interfere with the purely motor result that is desired. That, however, such a motor result is capable of being elicited from the motor neurones in question is shown by the experiment of pinching the skin and obtaining the enormous contraction of the muscle, which represents the natural protective reflex from such a stimulation, namely, the flexion of the leg at the knee joint, and the pulling of the leg under cover of the carapace. In view of the possibility of this enormous reflex, it is evident that direct stimulation of the cord is very inefficient as a means of bringing into action the latent powers of the motor neurones in question, and the picture of fatigue as represented by such experiments as

those of Figs. 1 and 2, is no true picture of fatigue of the spinal cord.

The two modes of stimulation above employed bring into play the same set of motor neurones, but the latter are reached by different paths through different synapses. Sherrington [3] has studied the " fatigue " of reflex motor mechanisms, and especially that of a mechanism in which a common efferent path may be reached by two or more afferent paths. When stimulation of one of the afferent paths has ceased to be effective, contractions have again occurred from stimulation of the other. The experiment shows that neither the common efferent path consisting of motor neurone (cell body and nerve fibre), nor the muscle, is the seat of the "fatigue." Sherrington believes it to be localized at the synapse between the afferent and efferent paths. One cannot help thinking that the " fatigue " of such an experiment, which follows a few contractions, is not genuine or complete fatigue at all, comparable to that resulting from the action of toxic fatigue substances or the loss of substance essential to activity. It is a temporary condition, from which recovery is easy and rapid. Perhaps it is due to a mild temporary asphyxia.

That a particular part of the spinal cord is capable of performing an enormous amount of work has been abundantly demonstrated by the newer ergographic methods. Beside the ergographic records we can place certain graphic records that we have obtained from the turtle, where the pinching of the skin in the ischial region occurred approximately every third second and the reflex contractions were confined, by severing efferent nerves, to a single ischio-caudali-tibialis. This is a crude method of experimentation, but we have continued it during more than one hour without observing any evidence of fatigue in the preparation. Before these and the ergographic records those obtained by Sherrington in his scratch reflexes and by ourselves through direct cord stimulation pale into insignificance and urge irresistibly the conviction that neither Sherrington's nor our " fatigue " is genuine or complete fatigue. We have chosen to call this condition *pseudo-fatigue*. Whether Sherrington's pseudo-fatigue and ours are identical in nature is not entirely certain, but the one fact of which we feel very sure is that, by no method yet

[3] Sherrington: The integrative action of the nervous system, New York, 1906; also, Address to the Physiological Section, Proceedings of the British Association for the Advancement of Science, 1904.

discovered of stimulating the spinal cord directly, has complete fatigue of the nerve substance of the cord been obtained. Hence neither our results nor Waller's can be considered as demonstrating in any way the early fatigability of the central nervous system. We find indeed, in both frog and turtle, that after the muscle has ceased to respond to direct cord stimulation, stimulation of the nerve or the muscle directly will elicit further contractions. If however the cord is then not really fatigued, such a result is of no significance. Therefore Waller's "probable conclusion, that the incidence of normal voluntary fatigue is in diminishing gravity from centre to periphery, — relatively greatest at the former, relatively least at the latter," cannot be said to be demonstrated.

Whether our pseudo-fatigue, as demonstrated in Figs. 1 and 2, is a condition localized at the synapses, is at present uncertain. It is clear, however, that it represents no fatigue whatever of the bodies of the motor neurones. The reflex contractions resulting from the stimulation of afferent nerves after long-continued cord stimulation and the production of pronounced pseudo-fatigue are usually more intense than before the stimulation of the cord has occurred. This fact is highly significant.

The problem whether the central nervous system is more or less resistant to fatigue in comparison with peripheral organs is still unsolved. In considering it the following few facts seem to be pertinent. Sensations of fatigue have their primary sources in the fatigue of tissues or organs which are situated outside the central nervous system. In starvation the brain and the spinal cord are the last part of the body to lose in weight; their substance and working power are maintained to the last at the expense of other tissues. In certain diseases, notably in syphilis, the brain and spinal cord are attacked only after other organs have become involved. Thus, under various untoward conditions the integrity of the central nervous system appears to be long preserved. It would seem to be entirely in harmony with this fact and with the hierarchical position of this system in the living body that it should be resistant to fatigue.

Conclusions.

1. Direct electrical stimulation of the tracts of the spinal cord does not bring into activity the total working power of the central nervous motor mechanism of an individual muscle.

2. The slight " fatigue " that may thus be demonstrated does not represent complete fatigue of the central nervous motor mechanism of the muscle, and is no measure of the working capacity of the mechanism.

3. The result of such an experiment does not justify the inference that the central nervous system succumbs readily to fatigue.

THE INNERVATION OF THE CORONARY VESSELS.

By CARL J. WIGGERS.

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

CONTENTS.

I. The value and limitations of the perfusion method 391
Errors arising from insertion of the perfusion cannula 392
Errors arising in measuring the venous outflow 394
Errors introduced by the beating heart 395
II. The reaction of the coronary vessels to adrenalin 396
Previous evidence 396
Methods employed in this research 397
Results and their discussion 398
III. The effect of nerve stimulation on the flow through the intact heart 399
Previous observations 400
Author's method 400
Conclusions 404

I. The Value and Limitations of the Perfusion Method.

THE criterion by which coronary vasomotion has generally been judged has been the reaction given by the coronary vessels when the heart was perfused and the flow through them was measured. Porter [1] was the first to employ this method in studying coronary innervation. He inserted a cannula into the aorta, perfused the heart with defibrinated sheep's blood, and measured the variations in flow from a cannula in the superior vena cava as a criterion of vasomotor changes. Maas,[2] in 1899, made similar experiments, using the outflow from the inferior vena cava as an index of coronary flow. In 1904 Schäfer [3] pointed out what he deemed an imperfection and source of error in this method, contending that the aortic valves were not effectively closed and that considerable leakage of fluid into the ventricle was liable to occur.

[1] Porter: Boston medical and surgical journal, Jan. 8, 1896.

[2] Maas: Archiv für die gesammte Physiologie, 1899, lxxiv, p. 281.

[3] Schafer: Archiv des sciences biologiques, 1899, xi, suppl. vol. p. 281.

For this reason he modified the perfusion method by pushing the cannula beyond the valves. This allowed the fluid to distend the left ventricle and pass by the side of the cannula into the coronaries, its back escape through the pulmonary veins being prevented by a ligature. As a measure of the coronary flow, Schäfer used the venous blood escaping from the caval openings into a funnel below.

Errors arising from insertion of the perfusion cannula. — The most convenient method of perfusing the heart is that utilized by Porter and by Maas. A cannula is inserted into the aorta or one of its branches, and the coronary arteries are perfused, since the pressure tended to close the aortic valves. As doubt has arisen concerning the perfect closure of these valves, a series of experiments was undertaken to test this point, for outflow changes obviously are in no wise trustworthy unless all the fluid passes through the coronaries.

The hearts of dogs, cats, and rabbits were perfused by this method, and the amount of fluid passing through the coronaries was measured by collecting the overflow from the filled right auricle and ventricle in the pan of a drip-recording apparatus, recently described.[4] The fluid entering the left ventricle was drained by a cannula pushed directly through its musculature, and was led by a thin rubber tube to a closed receptacle, which in turn was connected with a bellows recorder; in short, the method employed by Brodie and Dixon[5] to continually record outflow was used. The bellows recorder differed, however, from those ordinarily used, in that it possessed a device for altering the magnification of the bellows movement and wrote a vertical line instead of an arc on the drum.

A series of 20 experiments showed that the leakage through the aortic valves varied markedly. In some preparations the leak could by adjustment be reduced to a very small quantity, but in other preparations it persisted after the most painstaking adjustment. I have records in which the leak equalled from 36 to 48 per cent of the flow through the coronaries. That these figures represent a leak through the valves rather than a flow through the Thebesian vessels into the left ventricle is evidenced by the fact that the flow decreased to from 3 to 8 per cent when the cannula was inserted into the coronaries. The fallacy in the method consists, however, not so much in the degree of leak as in its tendency to vary. Thus the leak

[4] Wiggers: This journal, 1908, xxiii, p. 23.

[5] Brodie and Dixon: Journal of physiology, 1904, xxx, p. 478.

has been observed to increase (1) as cooled hearts were gradually warmed; (2) when the pressure rose; (3) after the introduction of adrenalin, probably because it reduced the tonus of the aortic ring; and (4) when no cause was apparent. Fig. 1 illustrates how such a change of leakage may exert an inverse effect on the flow through the coronaries, without materially changing the perfusion pressure.

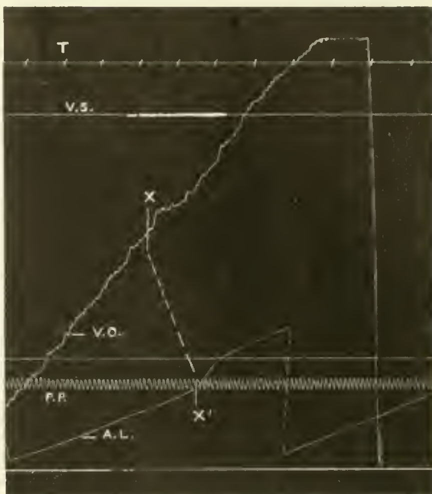


FIGURE 1. — Three sevenths the original size. *P.P.*, perfusion pressure; *T*, time in ten seconds; *V. O.*, flow through coronaries recorded by author's apparatus; *A. L.*, leak through aortic valves recorded by Brodie's method. *V. S.*, vagus stimulation. Published to show at *X'* sudden increase in leak causing change in flow through coronaries at *X*. Taken on non-beating heart.

These results seem to warrant the following conclusions: 1. The method cannot be utilized to study the effect of adrenalin or any other drug which affects the tonus of the aortic ring. 2. The method may be used to test the effect of nerve stimulation on the coronaries, but to render results free from criticism, they should be accompanied by records showing that the amount of fluid entering the left ventricle has not changed during stimulation.[6] Several times I have obtained what seemed perceptible changes in the outflow record during nerve stimulation which might be attributed to vasomotor changes, only to find that the increased leak through the valves counterbalanced the decrease, making the total effect nil.

Schäfer's modification is, however, no less open to criticism. In this procedure the pressure under which the coronaries were perfused, as well as the rhythm of the pressure, was determined by the contraction of the left ventricle. As long as the heart beat regu-

[6] This cannot be accomplished by recording the constancy of the perfusion pressure alone. Fig. 1 shows that this need not be an index of competent valves. Either the outflow from the left ventricle must be recorded, as was done by Magrath and Kennedy (Journal of Experimental Medicine, 1897, ii, p. 13) or by recording the changes in intraventricular pressure with a sensitive membrane manometer, as was done by Miss Hyde (This journal, 1898, i, p. 215).

larly the pulsating pressure remained constant, but, when nerves were stimulated or drugs introduced which affected the rate, rhythm, or tonus of the heart, the vessels were no longer supplied by the same pressure. The curves published in Schäfer's article, as well as that produced in Fig. 2, give evidence of this. It seems that this procedure violates a principle in the perfusion of organs, namely, that the supplying pressure must remain constant or vary within fixed limits if the outflow changes are to be used as a criterion of vasomotor changes.

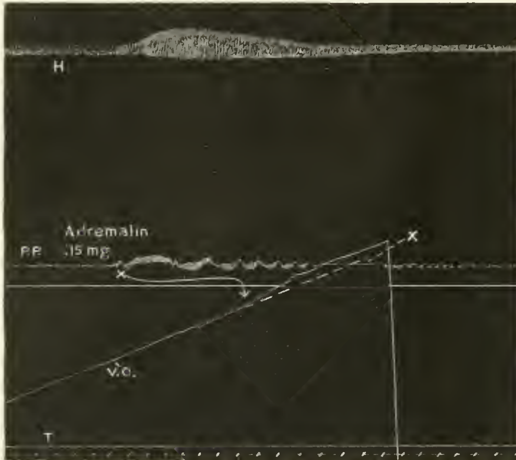


FIGURE 2. — One half the original size. Schäfer's method of perfusion. *H*, heart beats recorded by straw lever; *P. P.*, perfusion pressure; *V. O.*, venous outflow; *T*, time in ten seconds. At *X* pointer caught. Effect of adrenalin, 0.15 mg. Heart rate increased, tonus decreased, systole augmented, perfusion pressure elevated, venous outflow thus mechanically increased.

The objections to both of these methods may be removed by inserting the cannula directly into the coronary vessels, but this technic has so far not been employed, to my knowledge, in order to test the effect of drugs or nerve stimulation on the coronary vessels.

Errors arising in measuring the venous outflow. — The fluid perfused through the heart returns by the coronary veins into the right auricle and by the Thebesian vessels into the right ventricle, only a small quantity passing by similar vessels into the left ventricle. As there is no single vessel into which a cannula may be inserted and the total outflow thus received, the right auricle and ventricle have been used as intermediary reservoirs between the place of venous discharge and the recording device. Thus Porter, as well as Maas, believed that the outflow from the right auricle and ventricle through a vena cava could be used as an index of the flow from the coronary veins. Maas, however, made no attempt to measure the flow from the Thebesian vessels, while Porter did so in one experiment only. The return flow through these Thebesian vessels is so considerable that it should always be recorded in esti-

mating changes in the flow of blood through the heart. Schäfer allowed fluid to fill the right chambers, and then recorded the overflow from the auricle and ventricle by allowing fluid to leak out of the venæ cavæ and pulmonary artery. This method adequately measures the blood returned to the right heart as long as its beat or tonus remains unaltered, for as much blood will drip away as is added by the heart vessels. When the beat of the heart is changed through the stimulation of nerves or the administration of drugs, the overflow no longer gives a true indication of coronary flow. I have observed, for example, that the first few augmented beats induced by adrenalin caused a greater amount of fluid to be expelled from the cavity of the right heart, which, when recorded, produced the erroneous impression that the coronary flow had been suddenly increased.

It is evident, then, that the flow from the right chambers gives reliable information concerning the coronary flow only when the fluid passing through the Thebesian vessels is recorded, as well as that from the coronary veins, and when such outflow records are accompanied by graphic records showing or warranting the assumption that the size of these chambers was not altered by changes in contraction or tonus.

Errors introduced by the beating heart. — Since fluid is pushed through the intramural vessels by the compressing action of systole, and the refilling of these vessels is determined to a great extent by the degree of relaxation during diastole, it follows that changes in outflow cannot be interpreted as due to vasomotor action unless it can also be shown that neither the tonus nor contractions have altered.[7] I have a number of records showing that nerve stimulation may cause a change in tonus without change in heart rate, and this may account for the changes in flow observed. Maas emphasizes the statement that in order to bring out vasomotor changes a strong current is necessary. I have been able to show that changes in outflow may indeed be obtained with strong currents when weak ones fail, but inspection of the cardiac tracings, if delicately recorded, reveal beautiful tonus changes, probably due to a spread of current to the heart. The curves published by Maas himself, as Figs. 10 and 15, show, when a line is drawn perpendicular to the lower portion of the heart contractions, that they rise (tonus

[7] Porter: This journal, 1900, xxiv, p. xxiv.

change?). Since Porter took no graphic records to rule out these changes, and as his observations on non-beating hearts were limited apparently to a single experiment, his results offer, as he himself words it, "probable rather than quite certain evidence" of a vasomotor influence.[8] Schäfer accompanied his experiments with tracings, but assigned all changes in flow to synchronous changes in heart beat. I have been able to corroborate Schäfer's results showing that adrenalin increases the flow through the beating heart. Such a reaction, however, need not necessarily be interpreted, as it was by Schäfer, as indicating the absence of an adrenalin action, for, by increasing the amplitude and rate of cardiac contractions, the vessels would be more vigorously massaged, and so an increased flow might occur in spite of a constrictor action that the adrenalin might have.

The effect of cardiac changes on the flow through the heart may be eliminated by inducing standstill. After trying various procedures to attain this end I have found that perfusion with a non-oxygenated sodium chloride solution accomplishes it. If the perfusion occurs immediately after death, the influence of nerves on the heart vessels may be tested, while, if an interval of several hours is allowed to elapse before perfusion, adrenalin and other drugs will not, as a rule, cause a revival of contractions, but will still affect the blood vessels.

This preliminary investigation has shown that any decrease or increase in outflow from the right heart cannot be interpreted as due to vasomotor changes unless it can be shown (1) that the amount of fluid supplied to the coronary vessels in a given time remains constant, (2) that the size of the right chambers, which exist as intermediary reservoirs between the heart veins and the registering apparatus remains constant, and (3) that the massaging effect on the intramural vessels has not altered.

II. The Reaction of the Coronaries to Adrenalin.

Previous evidence. — Adrenalin has come to be regarded as a convenient agent for obtaining presumptive evidence of the innervation of blood vessels, for, even if a constrictor reaction is not universally accepted as a proof of nerve control, the lack of such a

[8] Porter: This journal, 1900, iii, p. xxiv.

reaction is enough to arouse a question as to the existence of such an innervation. So far a constrictor reaction of the coronary vessels has not been demonstrated. In fact, Schäfer [8] not only failed to obtain any evidence of constriction, but showed that the outflow from the coronaries was often increased. In 1907 Langendorff [9] pointed out the difficulty of determining accurately by the perfusion of hearts whether or not adrenalin exerted any action. He accordingly abandoned the method, and recorded instead the movements of a strip of artery from an ox heart, which was suspended in warm Locke's solution. Adrenalin, tested by this method, caused only a relaxation, while an induction shock caused a contraction.

Methods employed in this research. — The coronary arteries were perfused by a stream of normal salt solution regularly interrupted by a stopcock which was actuated through a cam and motor. The perfusion cannulas were inserted into the mouths of the two coronaries, or simply into one while the other was clamped or ligated. This procedure was found necessary, for sometimes the terminal nature of the coronaries did not prove true when the isolated and suspended heart was perfused at various intervals after death with a solution having less viscosity than the blood. Perfusion with normal salt solution instead of the customary Locke's solution or defibrinated blood brought the heart to complete standstill, so that the simultaneous effect of the drug on the heart was entirely eliminated. The venous outflow was generally determined by recording the overflow from the right auricle, right ventricle, and left ventricle after all of these chambers had previously been filled with saline solution. In this way the total flow from the Thebesian vessels as well as from the coronary veins was obtained. This overflow could be taken as evidence of the amount flowing through the coronaries, since the size of the chambers did not vary owing to the cessation of the heart. In a number of experiments the flow from the right ventricle was drained by a cannula passed through its musculature, and in still others the coronary sinus was incised and the flow allowed to drip away directly.

The changes in the diastolic portion of the perfusion-pressure oscillations were simultaneously utilized to corroborate the changes in calibre of the vessels. As the vessels were supplied by a stream of fluid rhythmically interrupted, it followed that the pressure in a laterally connected manometer rose whenever the stopcock was open,

[9] LANGENDORFF: Centralblatt für Physiologie, 1907, xxi.

and fell whenever it was closed. The extent of the fall depended entirely on the amount of fluid passing through the heart vessels during the interval in which the stopcock remained closed. The effect of a constriction would, by diminishing the amount of fluid passing through an organ, cause less of a fall, and so give the appearance of a rise in the diastolic pressure in a series of such oscillations long before the systolic pressure could be influenced. In a

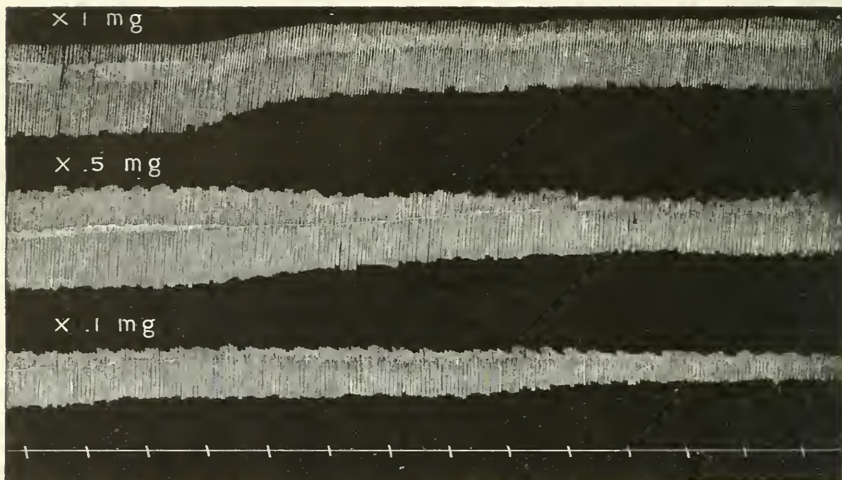


FIGURE 3. — Effect of 3 various-sized doses of adrenalin on dog's coronaries as indicated by change in diastolic portion of perfusion pressure. Recorded by membrane manometer, March 15, 1908.

number of experiments a membrane manometer was substituted for the mercury manometer, and the oscillatory changes thus magnified. In this way the slightest vascular changes could be determined.

The adrenalin used was the crystalline product supplied by Parke, Davis, & Co., or tablet titurates made of the same substance. This was dissolved in salt solution in the desired quantities just before use, so that deterioration of the solution and the presence of preservatives were avoided.

Results and their discussion. — By these procedures it was determined that adrenalin was capable of constricting the coronary vessels in the dog, cat, and rabbit, while a dilatation was never obtained. In dogs doses ranging from 0.1 to 3 mg. caused a decreased flow and a rise in the diastolic pressure. The reaction became weaker as the dose was diminished, but a dilation never occurred (Fig. 3).

Adrenalin failed to react more than eighteen hours after death, although digitalein was still found to respond. In the cat and rabbit a similar constriction was observed. Though the change in outflow and oscillations was never very great, owing to the small size of the coronary vessels, it was always clearly evident when the records were properly magnified (Fig. 4).

A word of explanation regarding the results of other workers seems necessary. In Schäfer's results the slight vasomotor change present in hearts as small as those of rabbits and cats were probably obscured by the greater effect of the drug on the heart (consult previous section). In regard to the result of Langendorff, it may be said that either the vessels of the ox heart behave differently to adrenalin, or he used an old solution which had lost the constricting power of the adrenalin, but retained the dilator influence of its preservative, the chloretone.

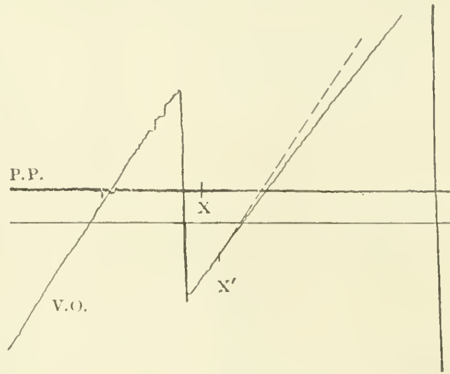


FIGURE 4. — One half the original size. Effect of adrenalin on cat's heart when not beating. *P. P.*, perfusion pressure; *V. O.*, venous outflow recorded by author's apparatus; *X*, *X′*, relative position of pointers.

III. The Effect of Nerve Stimulation on the Flow through the Intact Heart.

By using the perfusion method I have been unable to obtain a change of flow during nerve stimulation which could not be equally well explained by some factor other than a vasomotor influence. If the perfusion cannula is tied into the aorta or one of its branches, as it has been by previous investigators, the nerve supply may be kept intact, but the results are complicated by the loss of fluid through the aortic valves. If the technic employed in the previous part of this research to study the action of adrenalin is used, the nerves supplying the vessels are probably included within the ligatures that tie the cannulas into the coronary vessels. Beset with these difficulties, I abandoned the perfusion method temporarily and

made an attempt to study the effect of nerve stimulation on the vessels of the intact heart. (See Addendum.)

Previous work. — Observations in regard to the effect of nerve stimulation on the vessels of the intact heart have been at variance with each other. Inspection of the vessels seems to have been the favorite procedure. Panum is reported in Schmidt's Jahrbuch of 1858 to have observed a constriction of the coronary vessels after vagus stimulation. In 1869, however, Meyer[10] thought he saw a dilation when the vagus was stimulated. Similar observations were reported in 1891 by Martin.[11] To what extent concomitant changes in the heart and general pressure were ruled out is not clearly indicated. Quite recently Dogiel and Archangelsky[12] reduced the heart to standstill by vagus stimulation, and then observed a constriction of the arteries on stimulating the annulus of vieussen and the inferior (middle) cervical ganglion. Their article is illustrated by photographic plates.

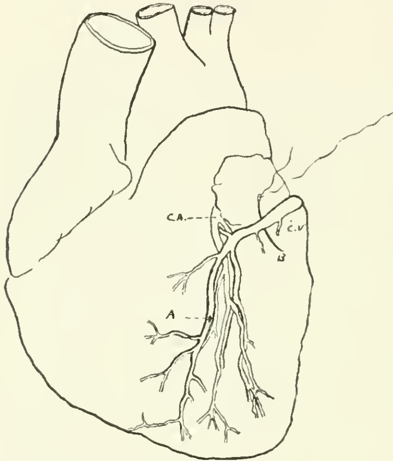


FIGURE 5. — Diagram showing relation of heart vessels. *C. A.*, coronary artery; *C. V.*, coronary vein. Vein wounded at *A* and ligature to prevent back flow applied at *B*.

Method employed in this research. — In this research the amount of blood flowing from one of the cut veins of the heart during nerve stimulation was compared with a similar flow when no stimulation occurred. A dog was anesthetized with morphine and chloretone, and the carotid artery together with the nerves to be stimulated was isolated. After instituting artificial respiration the thorax was opened and the heart exposed. The animal was then inverted and suspended in a hammock arrangement above an outflow-recording apparatus, recently described.[4] When the carotid pressure or the

[10] MEYER, quoted by DOGIEL and ARCHANGELSKY: Archiv für Physiologie, 1907, p. 482.

[11] MARTIN: Transactions of the Medical and Chirurgical Faculty of Maryland, 1891.

[12] DOGIEL and ARCHANGELSKY: Archiv für Physiologie, 1907, p. 482.

contractions of auricles and ventricles were being satisfactorily recorded, one of the veins accompanying the descending branch of the left coronary artery (Fig. 5) was wounded with the point of a pin or scalpel, and the blood leaving the wound was caught in the pan of the registering apparatus below. As coagulation over the vessel was mechanically delayed by the beating of the heart, the quantity of blood flowing from the vein during equal-time intervals remained constant for a considerable length of time, with the result that it was recorded as a straight oblique line on the moving drum.

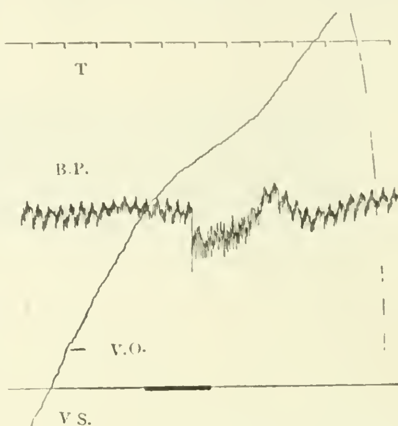


FIGURE 6. — One half the original size *V. S.*, stimulating the vagus with a moderate current; *B. P.*, effect on blood pressure and heart rate. These factors cause change in venous outflow (*V. O.*).

That the method may be used to indicate changes in the amount of blood flowing through the heart veins was shown by the fact that when the vagosympathetic of either side was stimulated the well-known diminution of flow was obtained, which gave place to an increased flow when stimulation ceased. Fig. 6 illustrates the nature of these results. This diminution was due in part to the fact that the blood pressure lowered, and in part to the removal or diminution of the normal squeezing action of the cardiac systôle. These were not the only factors concerned, however, for a decreased flow still occurred when the effect of the vagus on the heart was prevented by the previous introduction of atropin or by the use of a weak current which had no effect on cardiac contractions (Fig. 7).

These results having been obtained from two to five times consecutively in 7 experiments, were presented before the American Physiological Society at Baltimore as probable evidence of a vasoconstrictor influence of the vagus on the heart vessels. The reasoning by which the conclusion was reached was somewhat as follows: The results certainly indicate that the quantity of blood contained in the large veins of the heart is decreased during such stimulation. Into these veins blood flows (1) from the arteries by the capillaries,

(2) from the ventricles (especially the right) by the Thebesian vessels, and (3) from the auricles by a back flow through the incompetent venous valves. When the outflow from an opening in one of the veins decreases after nerve stimulation, in spite of the fact that changes in the blood pressure and in the contraction of auricles and ventricles have not occurred, there is no reason to suppose that

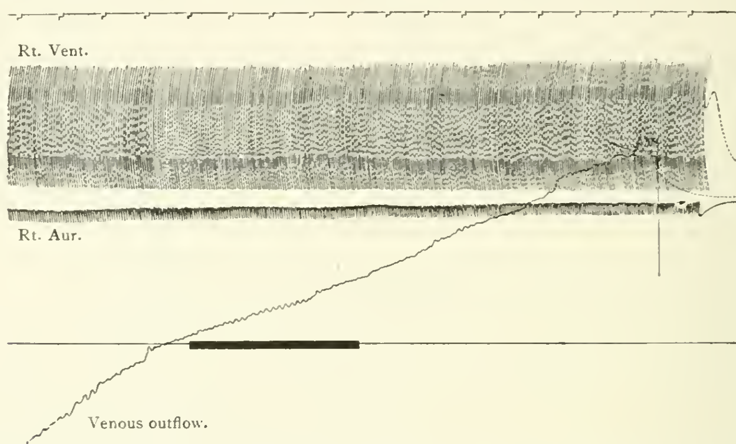


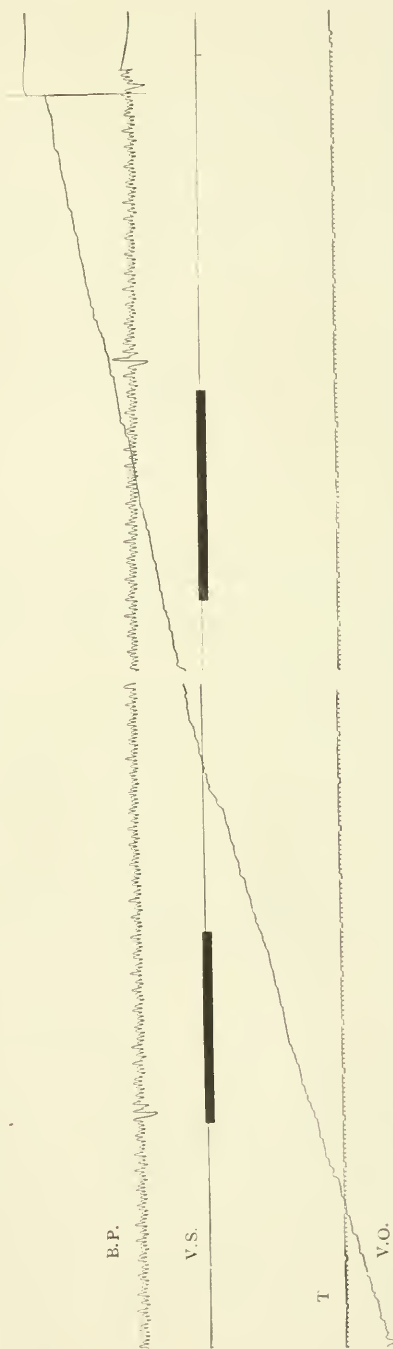
FIGURE 7. — One half the original size. Effect of stimulating vagus (weak current) on venous outflow when no change in contractions of auricle and ventricle were present.

the back flow has diminished unless the large veins have themselves been constricted. It cannot be so certainly said that the flow through the Thebesian vessels was not diminished, but, making this assumption, one can only conclude that these must have been constricted by nerve stimulation. Since, then, the decrease is most probably not due to these factors, it only remains to assume that the arterioles were constricted.

Such evidence, however, remains open to objection as long as the back flow from the auricles is not actually controlled. Theoretically this back flow should be easily prevented by ligating one of these veins and wounding it peripherally. Practically obstacles to the employment of this technic immediately arose, — a first in including the vasomotor nerves within the ligature, a second in producing an undue venous stasis in the heart vessels. After some experimenting, however, it was found that a place could be located where a ligature might be applied by a needle and yet not cause either of these effects. This place was where the veins and artery parted company, the

former assuming a horizontal direction (Fig. 5). When a ligature was so placed, the end of the vein toward the auricle was seen to swell markedly, while the veins of the heart diminished in size. The outflow from a wounded vein which before had been a spurting stream was now transformed into a dropping one. This introduced a further obstacle. The beating heart which adequately prevented clot formation as long as a spurting stream left the vein was no longer able to do so when the outflow was reduced, thus clot formation interfered seriously with accurate records. It was consequently necessary to render the blood non-coagulable. Accordingly 150 to 250 c.c. of blood were withdrawn at half-hour intervals, defibrinated, and then reinjected until little or no coagulation occurred.

In the first experiments in which this procedure was adopted the results after vagus stimulation were negative. In all of these cases, however, a great deal of hemorrhage had occurred on opening



CURVE 8. — About two thirds the original size. Effect of stimulating the vagus with a weak current twice consecutively. Blood of animal rendered non-coagulable, back flow from auricle prevented by ligature of vein. *V. O.*, venous outflow; *B. P.*, blood pressure; *T*, time in ten seconds and in seconds.

the thorax, because of the non-coagulability of the blood, and often the heart had to be kept beating by the use of strychnine and digitalis. At other times a marked diffusion of hemoglobin had taken place throughout the entire heart. In later experiments the thorax was always opened before defibrination of the blood, thus utilizing its coagulating power to check hemorrhage. Great care was also taken not to whip the blood too vigorously nor to heat it above 38° C. before injection. By attention to these details the animal with its blood defibrinated was in as good a condition as before. In such cases stimulation of the vagus caused the flow from the peripheral end of the vein to decrease. This decrease could be obtained many times in succession. In Fig. 8 is presented such a decreased outflow, obtained six consecutive times without any change in blood pressure or heart action.

Conclusions.

The conclusion that the coronary vessels are not influenced merely in a passive manner by the blood pressure and the massaging action of the heart, but that they possess a nerve control, is supported by the following evidence:

1. The coronary vessels react to adrenalin by constricting, when the simultaneous effect on the heart is eliminated.

2. Stimulation of the vagus nerve causes in the dog a decreased outflow from a wounded heart vein. This happens in spite of the fact that changes in the blood pressure, contractions of the auricle and ventricles, and back flow do not occur.

It is with pleasure that I acknowledge the suggestions made by Professor Schäfer during his visit to the laboratory last summer.

ADDENDUM.

Preliminary Note: — Recently the writer and Mr. H. Cummings have devised a method of perfusion which promises to determine whether nerve stimulation can cause changes in flow through the perfused heart which may be attributed only to vasomotor changes. The mouth of the left coronary artery is closed by approximating the intima around it with a stitch. The right coronary is ligated and a cannula is inserted into the central end of one of the

descending rami of the left coronary artery. By this backward perfusion through the arteries, the nerve supply of the vessels is not included within the ligature tying the cannula into the coronaries. Experimentation by this method will be resumed next autumn and a report on the results made later.

THE COAGULATION OF BLOOD.

By L. J. RETTGER.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

RECENT investigators of the phenomenon of blood coagulation have come to rather widely divergent conclusions. Morawitz,[1] attempting to harmonize the older views of Schmidt with the later work of Pekelharing, Hammarsten, and others, gives four factors entering into the process. Three of these pre-exist in the circulating blood, — the fibrinogen, the thrombogen or proferment, and the salts of calcium. The fourth factor arises at the moment of coagulation, and is produced by the disintegration of the formed elements of the blood, — the platelets and probably also the leucocytes. This substance is the thrombokinase, which activates the thrombogen in the presence of calcium salts to form thrombin. The thrombin then attacks the fibrinogen molecule and in the manner of a ferment or catalyzer changes it to fibrin. The thrombokinase may also be derived from the various tissues of the body, and when so extracted serves to activate the thrombin and initiate coagulation. The suggestion of a "kinase," as made by Pawlow in the study of the entero-kinase in the intestines, is here applied to explain the action of the "zymoplastic" substances of earlier workers.

On the other hand, Nolf,[2] impressed by the fact that a clot is soon re-dissolved by an act of autolysis, especially in the case of clots of fibrinogen produced by thrombin, sees in the phenomenon of coagulation, as the paramount question, not what makes blood clot, but what makes the clot persist. According to his view coagulation is a normal process in nutrition and assimilation, whereby the fibrinogen of the blood is rendered available as food for the tissues.

[1] MORAWITZ: Beiträge zur chemischen Physiologie und Pathologie, 1904, iv, p. 381. MORAWITZ: Ergebnisse der Physiologie, 1905, iv, p. 307; contains a full bibliography.

[2] NOLF: Archives internationelles de physiologie, 1906, iv, p. 165. *Ibid.*, 1908, vi, pp. 1, 115, 306. *Ibid.*, 1909, vii, pp. 281, 380.

Coagulation is normally and continually going on in the blood. It consists of a quantitative and mutual precipitation of two colloids, the thrombin and the fibrinogen. The thrombin is formed by the union of the "hepato-thrombin," which has its source in the liver and corresponds in a way to the thrombogen of Morawitz, and "leuco-thrombin," which is derived from the leucocytes and platelets of the blood. In the circulating blood small amounts of thrombin are formed around the leucocytes in the manner described. This precipitates a small amount of fibrinogen as fibrin, which covers the leucocyte with a thin ultra-microscopic layer of fibrin. This layer of fibrin renders the leucocyte neutral to some extent, in the matter of further production of leuco-thrombin. This precipitated cuticle of fibrin is now attacked by the real enzyme of the thrombin, the "thrombozyme," and dissolved. This autolytic action is a true fermentative process, and serves primarily purposes of tissue nutrition. In abnormal conditions, such as produce ordinary clots, the leucocytes and platelets are destroyed in great numbers, and the excessive amounts of leucothrombin so formed unite with hepatothrombin to form the active thrombin that produces the clot. The real question here is, what makes the clot persist? This anti-fibrinolytic substance he believes is the hepato-thrombin, to the inhibiting action of which on the fibrinolysis the clot owes its persistence. Coagulation is therefore, according to Nolf, a late phylogenetic adaptation. It is a secondary modification, for preventing hemorrhage, of a primitive nutritive and assimilative process.

Loeb,[3] approaching the problem from a study of blood coagulation in the invertebrates, assigns the primary rôle in the process to the *coagulins*. These coagulins may be extracted from the tissues, and when activated with calcium salts have an effect on the fibrinogen similar to ordinary thrombin. These coagulins are not merely zymoplastic substances, nor are they kinases. They do not activate any proferment, but independently attack the fibrinogen molecule. Neither are the coagulins identical with ordinary thrombin. They have a much greater specificity than thrombin, and are more stable.

The fact that tissue extracts properly irrigated will not clot carefully prepared fibrinogen solutions, even when calcium chloride is

[3] Loeb, Leo: Archiv für pathologische Anatomie, 1906, clxxxv, p. 160; Loeb: Beiträge zur chemischen Physiologie und Pathologie, 1906, viii, p. 67; *Ibid.*, 1907, ix, p. 185. Loeb: Biochemisches Centralblatt, 1907, vi, pp. 829, 889, excellent bibliography given. Loeb: Beiträge zur chemischen Physiologie und Pathologie, 1909, xvi, p. 157.

added, is explained by assuming that the repeated salting out has changed the character of the original fibrinogen.

Mellanby,[4] working with the blood of birds, assigns the main rôle in the coagulation of blood to the *kinases.* These kinases, extracted from the tissues, activate the proferment and form fibrin. The proferment seems bound in a way to the fibrinogen, so that the amount of proferment varies directly with the amount of fibrinogen present. As the source of his kinase he used the macerated testes. The fibrinogen solutions were made by diluting the clear bird's plasma with twenty times its volume of water, acidulating, centrifuging, and re-dissolving the precipitate in the original volume of water.

The present investigation was carried on under the direction of Dr. W. H. Howell, to whose stimulating interest and many valuable suggestions most of any credit which this work may have is largely due.

The first requisite in the investigation of blood coagulation is a trustworthy testing solution. The ideal solution would be the circulating blood, if it could be kept in its normal condition. This is of course impossible, at least with mammalian blood, inasmuch as it begins to clot immediately after taking. Recourse must therefore be had to artificial plasmas. It is believed, for reasons to be stated further on, that the most satisfactory testing plasma is a solution of fibrinogen, prepared essentially after the manner of Hammarsten, with two modifications.

The method of preparation of the fibrinogen solutions used in this work was as follows:

On account of the fact that the blood of the cat does not "lake" so readily as that of many other mammals, this animal was used in the majority of experiments. The blood was taken under careful conditions from the carotid artery, through a cannula and short tube either oiled with some neutral oil or washed out with a 1 per cent solution of sodium oxalate. A 1 per cent solution of sodium oxalate was added in the proportion of one part of oxalate solution to nine parts of blood. The blood was then immediately centrifuged, until a clear supernatant plasma was obtained. Sodium chloride was added to a little more than 50 per cent saturation, and the precipitate after centrifuging, and washing in a saturated solution of sodium

[4] MELLANBY: The journal of physiology, 1909, xxxviii, p. 28.

chloride by decantation, was re-dissolved in a 2 per cent solution of sodium chloride of volume equal to the original plasma.

A second and a third precipitation in the same manner produced a final precipitate, which was dissolved in 0.9 per cent solution of sodium chloride. If such a solution of fibrinogen still remained distinctly opalescent, 1 c.c. of a dilute solution of barium chloride and 1 c.c. of a dilute solution of sodium phosphate were added. The dilution of the two solutions was approximately such that 1 c.c. of the barium chloride and 1 c.c. of the sodium phosphate neutralized each other. The precipitate of barium phosphate so formed gradually fell to the bottom, and with it were swept out the particles giving the opalescence, leaving finally a clear solution of fibrinogen, not very different from the clear appearance of distilled water.

The solution of fibrinogen was then placed in a collodion tube and dialyzed against a large volume of 0.9 per cent solution of sodium chloride for about eighteen hours. This insures a practically complete removal of any oxalate remaining, or of any excess of barium chloride or sodium phosphate. It also insures to the fibrinogen solution a known concentration of sodium chloride.

Such test solutions of fibrinogen do not clot spontaneously, even when kept until putrefactive changes arise. They give no appearance of a clot on the addition of calcium salts, showing that no pro-stage remains in the fluid which can be activated by calcium. No clotting occurs either when Ringer's solution is added, such as occurs frequently in fibrinogen solutions, which have been precipitated only once or twice and which remain strongly opalescent. Probably in such opalescent solutions some of the fibrinogen has already been affected by the thrombin present. It is believed therefore that by this method a solution of fibrinogen was obtained which was entirely free from any substance which could give rise to the thrombin. Only with the aid of such solutions can reliable experiments be made upon the presence or absence of a coagulating agent.

That solutions of fibrinogen prepared as here described are, however, not materially changed from the condition found in the blood is shown by the fact that with serum or thrombin they form a typical clot, which resembles in every essential the normal blood clot. The promptness of the action and its similarity in every way to clots of other forms of test solutions prove clearly that the fibrinogen in question has retained its characteristic properties, and by its purity

and stability is peculiarly fitted to study the real coagulating effects of other factors entering into the process. With this fibrinogen solution the main constituents of normal plasma were tested as to their coagulating properties. As already pointed out in describing the method of preparing fibrinogen, the inorganic salts of the blood in normal (Ringer) concentrations had no effect, either added singly or together or in other possible combinations. Similar negative results were found in the case of solutions of lecithin and cholesterin. Carbon dioxide and oxygen gave negative results. The experiments showed, as has long been known, that the initiating factor of coagulation is not among the simpler chemical substances which exist preformed in the circulating blood.

The Thrombin.

The observation that serum added to a solution containing fibrinogen will induce clotting dates back to the work of Buchanan. Later Alexander Schmidt succeeded in getting the active substance in somewhat purer form by treating the serum with alcohol and extracting the dried residue with water. This substance he designated *fibrin ferment,* or *thrombin.*

The thrombin used in these experiments was prepared after Schmidt's method. Blood was drawn from an animal and allowed to clot. As soon as the serum was sufficiently pressed out of the clot, it was diluted with twenty times its volume of alcohol (95 per cent) and allowed to stand for a period ranging from several days to several months. The duration of the action of alcohol seemed to have no very appreciable effect on the quantity or strength of the thrombin extracted. After the proteins had been precipitated by the alcohol they were filtered off, dried, and extracted with water. Into this aqueous extract other things as well as thrombin will enter. Evidently the free salts of the serum will be dissolved. Some of the proteins also go into solution, especially if the aqueous extraction is continued for any considerable length of time. Unfortunately therefore such *thrombin* solutions are not very pure, and offer serious obstacles to a study of the chemical nature of this substance.

If however the extraction is made of short duration, it is possible to secure a thrombin solution which is very active in producing coagulation and which nevertheless gives only the faintest traces of

protein. The excess of salts may be removed by dialyzing the thrombin solution in a collodion tube against pure water. Experiments soon showed, however, that the dissolved proteins interfered in no way with the thrombin action, and some of the strongest thrombin solutions were secured by submitting the dried residue not only to prolonged aqueous extraction, but even to prolonged putrefaction, until the protein residue disappeared. Such solutions usually gained in amount of thrombin, as the processes of putrefaction continued, the possible explanation for which will appear in another connection. It is evident that the thrombin extracted from the serum is of the greatest importance in the process of coagulation. It appears in the plasma at the time of coagulation and is the agent which effects the change from the liquid fibrinogen to the solid fibrin. It seemed desirable therefore to determine the conditions under which it acts, its origin, and as far as possible its chemical nature.

Rapidity of coagulation with thrombin. — The rapidity of coagulation was found to be directly proportional to the amount of thrombin added, as indicated in the following experiment:

20 c.c. fibrinogen + 40 drops thrombin extract, 14 min.
20 c.c. fibrinogen + 20 drops thrombin extract, 26 min.
20 c.c. fibrinogen + 10 drops thrombin extract, 50 min.
20 c.c. fibrinogen + 5 drops thrombin extract, 90 min.

The time required for coagulation was determined by the moment when the glass could be inverted without spilling the contents. Such a rate, however, holds good only below certain limits. If the amounts of thrombin become excessively large, the rapidity of coagulation does not vary in similar proportion. There is soon reached a maximum amount beyond which the further increase of the amount of thrombin no longer increases the rapidity of coagulation. The evident reasons for this appear in a study of the quantitative relations of thrombin and fibrinogen.

Quantitative relations of thrombin and fibrinogen. — The original statement of Alexander Schmidt that minimal amounts of his "fibrin-ferment" always produced complete coagulation was soon questioned. It was found that partial clots could arise, or that a second or even third successive clot might form in the same fibrinogen solution or plasma. Careful quantitative experiments seem, however, not to have been made. For the experiment on this point

500 c.c. of clear plasma of four cats were taken, and from this plasma 500 c.c. of fibrinogen solution were prepared. This amount was divided into four equal parts. To part one 5 drops of a tested thrombin solution were added; to part two, 10 drops; to part three, 20 drops, and to part four, 40 drops. The glasses of fibrinogen were then allowed to stand untouched for twenty-four hours at room temperature, to allow ample time for coagulation. The fibrin from each glass was then collected on a filter, washed, and placed in a weighing tube, which together with the filter had been previously heated several times at a temperature of 110° C. to approximately constant weights. The weighing bottles with the several amounts of fibrin were then heated for a number of hours at 110° C., weighed, and reheated until fairly constant weights resulted. Final weighing gave the following results:

5 drops thrombin . .	0.2046 gm. fibrin.
10 drops thrombin . .	0.3573 gm fibrin.
20 drops thrombin . .	0.6089 gm. fibrin.
40 drops thrombin . .	1.5872 gm. fibrin.

It will be seen, from these results, that while they fall short of exact mathematical proportionality, yet they reveal very clearly that there is in the formation of the fibrin a definite and direct quantitative proportion. The bearing of such facts on the supposed fermentative nature of thrombin is treated farther on. It need not be pointed out that such quantitative relationships will appear only when the amount of thrombin is below the maximum amount required to affect the entire fibrinogen. Adding thrombin beyond this maximum amount can of course be of no moment, there being no further fibrinogen available.

Effects of temperature on coagulation with thrombin. — Similar amounts of fibrinogen and thrombin submitted to different temperatures showed that temperatures between 17° C. and 41° C. had practically no varying influence on the time of coagulation. In one experiment on this point the following results were obtained:

Temperature.	Time of clotting.
1° C.	2 hours
17° C.	28 min.
21° C.	28 min.
31° C.	27 min.
41° C.	29 min.

These results agree very closely with the determinations made by Mellanby on this point. The temperature coefficient of coagulation between the temperatures of 17° C. and 41° C. is practically 1. Whether this is to be interpreted to mean that the process of coagulation is essentially a physical or physico-chemical one, or at least is overshadowed by physical moments, is perhaps an open question. It is well to recall, however, that in the experiments here noted the thrombin was ready formed. The time element in the coagulation, as determined by a coagulometer, on freshly drawn blood is a different matter. In this case there is a definite relation between the temperature and the rapidity of the coagulation, as indicated in a few figures taken from Addis:[5]

Temperature. ° C.	Coagulation time. min.	sec.
10.25	21	30
12.25	16	30
13.5	14	32
16.5	10	10
18.5	7	34
19.5	6	2
20.5	5	22

It is obvious that in these latter experiments additional factors are concerned, which give rise to the thrombin, — either processes of secretion, or disintegration of formed elements, or chemical activation, which drop out when ready-formed thrombin is used.

Properties of Thrombin.

1. Its stability with reference to heat. In Alexander Schmidt's reports of the fibrin-ferment discovered by him, as given in his book "Zur Blutlehre," he cites, as one proof of the fermentative nature of the substance, that it is completely destroyed by boiling. A footnote, however, shows that he had overstated this point, inasmuch as he calls attention to the fact that "ordinarily one finds that boiling does not completely destroy the ferment." Howell also notes

[5] Addis: Quarterly journal of experimental physiology, 1908, i, p. 305. Addis: Quarterly journal of medicine, 1909, ii, p. 149.

the same fact. In the experiments made upon this point in the present investigation it was found that in aqueous solutions fairly free from proteins the thrombin is not wholly destroyed, and the freer the solution is from proteins, which coagulate on heating, the greater is the amount of thrombin which can bear boiling. Ordinary fresh thrombin solutions, after several minutes of boiling, still cause typical clotting. It is therefore not so thermolabile as often asserted, and its reaction to heat can hardly be cited as proof of its essential similarity to other enzymes.

In dried form it withstands a temperature of at least 135° C. for half an hour. In regaining the thrombin from such dried extracts it is necessary to submit it for several days to the dissolving action of water. The necessity for this no doubt lies in the excessively hard baking to which the mass has been exposed, and the difficulty with which subsequent extraction takes place. The extraction may be facilitated by allowing putrefactive changes to arise, which probably, in a manner to be described later, liberate the thrombin enclosed. The thrombin is, however, completely destroyed when charred or ashed. Extracts after such treatment show no trace of its action. Moreover, thrombin is only slightly diffusible. Placed in a collodion tube, it may be dialyzed against pure water for six or seven hours without serious loss. The conclusion is warranted therefore that thrombin is not a simple inorganic compound.

Recovery of inactivated thrombin. — While aqueous solutions of thrombin withstand, in large part, the boiling temperature, solutions of thrombin containing proteins are at first completely inactivated by that temperature. That the thrombin has, however, not been really destroyed is shown by the fact that its action may be recovered by simple processes. This recovery may be accomplished by submitting the solution of inactivated thrombin to the action of a small amount of sodium hydroxide. Not quite so effective is treatment with a dilute mineral acid for a few minutes. In this way amounts of thrombin may be regained depending upon the maximum temperature used. Instead of the action of the sodium hydroxide or acid, similar results are obtained by submitting the inactivated thrombin solution to putrefactive changes. Such a protein-containing solution of thrombin we have in the serum of the blood. Heating serum to 56° or 57° C. is usually stated to be sufficient to destroy the thrombin present and to insure a thrombin-free serum. This was found not to be strictly true. Active serum may be heated

above 57° C. and its thrombin action regained in part at least. If serum is heated to 65° C. for several minutes, it is found to be inactive when added to a fibrinogen solution. If however such an inactivated serum is treated with a small amount of sodium hydroxide for a few minutes and then neutralized, it will coagulate fibrinogen, whereas a control of heated serum not so activated has no coagulating effect on solutions of fibrinogen.

It is an old observation that serum soon loses its active thrombin on standing, and becomes ineffective in producing a clot. According to older observers the thrombin was supposed to disappear entirely. When later such inactive serum was activated with sodium hydroxide or mineral acids, and a thrombin activity as much as thirty times the original strength of fresh serum was secured, it was explained as being due to a proferment in the blood which had been activated and from which new thrombin had been formed. Such was the β proferment of Morawitz, in distinction from the a proferment which is activated by calcium alone. Later the disappearance of the thrombin from serum was explained as due to the formation of a *metathrombin,* which may be changed back to thrombin by the alkali or acid activation referred to.

The parallelism between the gradual disappearance of thrombin from serum on standing and its recovery by activation, and the disappearance of thrombin from serum on heating to 65° and its recovery by a similar activation, strongly points to the conclusion that these two things are essentially the same phenomenon, and that the disappearance of the thrombin is probably due to its combining in some loose way with some substance, possibly the proteins of the serum, from which it may in turn be liberated by agencies which tend to destroy this combination. That the disappearance of the thrombin is due to such a loose combination with some substance, and that activation by alkalies is due to the breaking apart of this connection, seems probable from the further fact that serum, which has become completely inactive on standing, becomes later, if submitted to putrefactive processes, quite active again. To procure such an active condition by putrefaction often requires a number of days, on account of the remarkable resistance shown by serum to putrefactive changes. Whether this union is of a chemical nature or not, is not easily told. It seems, however, clearly more than mere mechanical adsorption, for neither in the standing serum nor in the serum inactivated by heating to 56° C. is there any precipitation to

make mere mechanical adsorption possible. The process is evidently here more nearly allied to a loose but truly chemical combination.

Thrombin solutions containing proteins, when heated to points above the coagulation points of these proteins, may no doubt carry down the thrombin in a more mechanically adsorbed way.

Experiments make it certain that thrombin does not unite with all proteins. If it is a protein with which it unites, then there is a marked specificity in this respect. If, for instance, serum be allowed to stand, not only will the thrombin originally in it disappear, but it will also inactivate large amounts of thrombin added to it. In some experiments serum was able to neutralize its own volume of a thrombin solution whose activity as tested on fibrinogen was greater than that of fresh serum. If however thrombin is added to a solution of egg-albumen and the mixture is allowed to stand for several days, while its coagulating power is tested from time to time on fibrinogen solutions, it is found that practically none of the thrombin has disappeared, whereas blood serum in a similar experiment loses its coagulating power, unless it is re-activated or is submitted to slight putrefactive changes. If serum be heated to 80° C. *before* the thrombin is added, and the mixture is then allowed to stand, it is found, on testing with fibrinogen solutions, that the thrombin does not completely disappear. Clotting is evident, although not so immediate and complete, if the mixture has stood for several days, as with fresh thrombin solutions. The partial disappearance of the thrombin in this case may be due to the fact that the preliminary heating has only partly destroyed or altered the substance which combines with the thrombin. If this substance consists of the proteins of the blood, we can understand why the serum after being heated to 80° C. still possesses some power to inactivate the thrombin, since it is well known that not all of the protein is coagulated at this temperature.

The view that the union of the thrombin takes place with some blood protein is strengthened further by experiments which showed that thrombin is not neutralized by other known constituents of the blood. Attention was especially directed to cholesterin. Cholesterin solutions, both aqueous and soapy, had however no effect on thrombin solutions after days of standing.

It has been pointed out that the thrombin and fibrinogen unite in quantitatively proportional amounts in the formation of fibrin, and that coagulation in this respect is the mutual precipitation of these

two colloids as suggested by Nolf. But the union seems a relatively loose one. If less thrombin is added to an amount of fibrinogen than is required to precipitate the entire fibrinogen, a partial clot will form. If this clot be removed, no thrombin remains in the fibrinogen solution, and it will remain fluid unless new thrombin is added. But the fibrin removed yields relatively large amounts of thrombin, when "digested" according to the method of Gamgee, in a 5 per cent solution of sodium chloride at 40° C. for several hours. In this "solution of the fibrin" the thrombin is liberated anew. The same result may be reached by submitting the fibrin to putrefactive processes. As the fibrin dissolves, tests with fibrinogen solutions indicate a larger and larger amount of thrombin liberated. The question naturally arises whether, if it is possible to regain the thrombin as thrombin from fibrin, it may not be possible by proper treatment to regain the fibrinogen as fibrinogen.

Isolation of Thrombin.

In approaching the problem of isolating the thrombin, experiments were made to determine the media which dissolve thrombin and those out of which it is precipitated. It is evidently not dissolved by alcohol. In Schmidt's method for obtaining thrombin it is the dried precipitate after the removal of the alcohol that yields the thrombin. That practically no thrombin is dissolved in the supernatant alcohol is shown by the fact that when the alcohol is slowly evaporated and the residue is extracted with water, no perceptible traces of thrombin are obtained.

It is equally insoluble in ether, in chloroform, and in acetone. The dried alcohol precipitate of serum extracted with these liquids gives no trace of thrombin when the extract is evaporated and re-extracted with water. Aqueous extracts, however, of the dried alcohol precipitate, after having been submitted to the ether, chloroform, or acetone for hours, yield the usual amount of thrombin, showing that none has been dissolved out.

But thrombin is very soluble in aqueous solutions. The dried material which had been precipitated from serum by excess of alcohol, when shaken with twice its volume of distilled water for fifteen seconds only, showed a considerable thrombin activity.

Attempts to extract the thrombin with water soon showed that

not only the free salts of the dried material, but some of the protein as well, went into solution, especially so if the extraction was continued a few hours. The salts are readily removed by dialysis against distilled water, and it was hoped the thrombin could be separated from the excess of proteins by reprecipitating the proteins with alcohol and extracting anew with water. In this manner it was found that the proteins were thrown out in large part in three reprecipitations, but the thrombin activity was greatly reduced, in fact practically destroyed. Whether this is due to the fact that the thrombin was coagulated along with the proteins is not certain. It is possible that the toxic action of alcohol is more marked in purer thrombin solutions, while in solutions containing considerable amounts of blood proteins it is protected in some unkown way. The method of reprecipitation gave in all cases finally negative results.

To reduce the amount of proteins to a minimum, the dried alcohol precipitate of the serum was extracted with water for a period not exceeding one minute. In this short time, as stated, considerable amounts of thrombin were extracted, as shown in tests with fibrinogen solutions. Such solutions of thrombin showed only the faintest traces of protein when treated with the ordinary color tests, and they remained clear on boiling. When evaporated only a small amorphous residue remained.

That thrombin is, however, not a nucleo-protein, as believed by Pekelharing, is indicated by the fact that a thrombin solution may be acidulated with acetic acid, and the nucleo-proteins precipitated. If this precipitate is removed by centrifuging or filtering, and the filtrate at once neutralized, the thrombin activity is not materially reduced. The neutralization may not be postponed too long, as acids and alkalies, if allowed to act sufficiently long, are destructive in their effects.

While this experiment shows that we are not dealing with an ordinary nucleoprotein, it is more difficult to arrive at conclusions concerning the protein nature of thrombin. There are some facts which argue against its protein structure. As pointed out by Schmidt and others, it is possible to secure thrombin solutions which are quite active and which give only the faintest traces of protein. As stated in an earlier part of this paper, it stands boiling, and so differs at least from the ordinary proteins associated with it in the blood.

More significant, however, is the resistance of thrombin to putre-

factive changes. Bottles of thrombin containing large amounts of dissolved proteins were allowed to stand for months, until the putrefactive changes had run their normal course. Such thrombin solutions lost none of their activity, in fact they gained materially in the initial stages of the process. It should be pointed out, however, that such solutions, even after several months of standing, still show very distinct protein reactions, so that it would be entirely wrong to hold that the cleavage due to putrefaction had been complete.

On the other hand, some of the properties of thrombin are similar to those of the proteins. It is not readily diffusible. Dialyzed against pure water, it may, as stated before, be separated from salts present without serious loss. It is, however, slightly diffusible through a collodion membrane, and when allowed to dialyze against a smaller amount of water, it may be shown that distinct traces of thrombin appear on the outside after several hours.

Like proteins, it seems to be broken down by peptic and tryptic digestion. Solutions of thrombin submitted to ordinary peptic (acid) or tryptic (neutral) digestion always lost their coagulating activity. But other possibilities than those of regular peptic or tryptic digestion exist. In the case of the pepsin-hydrochloric experiment it may be that the prolonged action of the acid is toxic. Control experiments seemed to show that this was true. In the case of the tryptic digestion there was the disturbing action of the trypsin itself upon the fibrinogen of the test solution. Relatively small amounts of trypsin (neutral) in fibrinogen solutions induce active lytic effects, before coagulation is effected. In some experiments the mixture of thrombin solution and trypsin after having been left at room temperature for eighteen hours was first heated to 40° C. for two hours, and subsequently in order to destroy the trypsin was heated to 65° C. Then by careful activation an attempt was made to restore the thrombin. But all such attempts led to negative results, and it seems probable that thrombin is destroyed by the digestive action of both pepsin and trypsin. While it is impossible therefore to speak definitively as to whether thrombin is a protein or not, it is clear that it is not a simple inorganic compound. Some of the reactions of thrombin suggest its possible relationship with histones or protamines. Attempts have been made to test experimentally whether relatively pure thrombin solutions give the characteristic histone and protamine reactions, especially the precipitation out of aqueous solution by ammonia. The work on this point has not proceeded far enough to warrant any definite conclusions.

Source of Thrombin.

Thrombin exists in serum, but what its antecedents are in the circulating blood is a question that has been variously answered by different investigators. It has been assumed by many that there exists in the circulating blood a proferment or thrombogen or hepato-thrombin, different names by different authors for essentially the same thing. This pro-thrombin Nolf believes is formed in the liver; according to Morawitz it is derived from the formed elements of the blood. Activated by calcium salts, and by a *kinase* derived from leucocytes or tissues, the pro-stage becomes the active thrombin. By others the existence of a definite thrombogen or proferment in normal blood is denied. A series of experiments was undertaken to determine the existence or non-existence of such a body. It was necessary in these experiments to treat the blood in such a manner, at the very moment of taking, as to prevent the usual changes which occur at that instant and which usher in coagulation. The procedure consisted in laying bare the carotid artery of the cat and inserting a cannula. The cannula and short tube were thoroughly oiled with a neutral sterile oil. On opening the artery the first few cubic centimetres of blood were rejected. About 50 c.c. of the blood were then led into a large jar of 95 per cent alcohol. Vigorous stirring of the alcohol brought the small stream of submerged blood into general and instantaneous contact with the greatly excessive amount of alcohol and so insured immediate precipitation and fixation. The remaining amount of blood drawn from the artery (50 c.c.) was led into a beaker and allowed to clot. The serum from this clot was precipitated with twenty times its volume of alcohol. It was thus possible to have two parts of blood from the same animal, — one part precipitated by alcohol, before any coagulative changes occurred, the other part after the process of coagulation had run its course. The two precipitates were then dried, and aqueous extracts were made in the usual way for preparing thrombin solutions. Such an extract from the serum showed, of course, the usual thrombin activity. Extracts from the blood, taken immediately under alcohol, showed *no traces* of thrombin. Tested with fibrinogen solutions, no evidence of clotting of any kind appeared. Many similar experiments by Schmidt and others had shown that, with greater and greater care in taking blood under

alcohol, less and less evidence of "ferment" appeared. It is certain, therefore, that in circulating blood no appreciable amounts of *ready-formed* thrombin exist. *Does a proferment or thrombogen exist?* To determine this, if possible, extracts of the blood caught immediately under alcohol were activated with dilute sodium hydroxide and dilute mineral acids without giving any traces of thrombin. Activation with calcium salts in varying proportion proved equally negative. No evidence of any kind could be found that there existed in the blood extract any substance which could be activated by calcium salts, alkalies, or acids to real thrombin activity.

But, as stated above, those holding to the view that a thrombogen exists in circulating blood assert that the activation is by a kinase in the presence of calcium salts. Extracts of the alcohol blood were therefore made and calcium salts were added in proper proportion. Assuming that a prothrombin was present in the circulating blood, these extracts lacked only the kinase to produce active thrombin. This kinase, according to Morawitz, Nolf, Mellanby, and others, may come from the tissues. Extracts of tissues which had been carefully irrigated with a Ringer's solution were added to the alcohol-blood extract, and after standing for varying lengths of time the coagulating power of the mixture was tested with fibrinogen solutions. In no case was any evidence of thrombin action observed, in spite of the fact that all the supposed elements entering into the formation of active thrombin were present. If it be urged that the treatment with alcohol has destroyed the proferment, it need only be recalled that in the preparation of alcohol plasmas by the alcohol-precipitation method, authors assert the existence of the proferment unimpaired.[4] It seems probable from such results that thrombogen does not exist in circulating blood. In fact, in reading the literature on this point, one is impressed with the fact that the reasons for the existence of a thrombogen in circulating blood rest more upon speculative considerations than upon experimental evidence. It would seem that the normal existence of such a body, whose assumed presence in quantity certainly tends to complicate the interpretation of the process of coagulation, should be granted only when definite experimental evidence makes such an assumption necessary. No such necessity exists to-day, and thrombogen as a normal constituent of blood is a theoretical rather than a demonstrable thing. But thrombin does not exist as such in plasma. It must have material antecedents from which it is produced. The

conclusion seems probable that it has its source under abnormal conditions in greatly accelerated processes which affect the leucocytes or platelets of the blood. Whether the substance formed is a result of secretory activity or a product of cell disintegration, it is impossible to say. But a number of facts point unmistakably to the formed elements of the blood as the source of the pro-stage. Plasma in which the corpuscles have been removed immediately after taking has a greatly increased resistance to coagulation in avian and reptilian blood. In centrifuged blood experiments showed that parts of the oxalated plasma taken from the lower part of the tube near the layer of corpuscles yielded a much more active thrombin solution than a similar amount taken from the top of the tube, where the plasma was much freer from corpuscles. Substances in actual solution in the plasma could scarcely have shown such an unequal distribution. The absence of thrombin or its pro-stage in the blood taken under alcohol as just described finds a ready explanation in the assumption that the corpuscles were in this case coagulated by the action of the alcohol before the normal processes of disintegration were possible. Whether the substance so formed is of an ordinary protein or histone or protamine character, or is a still simpler organic substance, is undetermined, but no matter what its nature may be it is unable to produce the normal formation of fibrin unless activated by calcium salts. It is possible that other as yet undetermined conditions assist in this activation. This activation may find its simplest explanation in assuming that the calcium enters chemically into the thrombin molecule, as suggested by Mellanby, and by Loeb for the coagulins. If therefore the free calcium is thrown out of the blood by chemical agents, it remains fluid because the prothrombin becomes an active coagulating agent only when combined with this minimal amount of calcium, for the rôle of which strontium alone may be substituted with any success.

Sodium-oxalate plasma. — The fluidity of oxalated blood is, as has often been pointed out, due to the precipitation of the calcium. It is not due to a mere inhibition exercised by the oxalate. This was shown by taking oxalated plasma and dialyzing the same in a collodion tube against 0.9 per cent solution of sodium chloride for hours. In this way any excess of oxalate is removed, as may be shown by chemical tests. Centrifuging will remove any precipitate, and a fluid plasma is thus obtained which shows no tendency to coagulate when allowed to stand for days. Additions of very slight amounts

of calcium chloride bring about a speedy clotting, showing that the prothrombin is lacking only in that salt. A point of interest is in the observed fact that such plasma solutions may stand for several days, and yet give immediate clotting upon addition of calcium chloride. The pro-stage of the thrombin does not disappear from the plasma readily. In serum solutions, however, where the activated thrombin is present, this latter disappears very rapidly, as was pointed out in an earlier part of this paper, possibly by combining with some specific proteins in the serum. The thrombin before activation with calcium salts seems not yet to have such combining properties.

The fluoride plasma. — The action of fluorides in preventing the coagulation of the blood has been variously interpreted by different authors. Thus, according to Arthus, to whom we are indebted for the introduction of this method, the calcium salts of the blood are precipitated. In this respect it acts like the alkali oxalates. But, unlike the oxalates, Arthus holds that fluoride prevents the formation of a proferment, because calcium salts in excess do not coagulate fluoride blood. This inhibiting action was believed to be due to a certain fixation of the formed elements of the blood. Bordet and Gengou, noticing the precipitate which is produced when calcium salts are added to fluoride plasma, held that the prothrombin together with some fibrinogen was thrown out of the solution and thus caused the plasma to remain fluid. Mellanby attributes his failure to have the fluoride blood clot with excess of calcium to the precipitation of the fibrinogen when calcium salts are added to fluoride plasma. Nolf considers the fluoride blood a definite indicator, not only for thrombin, but for the presence or absence of his thrombozyme. Fluoride blood does not clot, according to Nolf, because there is no thrombozyme present, its formation having been prevented by the action of the fluoride presumably on the leucocytes and platelets.

In the following experiments it will be shown that none of the foregoing interpretations is entirely correct, but that the action of the fluoride is primarily identical with the action of the oxalate.

If a solution of fibrinogen is made from fluoride plasma by precipitating the fibrinogen several times with a 50 per cent saturated solution of sodium chloride, according to the method described earlier in this paper, and this fibrinogen solution is dialyzed in a collodion tube against an excess of 0.9 per cent sodium chloride solu-

tion, the fibrinogen clots. The clot is firm and typical and the "serum" expressed is peculiarly rich in thrombin. This makes it improbable that the calcium salts are definitely precipitated out of the solution. It is further evident that the prothrombin is present in a fluoride plasma. The prothrombin remains inactivated because the calcium is bound in a peculiar way by the fluoride. This loose connection is broken in the dialysis, the calcium thus freed activates the prothrombin, and active thrombin results. As this occurs in a clear plasma, it is not due to secondary changes of the leucocytes, brought about by the dialysis. It is possible that the calcium and the fluoride are bound to some protein of the plasma in such a way that the calcium activation of the proferment is prevented, and yet loosely enough to be dissociated by the dialysis.

If a solution of calcium chloride, of a concentration of 1 gm. anhydrous calcium chloride to 25 c.c. of water, be added to a fluoride plasma, a copious precipitate appears, which is largely composed of protein material thrown down together with the calcium fluoride. If the calcium chloride solution be added carefully, drop by drop, until the point is reached when the addition of a small drop produces no further precipitate, it will be found that the fluoride plasma so treated clots promptly and normally. Such results show that the statements usually made that calcium salts added in excess to fluoride plasma produce no coagulation are not strictly correct. If in the manner described the fluoride is precipitated by the calcium chloride solution and a slight excess of calcium salts remains, the clotting is similar in every way to that produced in oxalate solutions under similar treatment. Which of the proteins of the plasma are thrown down with the calcium fluoride precipitate has not yet been determined, but it is certainly not the fibrinogen which is primarily precipitated. This is evident from the fact that fluoride plasmas still clot, normally after the heavy precipitate which forms on addition of calcium chloride is removed. To test more directly whether fibrinogen suffers any material loss, sodium fluoride was added to a fibrinogen solution to a proportion similar to that in fluoride plasma. Calcium chloride of the concentration described gave, when added, only a slight precipitate, even after standing several hours. Addition of thrombin to the fibrinogen solution so treated with sodium fluoride and calcium chloride clotted in a manner similar to the control of pure fibrinogen. It is probable, therefore, that the protein precipitate in the experiment is not to any considerable ex-

tent fibrinogen. If however the calcium chloride is in excess beyond a certain point, the coagulation is prevented, and if the excess be considerable, thrombin added to the fluoride plasma remains ineffective. The inhibition is therefore of the formed thrombin itself. That it is the great excess of the calcium chloride which inhibits the thrombin is shown in the further experiment that when sodium fluoride is added to remove the extra excess it brings about a prompt clotting.

It would seem therefore clear that the action of the fluoride is essentially the same as that of the oxalate, with the difference that the fluoride is bound in some way with the calcium and protein and remains in solution in ordinary fluoride plasma. The inability of the calcium so bound to activate the proferment present as in oxalated blood explains the fluidity of the plasma. That the fluoride cannot bind ready-formed thrombin is shown by the fact that such ready-formed thrombin added to fluoride blood produces a normal coagulation. Dialysis breaks the loose combination, liberates the calcium, activation of the proferment follows, and thrombin results.

On the other hand, the addition of a solution of calcium chloride of the concentration given to a fluoride plasma precipitates this fluoride-calcium-protein compound, leaving the proferment and the fibrinogen practically unaltered. A slight excess then of calcium salts suffices to activate the proferment, and the resultant thrombin clots the fibrinogen. Excess of calcium salts, however, beyond a certain point inhibits and finally prevents the action of the thrombin formed. Removal of the excess makes clotting again possible.

The essential identity in the action of oxalate and fluoride plasmas not only explains both as due to their action on the calcium, but it renders evident the fact that fluoride plasma cannot be used either as an indicator for thrombin or for thrombozyme, and inferences drawn from its use in this way must remain untrustworthy.

Do the Tissues yield a Coagulating Agent?

One of the most involved chapters in the study of blood coagulation has been the question, whether there are derived from the tissues, as extracts or otherwise, substances which either clot the blood directly, as thrombin does, or which exert a favorable and accelerating influence in the process. Schmidt's "zymoplastic sub-

stances" were supposed by this investigator to greatly facilitate, if not to make possible, the ordinary process of clotting. The work of Hammarsten made clear, however, that coagulation was a property belonging to the fibrinogen and thrombin alone, and that Schmidt's zymoplastic substances were not necessary to produce a normal clot. Perhaps this fact induced investigators who saw a distinctly favorable influence exerted by the extracts of tissues to interpret this effect, not from the side of fibrinogen, but rather from the side of the thrombin, and the effects were ascribed to tissue thrombins. This is the view of Nolf, who holds that, in addition to the leucothrombin of the blood, the tissues produce histo-thrombins. The extract from the walls of blood vessels he designates as vasothrombin. These views are essentially the same as those of Morawitz, who designates the active substance in the tissue extracts as thrombo-kinase. They do not directly induce clotting, but serve to activate the proferment in the blood. This is further the view of Mellanby, who in his experiments on coagulation used the macerated testes of the cockerel as his source of "kinase." This "kinase" is held to activate the proferment of the blood and so produce the active thrombin which clots the fibrinogen. If this view differs at all from the views of Morawitz, it is perhaps in the emphasis it lays on the kinase.

On the other hand, Loeb, approaching the question from the study of coagulation in invertebrate blood, sees in the extracts of tissues, not kinases, but definite "coagulins" which are able to attack the fibrinogen molecule directly and form fibrin. These coagulins are not identical with thrombin, but have several well-marked characteristics, especially that of their specificity. The question as to the existence of such "kinases" or "coagulins" or "zymoplastic substances" was submitted to examination in the following experiments:

Fibrinogen solutions were made from the blood of an animal in the manner already described. The organ or tissue from the same animal, which was to be studied for the presence of active coagulating substances, was then irrigated by insertion of a cannula in its main artery and the ligaturing of neighboring arteries. The fluid used was Ringer's solution ($NaCl$, 0.9 per cent; $CaCl_2$, 0.023 per cent; KCl, 0.03 per cent; $NaHCO_3$, 0.024), with which the irrigation was continued for several hours. In this way the organ or tissue was thoroughly freed from its contained blood. The pro-

portion of salts was but slightly disturbed, and the tissue cells were not seriously modified. That such is the case is fairly evident from the fact that the mammalian heart, so irrigated, beats for hours. There is therefore no reason to believe that the careful and prolonged irrigation has materially changed the tissues. Muscles, for instance, that still contract have also probably retained the power to produce a kinase or coagulin, if such exist. Moreover, it is not probable that any such "thrombic" substance, if it exists ready-formed in the cells of the tissue, would be washed out by the irrigation, for experiments have shown that thrombin, and so probably similarly acting substances, are only slightly diffusible. After such prolonged irrigation, to remove every trace of blood or serum, bits of the organ or tissue were macerated in 0.9 per cent solution of sodium chloride, and portions of this macerated tissue were put into fibrinogen solutions. Of another part of the irrigated tissue filtered saline extracts were made, and these were tested from time to time on fibrinogen solutions. In not a single case in the repeated series of experiments was there found any evidence of coagulation. All such fibrinogen solutions remained fluid, like the controls beside them, while the addition of thrombin induced the ordinary clotting at once. The sweeping statement just made needs a slight modification. In the case of two tissues the results were not completely negative. One of these was the macerated red marrow of bone. This extract produced a slight coagulum over its surface, which did not however extend very far into the solution, and there never formed anything approaching a typical clot. Whether this is to be interpreted to mean that there exists in red bone marrow a source of thrombin is open to question. The marrow remained red after the irrigation, and its extracts were slightly discolored by the hemoglobin. It is possible, remembering the finer structure of this tissue, that perfect irrigation under the circumstances was impossible and that the tissue still contained blood or serum. The other tissue was the spleen. Macerated and extracted, it produced in fibrinogen solutions small flocculi, which soon settled to the bottom as a white precipitate. There was no evidence of clotting. With these two qualifying statements the experiments in every case gave not the slightest evidence of the presence of any substance inducing coagulation. There were, then, evidently no thrombins or coagulins present. Were "kinases" present? These would require prothrombin and calcium salts to produce an active thrombin. The prothrombin

being supposed to exist in circulating blood, it ought to be extracted when blood taken immediately under alcohol and dried is subjected to the dissolving action of water. It has been pointed out that the supposed prothrombin in alcohol plasmas has been procured in essentially the same way. Such extracts when combined with calcium and extracts of tissues thoroughly irrigated gave no evidence whatever of the presence of a coagulating agent. Evidently in the chain of argument as usually presented there is a missing link. Either the prothrombin of the circulating blood or the kinase, or perhaps both, are non-existent. Thus throughout these experiments no hint of a coagulin or a kinase could be discovered. They left unexplained, however, the obvious facts that some forms of blood or plasma do react immediately and definitely to tissue extracts. The experiments on the blood of terrapins and birds noted by previous observers were repeated. Terrapin's blood taken carefully through an oiled cannula and put into a perfectly clean beaker will remain fluid for days. The blood may be centrifuged, and the clear supernatant plasma is then equally or more resistant to spontaneous clotting. If however tissue extracts or pieces of tissue be added, the coagulation is pronounced and immediate. The most apparent explanation is that of a thrombin or coagulin or kinase present in the extract. But this blood or plasma can be made to clot equally well and equally rapidly in ways which preclude the presence of such a definite agent. If, for instance, dust particles, loose sweepings, or linty shreds be added, the coagulation is equally prompt, and in a number of experiments was more rapid than with tissue extract. Boiling the tissue extracts was found not to decrease the coagulating action in the least. Tissue extracts of terrapin were dialyzed against distilled water for twenty-four hours. In this way not only were the free salts removed, but much of the dissolved protein (globulin) was precipitated and the solution seemed relatively much clearer. Such solutions showed a very much diminished effect in producing clotting. It is therefore hard to escape the conclusion that simple physical factors take part in facilitating the coagulation of the terrapin's blood.

Similar experiments were made on birds. The blood was taken, under precautions against foreign contamination, from the femoral artery of a chicken. Such blood, as pointed out by Delezenne, remains fluid for a long time. Centrifuged, it gives a clear plasma which will stand uncoagulated for days. If to such blood tissue

extracts of either the bird or the terrapin be added, clotting as in the case of terrapin's blood is immediate, and the clot is firm. If the clear plasma is taken, the clotting is not quite so rapid. It is held that these tissue extracts contain a "kinase" which activates the prothrombin to thrombin. But the bird's blood or plasma clots with practically the same rapidity and firmness if dust particles are generously added. Bits of down-feathers introduced bring about a speedy clotting. Surely there can be no question of a "kinase" in these instances. Ringer's solution hastens the coagulation time. On the other hand, macerated testis when dialyzed against distilled water for twenty-four hours and then filtered gives a solution which has lost much of its accelerating property. Whether this loss is due to the absence of the mineral salts in the extract, or to the precipitation and removal of the cellular debris from the same, is not clear. Probably both factors enter into the result. Boiling the testicular extract, or other tissue extracts, had no effect, showing that the accelerating substance is not in the least thermolabile, and so differs very greatly from thrombin, which boiled in protein solutions suffers a marked diminution in strength. Mellanby states that he could find no kinase in the muscular tissue of birds. In my experiments in which the fresh plasma of the bird was used muscle extract was practically as effective as extract of testes. In Mellanby's experiments the fibrinogen had been prepared by dilution of plasma with water, acidulation, the centrifuging of the precipitate, and its final re-solution *in toto* in water. Such solutions clotted slowly with calcium salts alone, and very rapidly with extract of testes. Extract of muscle, however, no longer sufficed to produce clotting. It is difficult to escape the conclusion that his fibrinogen solution still contained large amounts of all the coagulating factors of the fresh plasma and showed essentially the same reactions. Remembering the ease with which bird's blood clots on the addition of a great number of entirely foreign substances, and that the rapidity and firmness of the clotting is not materially below that produced by tissue extracts, further investigation would seem necessary before the effect caused by tissue extracts can be attributed to a specific kinase. The supposed absence of such a kinase in muscular tissue, where it would appear to be of the greatest significance and value, if kinases play the important rôle assigned to them, is not easily understood. It has been suggested that the resistance of the blood of bird and reptile to coagulation may be due to the relatively greater

difficulty with which the formed elements disintegrate, and that the explanation of the action of extracts, especially those containing cellular detritus, and of dust particles, lies in the fact that these facilitate the breaking down of the corpuscles. On the other hand, it may be that the foreign substances added form simply "nuclear" points which by some physico-chemical reaction facilitate or initiate the process of clotting. That the tissue extracts of bird or terrapin do not contain an active coagulating agent at all similar to thrombin is shown by the following experiment: Extracts of carefully irrigated tissues of bird or terrapin when added to fibrinogen solutions prepared from cat's blood show no coagulating property whatever, while the fresh serum of bird or terrapin when added causes a prompt clotting, showing that in the latter the thrombin is present in its usual form. This experiment also calls attention to the fact that tissues not thoroughly irrigated will of course show clotting properties, in direct proportion to the amount of blood remaining, and that extracts made from such unirrigated tissues must give untrustworthy results.

Anti-thrombins.

The question whether or not anti-thrombins exist in the blood has been variously answered by different observers. In this series of experiments only one clear evidence of anti-thrombic action was met. This was the neutralizing effect of serum. Fresh serum soon becomes inactive on standing, and relatively large quantities of a strong thrombin solution added to such a serum disappear entirely in a short time. The thrombin may be recovered, in part at least, by proper alkali or acid activation. There is no reason why a similar neutralizing action might not be exercised by the circulating plasma, and in this manner the small amounts of thrombin which probably are being formed all the time may be destroyed. Direct experimental evidence on this point is lacking. The argument of Morawitz, for the existence of an anti-thrombin in blood, because very small amounts of thrombin produce only partial clots, which do not proceed farther, in oxalate or fluoride blood, is to be differently interpreted. This partial clotting is due to the quantitative relations which hold between thrombin and fibrinogen.

Bordet and Gengou, following the general methods of preparing immune sera, injected rabbit serum into the guinea-pig, and so pro-

duced a serum which retarded or destroyed the coagulating action of rabbit serum. This anti-action was upon the thrombin and not upon the fibrinogen. They found that when fresh rabbit serum was added to the fresh serum of the guinea-pig, this latter serum having been previously heated to 58° C. and this mixture was added to the bird's plasma, the latter clotted. If however the serum of a guinea-pig, previously injected as described, was heated to 58° C. and then added to the serum of the rabbit, and this mixture added to bird's plasma, there was no clotting. Accordingly it was assumed that there existed in this immune serum a specific anti-body, not destroyed by a temperature of 58° C., which had inhibited the action of the thrombin in the rabbit's serum.

The immune serum when not heated will, however, at once clot bird's plasma. The anti-body is therefore not effective against the thrombin of its own serum. Whether we are dealing here with the formation of a real anti-body, or whether the results are more involved and due to the play of other factors, further investigation must show. As Morawitz states, the experiments are not fully conclusive and admit of different interpretation. With the more recent production of various specific anti-bodies, such for instance as the anti-chymosin of Morgenroth, it seemed desirable to repeat the work on the possible production of an anti-thrombin which should be effective for the animal's own blood. The presence of such an anti-thrombin in excess would be indicated by a condition of hæmophilia. In the first experiment a medium-sized dog was taken, and the time of coagulation of its blood was determined, by taking a small amount from the vein of the ear. Subcutaneous injections of a thrombin solution were then made every other day, in doses which beginning with 2 c.c. were increased 2 c.c. each succeeding time. The thrombin solution was made by extracting the heated dried alcohol precipitate of serum with distilled water. To insure an active solution, the thrombin was tested on solutions of fibrinogen. After such injections, covering a period of two weeks, the animal was killed. The blood clotted in the normal time, and the serum, when tried on fibrinogen solutions, was found to have the usual coagulating strength. There was therefore no evidence of any kind of the existence of an anti-thrombin. Nor was there any evidence of intravascular coagulation.

In the second experiment a rabbit was taken and active thrombin was injected subcutaneously in amounts which beginning with 2 c.c.

were gradually increased to 20 c.c. The coagulation time of the blood was determined from time to time. It was found that the injection of the thrombin was entirely without effect. There was no evidence of hæmophilia, and the serum after clotting contained the usual amount of thrombin. These results seem to indicate that the organism cannot produce an anti-body for thrombin, or, if it does, the anti-body is not produced in the great excess usual with antibodies. It has been pointed out, however, in an earlier part of this paper that fresh serum is able to neutralize not only its own active thrombin but added excessive amounts. The neutralization is believed to be due to a loose combination with some element of the blood, most probably the proteins. It is possible that the disappearance of thrombin from the circulating blood may be due to the same action. The observed fact that anti-bodies cannot be artificially produced by the injection of relatively simple organic substances, but are limited to substances which are essentially protein, may mean that the thrombin is of the former class, although this observation unsupported by other evidence would be inconclusive as to its non-protein structure.

General Conclusions.

The experiments here reported point to the following general conclusions:

1. The coagulation of blood is probably not a fermentative process. Definite statement on this point is complicated by the present unsatisfactory knowledge of the exact nature of ferments and their action. As usually understood, however, ferments act in the manner of a catalytic agent, and they are thermolabile. The coagulation of blood, on the contrary, is, in the first place, clearly a quantitative reaction, and is probably, as stated by Nolf, a mutual precipitation of two colloids, fibrinogen and thrombin. The partial clots which never proceed farther, the successive clots which may be formed by successive additions of definite amounts of thrombin, and the approximate equality in amount between the sum of these successive clots and a maximum initial clot, indicate a definite quantitative proportionality. Careful quantitative weighings of the fibrin formed, as reported in the earlier part of the paper, showed such a direct proportionality between fibrinogen and thrombin as would be required in a definite chemical combination of two substances.

The further resistance of the thrombin in aqueous solution relatively free from dissolved proteins to boiling temperatures is a property not usual with the ordinary catalytic ferments. The recovery of thrombin from protein solutions, such as serum, which have become inactive on standing, or by being heated to 60° C., points toward the same conclusion. Such a recovery, whether by activation with alkali or acid or by putrefactive changes, is believed to depend upon the dissociation of the combination of thrombin with some protein other than fibrinogen. In a similar way the partial recovery of thrombin from fibrin, according to Gamgee's method, is more than the solution of mechanically adsorbed amounts. The three main reasons given by Schmidt originally as proofs of the truly fermentative nature of his ferment are as follows:

(*a*) Minimal amounts of "fibrin-ferment" will produce a complete coagulation.

(*b*) The artificial serum contains after coagulation unused ferment with which several successive clots may be produced.

(*c*) The ferment is destroyed at 100° C.

The first point has been shown to be incorrect. Minimal amounts produce correspondingly small clots. The quantitative relationship holds with all submaximal amounts of thrombin added to a fibrinogen solution. Nor is it possible to find unused thrombin in a fibrinogen solution if a submaximal amount has been added. If the clots are removed after four to twenty-four hours, the remaining fibrinogen solution remains fluid, but clots promptly again on addition of further amounts of thrombin. The probabilities are therefore that the action of the thrombin is in no way that of an ordinary catalytic agent, but that the formation of the fibrinogen is essentially the mutual precipitation of two colloids from the plasma.

2. It has been impossible to find experimental evidence for the assumption that there exists preformed in the blood a thrombogen or proferment.

3. The existence of "kinase" or "coagulins" in the various tissues is improbable. Using carefully prepared fibrinogen solutions, extracts of tissues, irrigated to remove every trace of thrombin-containing blood, cause no clotting. When the addition of such extracts produces coagulation in bloods of bird or reptile, similar results can be secured by the addition of substances such as dust, lint, shreds, etc., which preclude the presence of definite coagulating agents. These results render very probable the assumption that in

such plasmas all the factors of coagulation are in reality present, and the addition of tissue extract or other foreign substance brings into the mixture nothing that can be regarded as a coagulin or as a kinase.

4. The active coagulating agent is thrombin, whose properties and reactions were the chief questions of inquiry in this work. It is derived from the formed elements, in all probability, at the moment of their rapid disintegration when placed under abnormal conditions. In this pro-stage it is inactive in the blood. To become an active precipitating agent it is believed that minimal amounts of calcium salts enter chemically into the molecular complex. Such calcium salts exist in the normal plasma, and the action of sodium oxalate depends on the precipitation of the calcium out of the solution. The action of sodium fluoride consists in preventing the combination of the calcium with the pro-stage of the thrombin, the calcium being bound but not actually precipitated.

5. An anti-thrombin cannot be produced artificially by subcutaneous injection of active thrombin, but the serum contains substances, possibly the proteins, which gradually neutralize the thrombin even when formed in excessive amounts, and this property is perhaps important in maintaining the fluidity of the circulating blood.

6. On the basis of the work here presented it is not necessary to assume the existence of a *kinase* in explaining the clotting of shed blood. The prothrombin formed from the platelets and leucocytes by secretion or by processes of disintegration is activated to thrombin by the calcium salts present, and the thrombin so formed combines in quantitative fashion with the fibrinogen to form fibrin.

7. The proferment found in the blood under abnormal conditions is not readily neutralized. It remains in the calcium-free serum for days, and when activated forms thrombin promptly. Thrombin, on the other hand, disappears very quickly from active serum unless it is re-activated. The ability of the thrombin to unite with other substances of the blood arises only after the calcium activation of the prothrombin.

8. The action of oxalates and fluorides in preventing coagulation is primarily the same. Both salts act by removing the calcium. In the oxalated plasma the free calcium is definitely precipitated, in the fluoride plasma the free calcium remains *bound* in solution. Dialysis or extreme dilution breaks the loose connection and thrombin results. Excess of calcium salts clots oxalated solutions at once. Excess of

calcium salts of sufficient concentration clots fluoride plasmas, after the fluoride-calcium-protein combination is precipitated by this method. In both plasmas great excess of calcium salts inhibits and finally prevents coagulation, although it would seem that fluoride plasma is in this respect more readily inhibited than the oxalate plasma, — a difference which may result from the formation of the heavy precipitate in the fluoride blood.

HYDROLYSIS OF OX MUSCLE.[1]

By THOMAS B. OSBORNE and D. BREESE JONES.

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

ALTHOUGH the muscle substance of the ox forms one of the most important foods for man, the only evidence at present recorded which in any way indicates the nature and proportion of the amino-acids which it yields on hydrolysis consists of a single determination, made by Abderhalden and Sasaki,[2] of the mono-amino-acids yielded by syntonin prepared by extracting this muscle with dilute hydrochloric acid, and determinations by Hart [3] of the basic amino-acids in the same substance. Whether or not these results represent approximately the proportions of the various amino-acids which would result from hydrolyzing the entire muscle substance can only be determined by comparing the amount of the products of hydrolysis of the two substances. We have therefore undertaken the work described in this paper in order to make this comparison and thereby increase our knowledge of this important food product, and also to obtain a comparison of its products of hydrolysis with those of the muscle substance of the chicken,[4] fish,[5] and scallop [6] already made in this laboratory. The results of these hydrolyses, together with those of syntonin above mentioned, are given in the table on page 438.

The ox muscle and the syntonin yield approximately similar quantities of some of the products of hydrolysis, but in respect to others marked differences exist, some of which, as for instance that

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

[2] ABDERHALDEN and SASAKI: Zeitschrift für physiologische Chemie, 1907, li, p. 404.

[3] HART: *Ibid.*, 1901, xxxiii, p. 348.

[4] OSBORNE and HEYL: This journal, 1908, xxii, p. 348.

[5] OSBORNE and HEYL: *Ibid.*, 1908, xxiii, p. 81.

[6] OSBORNE and JONES: *Ibid.*, 1909, xxiv, p. 161.

shown by glycocoll, may be explained by the presence of connective tissue in the entire muscle, while others cannot be thus explained, as for example the smaller quantity of leucine, proline, phenyl-

HYDROLYSES OF THE MUSCLE SUBSTANCE OF DIFFERENT ANIMALS.

Substance.	Scallop.	Fish.	Chicken.	Ox.	Syntonin Ox.[1]
Glycocoll	0.00	0.00	0.68	2.06	0.50
Alanine	+ ?	+ ?	2.28	3.72	4.00
Valine	+ ?	0.79	+ ?	0.81	0.90
Leucine	8.78	10.33	11.19	11.65	7.80
Proline	2.28	3.17	4.74	5.82	3.30
Phenylalanine	4.90	3.04	3.53	3.15	2.50
Aspartic acid	3.47	2.73	3.21	4.51	0.50
Glutaminic acid	14.88	10.13	16.48	15.49	13.60
Serine	?	?	?	?	?
Tyrosine	1.95	2.39	2.16	2.20	2.20
Arginine	7.38	6.34	6.50	7.47	5.06[2]
Histidine	2.02	2.55	2.47	1.76	2.66[2]
Lysine	5.77	7.45	7.24	7.59	3.26[2]
Ammonia	1.08	1.33	1.67	1.07	0.83[2]
Tryptophane	present	present	present	present	?
Total	52.51	50.25	62.15	67.30	47.11

[1] ABDERHALDEN and SASAKI: Zeitschrift für physiologische Chemie, 1907, li, p. 404. [2] HART: *Ibid.*, 1901, xxxiii, p. 348.

alanine, and aspartic acid yielded by syntonin and also the very much smaller amount of lysine.

From the results given in the table it is seen that the entire muscle substance of different types of animals is similar in respect to the proportion of phenylalanine, aspartic acid, tyrosine, arginine, histidine, and lysine. The muscle of the fish appears to yield distinctly less glutaminic acid than the others, and, as was shown in a previous

paper, there is good reason to believe that an actual difference exists between them in this respect. The amount of glutaminic acid obtained from the scallop, chicken, and ox muscles agrees so closely that no actual difference in the proportion of this amino-acid can be assumed to exist between them. Although the chicken, fish, and ox muscles yielded nearly equal quantities of phenylalanine, the scallop muscle yielded so much more than the others that it is practically certain that in this respect it actually differs from them. Glycocoll, alanine, valine, leucine, and proline increase in proportion as we go from the lower to the higher forms of life. This difference is so marked for the three acids first mentioned that it does not seem probable that it can be explained by imperfections of the methods of analysis or by faults in carrying them out. Furthermore, the amount of mixed amino-acids, which could not be separated in a state of sufficient purity to weigh, was distinctly greater in the case of the chicken and the ox muscle, in which the proportion of these acids actually weighed was decidedly greater than in the analyses of the scallop and fish muscles. The results of these hydrolyses of different muscles, while giving evidence that differences in the proportions of their products of hydrolysis exist between them, show no such wide differences as are found between some of the different kinds of protein of vegetable origin which are used for human food. Thus, as an example, the proteins of the cereals differ in a very marked degree in the high proportion of glutaminic acid, proline, and ammonia which they yield, the small proportion of arginine and histidine, and the entire absence of lysine from some of them. The proteins of many of the nuts and oil seeds which are used for food differ from the muscle proteins notably in the very large proportion of arginine which they yield and the very much smaller amount of lysine. Many of the proteins of the leguminous seeds show a great similarity to these muscle substances in the close agreement in the proportions of the various amino-acids which they yield, and it is possible that for this reason these seeds have their high reputation as sustaining food.

Experimental Part.

The material used for this hydrolysis consisted of a piece of very lean muscle cut from the flank of an animal immediately after slaughtering. This was at once reduced to small pieces in a meat-

chopping machine and extracted repeatedly with large quantities of water saturated with toluol. It was then extracted several times with 95 per cent alcohol and finally with absolute alcohol. After extracting several times with large amounts of ether the substance was air-dried and ground to a coarse powder. In this condition it contained 8.01 per cent of moisture, when dried at 110°, and 0.53 per cent of ash. The ash and moisture free material contained 16.18 per cent of nitrogen.

Of the ox muscle thus prepared 500 gm., equivalent to 457.3 gm. of ash and moisture free substance, were hydrolyzed in three separate portions of 100, 100, and 300 gm. each by heating with 1000 c.c. of hydrochloric acid, sp. g. 1.11, for two hours on a water bath, and then boiling for twenty-one hours in an oil bath.

Glutaminic acid was separated from each portion by saturating the somewhat concentrated solutions with hydrochloric acid gas and allowing them to stand in an ice box for several days. There separated 13.50, 12.05, and 37.85 gm., respectively, of glutaminic acid hydrochloride, which after deducting the ammonium chloride present was equal to a total of 50.20 gm. of free glutaminic acid, or 10.98 per cent of the muscle. The free acid gave the following analytical results:

Carbon and hydrogen, 0.2008 gm. subst., gave 0.3005 gm. CO_2 and 0.1105 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.79; H 6.17 per cent.
Found . . . . . . = C 40.81; H 6.16 " "

The filtrates from the glutaminic acid hydrochloride were united and concentrated under diminished pressure to a thick syrup and esterified by the method of Emil Fischer. The free esters were then liberated, shaken out, and dried over anhydrous sodium sulphate. The aqueous layer was freed from inorganic salts and the esterification repeated. The total esters thus obtained yielded the following fractions when distilled in the usual way:

Fraction.	Temperature.	Pressure.	Weight.
I	100°	18.00 mm.	31.23 gm.
II	70°	0.80 "	48.88 "
III	100°	0.33 "	98.77 "
IV	110°	0.25 "	48.76 "
V	190°	0.28 "	64.78 "
	Total	. . . .	292.42 gm.

The undistilled residue weighed 79.00 gm.

Fraction I. — The esters of this fraction were saponified by boiling with water for eight and one-half hours, the solution evaporated to dryness under diminished pressure, and proline extracted with boiling absolute alcohol. The part remaining undissolved when subjected to fractional crystallization yielded a fraction containing the more soluble amino-acids from which 0.91 gm. leucine and 2.30 gm. of a mixture of leucine and valine were obtained. The leucine had the following composition:

Carbon and hydrogen, 0.1799 gm. subst., gave 0.3632 gm. CO_2 and 0.1561 gm. H_2O.

Calculated for $C_6H_{13}NO_2$ = C 54.92; H 9.99 per cent.
Found . . . . . = C 55.06; H 9.71 " "

In order to separate the mixture of leucine and valine, use was made of the method of Van Slyke and Levene [7] by which the insoluble lead salt of leucine is precipitated from an ammoniacal solution under definite conditions. There were thus obtained, after regenerating the free acids, 1.04 gm. of leucine and 1.01 gm. of valine.

The rest of this fraction was then esterified, and 1.8 gm. of glycocoll ester hydrochloride, equivalent to 0.98 gm. of free glycocoll, isolated. The glycocoll ester hydrochloride crystallized from alcohol in prisms which melted sharply at 144°.

Chlorine, 0.1114 gm. subst., gave 0.1138 gm. AgCl.

Calculated for $C_4H_{10}O_2NCl$ = Cl 25.41 per cent.
Found . . . . = Cl 25.26 " "

After removing the hydrochloric acid from the filtrates from the glycocoll ester hydrochloride, 6.65 gm. of alanine were obtained, which crystallized in the characteristic prisms and gave the following results on analysis:

Carbon and hydrogen, 0.1351 gm. subst., gave 0.2016 gm. CO_2 and 0.0921 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.42; H 7.92 per cent.
Found . . . . = C 40.70; H 7.63 " "

Fraction II. — After saponifying the esters of this fraction with boiling water as usual and evaporating the solution to dryness under

[7] Van Slyke and Levene: Proceedings of the Society for Biology and Experimental Medicine, 1909, vi, p. 54.

diminished pressure, the proline was extracted with boiling absolute alcohol. From the residue which was insoluble in alcohol, there were isolated 14.30 gm. of leucine, 0.91 gm. of valine, and 10.38 gm. of alanine.

The alanine crystallized from water in hard dense prisms.

Carbon and hydrogen, 0.1571 gm. subst., gave 0.2344 gm. CO_2 and 0.1088 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.42; H 7.92 per cent.
Found . . . . . . = C 40.69; H 7.75 " "

A fraction was also obtained consisting of a mixture of leucine and valine from which 0.61 gm. of leucine and 1.8 gm. of valine were isolated by the lead method. Analysis showed the leucine to have the following composition:

Carbon and hydrogen, 0.1696 gm. subst., gave 0.3404 gm. CO_2 and 0.1487 gm. H_2O.

Calculated for $C_6H_{13}NO_2$ = C 54.92; H 9.99 per cent.
Found . . . . . . = C 54.74; H 9.81 " "

The valine gave the following results when analyzed:

Carbon and hydrogen, 0.1230 gm. subst., gave 0.2311 gm. CO_2 and 0.1040 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.24; H 9.47 per cent.
Found . . . . . . = C 51.24; H 9.46 " "

The different fractions of valine were united and racemized by heating with baryta in an autoclave for twenty-seven hours at 175°–180°. The baryta was removed quantitatively from the solution, and the valine converted into the phenyl-hydantoic acid derivative, which crystallized from water in hexagonal plates and decomposed at about 160°.

Carbon and hydrogen, 0.1380 gm. subst., gave 0.3095 gm. CO_2 and 0.0846 gm. H_2O.

Calculated for $C_{12}H_{16}O_3N_2$ = C 60.98; H 6.83 per cent.
Found . . . . . . = C 61.17; H 6.86 " "

Fraction III. — The amino-acids of this fraction consisted almost entirely of leucine and proline. After saponifying the esters and evaporating the solution to dryness, the proline was extracted with

boiling absolute alcohol. There were isolated from the residue insoluble in alcohol 36.41 gm. of leucine.

Carbon and hydrogen, 0.1486 gm. subst., gave 0.2788 gm. CO_2 and 0.1193 gm. H_2O.

Calculated for $C_6H_{13}NO_2$ = C 54.92; H 9.99 per cent.
Found . . . . = C 54.66; H 9.75 " "

The filtrate from the leucine was boiled with copper hydroxide, and 2.57 gm. of copper aspartate were separated.

Copper, 0.1069 gm. subst., air dried, gave 0.0311 gm. CuO.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07 per cent.
Found . . . . = Cu 23.24 " "

The united alcoholic extracts containing the proline from Fractions I, II, and III were evaporated to dryness under diminished pressure, and the residue redissolved in absolute alcohol. After standing for several days an insignificant amount of substance separated, which was filtered off and the filtrate evaporated to dryness and the proline converted into its copper salt. No appreciable amount of racemic-proline copper could be separated. The l-proline, after drying to constant weight at 110°, weighed 33.73 gm., equivalent to 26.61 gm. of free proline.

For identification the copper salt was decomposed with hydrogen sulphide, and the free proline converted into the phenyl-hydantoine derivative. It crystallized from water in beautiful prisms which melted sharply at 144°.

Carbon and hydrogen, 0.1840 gm. subst., gave 0.4473 gm. CO_2 and 0.0929 gm. H_2O.

Calculated for $C_{12}H_{12}N_2O_2$ = C 66.64; H 5.59 per cent.
Found . . . . = C 66.30; H 5.65 " "

Fraction IV. — After extracting the phenylalanine ester from this fraction by shaking out with ether and saponifying the ether soluble esters with strong hydrochloric acid, 3.41 gm. of phenylalanine hydrochloride were obtained.

The aqueous layer which remained after the extraction with ether was saponified by boiling with baryta, and 7.43 gm. of aspartic acid were isolated in the form of a barium salt. Analysis of the free aspartic acid obtained by decomposing the barium salt with sulphuric acid showed it to have the following composition:

Carbon and hydrogen, 0.2327 gm. subst., gave 0.3107 gm. CO_2 and 0.1093 gm. H_2O.

Calculated for $C_4H_7O_4N$ = C 36.07; H 5.30 per cent.
Found . . . . . . = C 36.41; H 5.26 " "

The filtrate from the barium aspartate, when freed from barium, was boiled with copper hydroxide, and 5.87 gm. of copper aspartate were obtained.

Copper, 0.1363 gm. subst., air-dried, gave 0.0394 gm. CuO.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07 per cent.
Found . . . . = Cu 23.10 " "

No serine or other definite product was obtained from the solution which remained.

Fraction V. — The phenylalanine ester was extracted from this fraction with ether and converted, in the usual way, into the hydrochloride, of which 12.02 gm. were obtained. By treating the filtrate from the phenylalanine hydrochloride with ammonia 1.75 gm. of free phenylalanine were separated.

Carbon and hydrogen, 0.1453 gm. subst., gave 0.3498 gm. CO_2 and 0.0907 gm. H_2O.

Calculated for $C_9H_{11}O_2N$ = C 65.42; H 6.72 per cent.
Found . . . . . . = C 65.66; H 6.98 " "

From the aqueous layer, after saponifying with baryta, 5.07 gm. of aspartic acid, in the form of the barium salt, were isolated. The free acid reddened but did not decompose at about 300°.

The barium was removed quantitatively from the filtrate from the barium aspartate, and the solution concentrated and saturated with hydrochloric acid gas. After standing for several days in an ice box 17.41 gm. of glutaminic acid hydrochloride separated, which decomposed at 197°.

Chlorine, 0.1833 gm. subst., gave 0.1421 gm. AgCl.

Calculated for $C_5H_{10}O_4NCl$ = Cl 19.35 per cent.
Found . . . . = Cl 19.17 " "

When the hydrochloric acid was removed from the filtrate with silver sulphate and the amino-acids converted into their copper salts, 8.40 gm. of copper aspartate separated, which without being recrystallized gave the following analysis:

Copper, 0.1555 gm. subst., gave 0.0448 gm. CuO.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.06 per cent.
Found . . . . = Cu 23.02 " "

After removing the copper from the filtrate from the copper aspartate and saturating the concentrated solution with hydrochloric acid gas, 1.21 gm. of glutaminic acid hydrochloride separated.

The Residue after Distillation.

The residue remaining after the distillation of the esters, which weighed 79 gm., was dissolved in boiling alcohol. On cooling, 1.15 gm. of crystalline substance separated. The filtrate from this was saponified by boiling with baryta, and after removing the baryta quantitatively with sulphuric acid, 7.13 gm. of glutaminic acid hydrochloride were isolated by the usual process.

The free glutaminic acid decomposed at 203° and had the following composition:

Carbon and hydrogen, 0.2476 gm. subst., gave 0.3713 gm. CO_2 and 0.1341 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.79; H 6.17 per cent.
Found . . . . . . = C 40.90; H 6.06 " "

Ether Distilled from the Esters at 760 mm.

The ether which was distilled from the esters at 760 mm. was treated with about 100 c.c. of a cold saturated solution of hydrochloric acid in absolute alcohol. After standing for several days a substance separated in fine needles which melted between 140° and 144°, thus showing it to be nearly pure glycocoll-ester-hydrochloride.

The ammonium chloride was removed by boiling the substance with baryta, and the baryta removed quantitatively with sulphuric acid. After concentrating the solution to a small volume a crystalline precipitate separated which weighed 10.51 gm. This, when recrystallized from absolute alcohol, separated in prisms which decomposed at about 190°. Analysis showed this to be the di-glycocoll hydrochloride described by Horsford[8] and later by Kraut and Hartmann.[9]

[8] Horsford: Annalen der Chemie und Pharmacie, 1846, lx, p. 1.

[9] Kraut and Hartmann: *Ibid.*, 1865, cxxxi, p. 99.

Chlorine, 0.1457 gm. subst., gave 0.1127 gm. AgCl.

Calculated for $(C_2H_5O_2N)_2$ HCl = Cl 19.00 per cent.
Found . . . . = Cl. 19.12 " "

Carbon and hydrogen, 0.1707 gm. subst., gave 0.1591 gm. CO_2 and 0.0946 gm. H_2O.

Calculated for $(C_2H_5O_2N)_2$ HCl = C 25.73; H 5.94 per cent.
Found . . . . = C 25.42; H 6.20 " "

Horsford, and also Kraut and Hartmann, obtained this compound of glycocoll by treating an alcoholic solution of the mono-hydrochloride with ammonia, a method analogous to the treatment by which the product described above was obtained.

We found that while the di-glycocoll hydrochloride was easily soluble in cold 95 per cent alcohol it could be recrystallized from hot *absolute* alcohol.

The 10.51 gm. of this compound of glycocoll thus obtained is equivalent to 8.46 gm. of free glycocoll, which, together with that isolated from Fraction I, amounts to 9.44 gm., or 2.06 per cent of the muscle hydrolyzed.

Partition of Nitrogen.

The partition of nitrogen in the beef muscle determined by the modified Hausmann method was:

Nitrogen as ammonia	0.89 per cent
Basic nitrogen	4.42 " "
Non-basic nitrogen	10.44 " "
Nitrogen in magnesium oxide precipitate . . .	0.43 " "
Total nitrogen	16.18 per cent

The nitrogen contained in the histidine, arginine, and lysine was equal to 4.32 per cent of the dry muscle, which agrees very closely with that precipitated by phosphotungstic acid, thereby indicating the probable accuracy of the determinations of these bases.

THE RATE OF DIGESTION IN COLD-BLOODED VERTEBRATES. — THE INFLUENCE OF SEASON AND TEMPERATURE.

By OSCAR RIDDLE.

[*From the Laboratories of Experimental Therapeutics and Zoölogy, University of Chicago.*]

ALTHOUGH the digestive glands of the lower vertebrates have been the subject of numerous histological researches, and the digestive activity of the secretions of these organs has often been tested, there has been until now no attempt to gather data on the actual and relative digestion power of the different *groups* of vertebrates. It is possible, moreover, to derive the latter kind of data neither from the findings recorded in the works just mentioned nor from the very vague natural history observations bearing on this subject.

Certain phenomena already reported by me[1] and having to do with digestion processes which occur periodically at the periphery of the growing ova of many vertebrates have primarily led me to make the present inquiry as to the digestion powers or capacities of members of three groups of lower vertebrates, and to determine the influence of season and temperature upon the rate of digestion in these animals.

These data promised to be of value, too, (1) as supplying a measure of the functioning capabilities of the digestive organs of forms occupying different positions in the vertebrate series; and the fixing of a definite value to the influence upon this process of (2) season and of (3) temperature. The results seem to justify this expectation. They give, furthermore, considerable support to a proposition that was quite unexpected, and which is possibly of considerable import, namely, that as the lower cold-blooded vertebrates were evolved into the higher cold-blooded forms the latter progressively *lost* in digestion capacity.

A full discussion of the importance of a gradual decline of diges-

[1] RIDDLE, O.: Paper before the American Society of Zoölogists, Chicago, Dec. 29, 1907; and Abstract in Science, 1908, xxvii, p. 945.

tion capacity within the cold-blooded vertebrate series, to a consideration of the determining factors of the evolution of the higher vertebrates, and a treatment of the phenomena of periodic digestions occurring in ova, as mentioned above, will, however, not be attempted here. These subjects can be best treated in another connection. We limit this communication to a presentation of such data as is suggested by its title.

The data are by no means exhaustive; the rate of digestion has been measured in only about 150 individuals — representing 6 species — and though there are some rather definite findings, the value of the data probably lies not so much in what is conclusively proved as in what is suggested, and in the light thrown upon topics which heretofore have remained quite unilluminated. It is hoped that some one may find oportunity to extend these observations, and to test the present result with data derived from other orders of the lower vertebrata.

Methods and Materials.

Representatives of three vertebrate classes were studied: fishes, Amphibia, reptiles. The fresh-water "dog fish" (Amia calva), the common frog (Rana virescens), the mud puppy (Necturus maculatus), and the turtle (Emydoidea blandingii) furnished the principal material. Several sun fish (Lepomis) and a few snapping turtles (Chelydra) were also used.

A tank of 70 gallons' capacity was so constructed and equipped as to provide running water of any desired constant temperature. The animals to be used in the experiment were transferred to this tank from large aquaria in which the main supply was kept (in water of outdoor temperature), and the temperature of the experimental tank then slowly brought to the desired point. The animals were brought in from their natural habitats during November, March, and July (no Amia or Amphibia in March).

The temperature of the tank was recorded at frequent intervals. The difference between the tank temperature and the stomach temperature was determined to the 0.1 of a degree, for each individual animal used; such comparative readings were taken at that particular tank temperature at which the animal was to live during the experiments. It was thus possible to calculate the average

stomach temperature of the animal — *i. e.*, the actual temperature at which the digestion occurred — for the whole period of the experiment.

Metts [2] tubes of lengths appropriate to the size of the animal's stomach were prepared and inserted directly into the stomach. After periods ranging from seventeen to one hundred and sixty-eight hours, the animals were killed, the tubes recovered, and the amount of digestion recorded; the amount of digestion being indicated by the number of millimetres of albumen that had been digested from the end of the tube. In all cases, too, a small piece of fresh beef was fed along with the tube. All of the measurements recorded represent *gastric* digestion. On a number of specimens of Amia and turtles, however, observations were also made on the rate of intestinal digestion.

Both the method and results of the study of intestinal digestion may be briefly stated here. Tubes short enough to pass easily the pyloric curve of the stomach into the intestine were fed to Amia and turtles. The time necessary for the tubes to enter the intestine was determined approximately. Tubes were then fed and recovered only after twenty-four or forty-eight hours beyond the time necessary for them to reach the intestine. Since the rate of gastric digestion per hour was known from the study of other specimens, and since a further control was in some instances obtained by feeding one long tube (which would remain in the stomach) along with the short one, it was possible to roughly calculate the rate of digestion

[2] The Metts tubes were made from glass tubing 2.5 mm. in diameter, filled with fresh egg-albumen which had been strained through fine cheese cloth and coagulated (within the tubes) at 95° C. In regard to the use of coagulated albumen in the Metts tubes it may be said that Yung (Recherches sur la digestion des poissons, Archives de zoologie expérimentale, 1899, vii, ser. 3, p. 121) and Van Herwerden (Zur Magenverdaung der Fische, Zeitschrift für physiologische Chemie, 1908, lxv, p. 453) have found this substance not well adapted for a study of digestion in fishes. They found that the albumen digests much more slowly than some other proteins, *e.g.*, fibrin. For comparative purposes, however, and for such work as easily permits an extension of the time of digestion there can hardly be objection to the use of egg-albumen.

It is of historical interest to note that Spallanzani (Expériences sur la digestion de l'homme et de différentes espéces d'animaux, avec des considérations sur sa méthode de faire des espériences, etc., by Jean Senebier, Geneva, 1783) studied digestion in fishes by filling tubes with the flesh of fishes, putting these into the stomachs of pikes, eels, barbels, and carp and recovering them at intervals.

in the intestine of these two forms. Eight apparently valid measurements on Amia showed a rate of intestinal digestion three to four times the rate of gastric digestion in Amia. Five good measurements on Emydoidea and two on Chelydra indicated likewise an intestinal digestion about four times more powerful than the gastric digestion of these turtles.

The Rate of Digestion.

The actual measurements of the rate of gastric digestion in Amia, Rana, Necturus, and Emydoidea are given in Tables I, II,

TABLE I.

Amia calva.

No. of specimen.	Temp.	Time. (hr.).	Digestion in mm.	Season.	No. of specimen.	Temp.	Time. (hr.).	Digestion in mm.	Season.
1	23.0	17	3.0	Nov.	14	2.5	168	trace	Nov.
2	23.5	24	4.0	"	15	2.6 8.0	168 48	0.4	Dec.
3	22.9	48	4.9	"	16	8.6	48	2.5	"
4	22.9	48	5.1	"	17	8.3	49	3.0	"
5	3.3	24	0	"	18	8.2	96	4.0	"
6	3.5	24	0	"	19	8.6	96	1.5	"
7	3.4	24	0	"	20	9.9	168	6.0	March
8	3.0	48	0	"	22	24.9	48	3.8	"
9	3.5	96	trace(?)	"	23	24.7	48	4.2	"
10	3.2	120	"	"	24	24.8	72	7.0	"
11	2.9	144	"	"	25	25.0	72	4.5	"
12	2.5	168	"	"	26	29.8	48	11.0	April
13	2.8	168	"	"	27	29.8	48	7.0	"

and III. These may be consulted for the main facts. It will be noted (1) that there is very considerable individual variation; (2) the rate of digestion is highest in the fish and lowest in the turtle; (3) season is an important factor; (4) temperature modi-

fies the rate of digestion very markedly; (5) the minimum temperature at which digestion is possible is not the same in the several

TABLE II.

Necturus.					Turtle (Emydoidea).				
No. of specimen.	Temp.	Time. (hr.).	Digestion in mm.	Season.	No. of specimen.	Temp.	Time. (hr.).	Digestion in mm.	Season.
1	22.8	25	1.3	Nov.	5	10.1	96	0.4	March
2	23.1	51	2.3	"	6	10.2	168	1.0	"
4	8.6	48	1.4	Dec.	7	10.3	168	0.6	"
5	8.8	49	1.5	"	8	24.7	48	3.0	"
6	8.7	72	3.0	"	9	24.8	72	2.5	"
7	8.6	96	3.6	"	10	24.9	101	6.1	"
8	9.9	96	2.3	March	12	17.7	24	0.8	July
9	10.2	168	2.4	"	13	17.7	24	2.0	"
10	10.0	168	5.0	"	14	17.7	24	1.2	"
11	9.9	168	4.4	"	15	18.0	48	4.2	"
12	10.0	168	1.0	"	16	18.0	48	1.8	"
13	24.9	29	2.0	"	17	18.2	72	4.0	"
14	24.6	48	1.2	"	18	28.7	24	3.2	"
15	24.6	48	2.0	"	19	28.7	24	1.6	"
16	24.9	72	1.5	"	20	28.6	24	3.2	"
17	24.9	101	6.1	"	21	28.7	24	0.5	"
18	25.0	101	3.8	"	22	28.7	24	9.0	"
19	24.9	101	3.1	"	23	28.6	24	1.7	"
20	24.9	101	3.7	"	..	...	...	...	...
21	17.8	24	0.5	"	..	...	...	...	...

forms, but is lowest in those forms which are lowest in the vertebrate series, the order being Amia, Necturus, Rana, turtle; (6) the maximum temperature at which digestion may occur appears to be

nearly the reverse, *i. e.*, it is highest for those highest in the vertebrate scale. This, however, does not seem to hold for the fish.

TABLE III.

Frog (Rana virescens).

No. of specimen.	Temp.	Time. (hr.).	Digestion in mm.	Season.	No. of specimen.	Temp.	Time. (hr.).	Digestion in mm.	Season.
1	22.8	24	1.7	Nov.	36	24.7	48	4.7	March
2	23.3	24	4.0	"	37	24.7	48	3.0	"
3	23.3	52	5.6	"	38	24.7	48	8.0	"
4	23.1	52	7.0	"	39	24.7	48	5.5	"
5	3.2	24	0	"	40	24.7	48	4.0	"
6	3.2	37	0	"	41	24.7	48	7.0	"
7	3.3	48	0	"	42	24.7	48	4.2	"
8	3.1	96	0	"	43	24.7	48	3.3	"
9	2.8	144	0	"	44	24.7	48	1.4	"
10	2.9	168	0	"	45	29.8	48	5.3	April
13	8.9	72	1.2	Dec.	46	29.8	48	7.5	"
15	8.9	96	2.0	"	47	29.8	48	9.1	"
16	8.6	96	1.2	"	48	29.8	48	8.0	"
17	10.4	48	1.2	March	49	29.8	48	6.0	"
18	10.4	48	0.4	"	50	29.8	48	7.6	"
19	11.0	96	1.6	"	52	18.2	24	3.0	July
20	10.6	96	4.2	"	53	18.2	24	4.5	"
21	10.7	168	3.5	"	54	18.2	24	5.5	"
22	10.7	168	3.0	"	56	18.2	24	5.5	"
23	24.8	26	2.3	"	57	18.2	24	1.3	"
26	24.8	26	2.0	"	58	18.2	25	7.5	"
27	24.6	40	2.2	"	59	18.5	48	4.5	"
28	24.7	48	7.0	"	60	27.7	24	2.4	"
29	24.7	48	5.0	"	61	27.7	24	4.5	"
30	24.7	48	2.9	"	62	27.7	24	5.5	"
31	24.7	48	7.5	"	63	27.7	24	4.2	"
32	24.7	48	1.2	"	64	27.7	24	3.3	"
33	24.7	48	4.0	"	65	27.7	24	7.1	"
34	24.7	48	5.5	"	66	27.7	24	4.7	"
35	24.7	48	3.5	"	67	27.7	24	2.3	"

These several results — with the exception of the first — are brought into still better relief by the summary presented in Table IV. There the rate of digestion is expressed in millimetres per

hour; the number given being the quotient obtained by dividing the total digestion in millimetres (for all the individuals of a given species kept at a given temperature) by the total number of hours of digestion. The temperatures indicated are the averages of the mean stomach temperatures of the several individuals used in the experiment.

The Influence of Season and Temperature.

Season. — A comparison of the figures given in Table IV shows the following facts concerning the influence of season upon diges-

TABLE IV.

Month.	Temp.	Amia.	Temp.	Frog.	Temp.	Necturus.	Temp.	Turtle.
November	23.1°	.1241	23.1°	.1204	23.0°	.0474	...	....
November	3.1°	.0	3.1°		....	....	...	....
December	8.2°	.0380	8.8°	.0167	8.7°	.0358	...	....
March . .	9.9°	.0357	10.6°	.0249	10.0°	.0197	10.2°	.0046
March . .	24.8°	.0812	24.7°	.0924	24.8°	.0389	24.8°	.0525
April . . .	29.8°	.1875	29.8°	.1510	...	....	...	....
July . . .	...	....	18.2°	.1642	...	....	17.9°	.0583
July . . .	...	....	27.7°	.1807	...	....	28.7°	.1333

Amia : Frog :: 7 : 6.[1]
Amia : Necturus :: 17 : 8.
Amia : Turtle :: 29 : 14.
Frog : Necturus :: 31 : 16.
Frog : Turtle :: 2 : 1.
Necturus : Turtle :: 117 : 113

[1] All comparable averages of the pair are considered; correction is made for the slight temperature differences.

tion: (1) There is a considerable reduction (about one third of total) of digestion power in all of the forms studied between November (23.1°) and March (24.7°). Other figures for December (8.2°) and March (9.9°) show the same thing, with the exception that the frogs show no such distinction (when correction is made for temperature differences the latter shows practically no change

in digestion power). Necturus has in December more than twice the digestion power (at low temperatures) which it possesses in March. (2) In July the digestion power of the forms studied is much greater than in March and April. The seasonal increase here represents about one third of the total digestion. (3) Apparently all of the animals are capable of digestion at all seasons provided they are given a suitable temperature. (4) Midwinter to March is the period when digestion capacities are at the lowest point; while midsummer (July) is the season most favorable to digestion.

It thus appears that the period of low digestion power falls within the fasting period of these animals, and the maximum of digestion power is attained during those months when a maximum of feeding is the rule. This relation of maximum and minimum digestion power to the maximum and minimum feeding periods of the animals agrees with Gillespie's (1898) results on the Scottish salmon, though the seasonal relations are there reversed. We note here the significant fact that the digestions which take place at the surfaces of ova occur during the period of lowest digestion power of the alimentary canal.

Another factor which doubtless influences the several digestion powers is the variation in acid content of the gastric juice. Richet has shown that in fasting fish (Scillium) the latter fluid is neutral in reaction, whereas a maximum of digestion efficiency is obtained only with a rather high acid content.

Our own figures clearly indicate that in all the cold-blooded forms studied a smaller amount of proteolytic ferment is secreted during the fasting (winter) than during the feeding period. Gillespie found that the amount of enzyme *extractable* from the gastric epithelium of the fasting (summer) salmon was much decreased.

Temperature. — The data indicate that the effects of temperature on the digestive processes must be considered under two heads: First, the accelerating action of increased temperature on the chemical processes involved; and second, the retarding action of very high or very low temperatures due (*a*) to the production by the animal of smaller amounts of digestive enzymes under these conditions or (*b*) to the actual destruction of the enzymes by these extreme temperatures. This retarding action will be considered first.

There is no doubt that at very *low* temperatures the quantity of pepsin secreted into the stomachs of these animals is decreased.

Since the rate of digestion at a given temperature, other conditions being equal, is a function of the amount of enzyme present, such a decrease in the amount of enzyme must cause a decrease in digestive power. At temperatures too *high* for the comfort and well-being of the animal, it may be that a diminished secretion also occurs. It is certain, however, from the work of other observers, that the ferment itself — *i. e.*, the pepsin containing extracts of the gastric mucosa of fishes — has its power diminished by a temperature of even less than 40° C. The statements in the literature on this point are rather vague and contradictory and should be reviewed in connection with the data presented in Tables I to V.

Brinton[3] says: "The same heat which is practically necessary for the function of this organ (stomach) in a warm-blooded animal annihilates the efficacy of the pepsin of many fishes." Fick and Murisier[4] declared that in the pike and the trout the peptic digestion is not more rapid at 40° than at 10° C. Hoppe-Seyler confirmed the statement. Luchhau,[5] however, states that in the salmon and pike the digestion was more rapid at 40° than at 15° C. Richet and Mourrut[6] say that at 40° the mammal digests faster than the fish, while at 32° the reverse is true. Krukenberg[7] is more definite in a declaration that the pepsin of a fish acts as well at 20° as at 40° C. Gillespie's[8] less definite statement is: "If the active secretion (pepsin) be tested outside the body at a higher temperature (no figures named), a very much more powerful action is exerted on proteids than by ordinary peptic extract obtained from the gastric mucous membrane of mammals." The last statement on this subject is apparently the following by Yung:[9] "une augmentation artificielle de la température d'un squale qui l'élèverait à la tempéra-

[3] Brinton, W.: Proceedings of the Royal Society, London, 1860–1862, xi, pp. 357–359.

[4] Fick, A., and Murisier: Verhandlungen der Würzburger physikalischen medicinischen Gesellschaft, 1873, iv, p. 120.

[5] Luchhau, E.: Inaugural dissertation, Königsberg, 1878.

[6] Richet, Ch., et Mourrut: Comptes rendus de l'Académie des Sciences de Paris, 1880, lxxxvi, p. 879.

[7] Krukenberg, C. F. W.: Untersuchungen aus dem physiologischen Institut der Universität zu Heidelberg, 1882.

[8] Gillespie, A. L.: Report Fishery Board for Scotland (Noël Paton, ed.), 1898

[9] Yung, E.: Recherches sur la digestion des poissons, Archives de zoologie expérimentale, 1899, vii, ser. 3, p. 121.

ture d'un mammifère, loin de gêner sa digestion, l'activerait au contraire."

I have not been able to carry through a sufficient number of experiments to determine the exact points at which high and low temperatures absolutely inhibit the action of the pepsin of the animals under consideration. The data obtained does, however, add to our information on these points; and at the same time some reason is furnished for the contradictory results of other workers.

A glance at our Table IV shows that for both Amia and the frog a rise of temperature from 25° C. to 30° is accompanied by a very marked increase in the rate of digestion. Again, in the turtle a rise of temperature (July) from 18° to 28° C. more than doubles the rate, while in marked contrast a similar elevation of the temperature of the frog at this season increases its rate by less than one fifth. These facts would indicate, first of all, that season is an important factor to be reckoned with in this connection, and is fully capable of supplying the data for the discrepant results of other workers cited above.

The destructive action of low temperatures on the pepsin of cold-blooded vertebrates receives scant attention in the literature. Fick and Murisier [4] furnish the statement that the pepsin of the trout and the pike can digest at a temperature "near zero." Yung [9] makes the same declaration for many species of fishes.

Our data show that 3 or 4° C. is the minimum for Amia in November and December. The point of more considerable interest which our data brings out, however, is that among the classes of cold-blooded vertebrates the minimum temperature at which pepsin will act differs widely, being lowest in the fishes, intermediate in the amphibia, and highest in the turtles. In other words, the study of six species indicates that as evolutionary development occurred within the vertebrate group the more highly evolved forms progressively lost their capacity for peptic digestion at low temperatures. Facts already known [10] enable us to extend this statement beyond the amphibia and reptiles to the warm-blooded forms — birds and mammals.

The action of temperature first mentioned above, namely, the accelerating action of increased temperature upon the chemical processes involved, is treated in the following section.

[10] See RICHET and MOURRUT, cited elsewhere.

THE TEMPERATURE COEFFICIENT OF DIGESTION.

The well-known rule of Arrhenius and van't Hoff concerning the velocity of chemical reactions in relation to temperature, namely, that each increase of 10° C. in temperature multiplies the speed

TABLE V.

TEMPERATURE COEFFICIENTS OF DIGESTION.

AMIA.			
November & December	8.2° = rate of .0380;	23.1° = rate of .1241;	= 2.19
March	9.9° = rate of .0357;	24.8° = rate of .0812;	= 1.55
March & April	9.9° = rate of .0357;	29.8° = rate of .1875;	= 2.55
March & April	24.8° = rate of .0812;	29.8° = rate of .1875;	= 4.53
FROG.			
November & December	8.8° = rate of .0167;	23.1° = rate of .1204;	= 5.71 [1]
March	10.6° = rate of .0249;	24.7° = rate of .0927;	= 1.94
March & April	10.6° = rate of .0249;	29.8° = rate of .1510;	= 3.16
March & April	24.7° = rate of .0924;	29.8° = rate of .1510;	= 3.20
July	18.2° = rate of .1642;	27.7° = rate of .1807;	= 1.19 [1]
NECTURUS.			
November & December	8.7° = rate of .0358;	23.0° = rate of .0474;	= 0.93 [1]
March	10.0° = rate of .0197;	24.8° = rate of .0389;	= 1.62 [1]
TURTLE.			
March	10.2° = rate of .0046;	24.8° = rate of .0525;	= 7.81 [1]
July	17.9° = rate of .0583;	28.7° = rate of .1333;	= 2.12
Average for eight valid measurements = ...			2.62

[1] See discussion in the text.

of such reactions by 2 or 3, should of course apply to the digestive process. It is of considerable interest to know whether in *living* animals this result is attained, within what limits it is attained, and whether the cold-blooded animals vary in this respect. Obviously, in the living, digesting organism the temperature changes may affect not only the action of the ferment present on the hydrolysis of food materials, but such other factors conditioning the digestion rate as the amount and acidity of the secretion, the rate of absorption, etc. The measurements and calculations recorded in Table V represent, I believe, the only attempt thus far made to answer the above-mentioned questions.

The tabulated [11] results indicate (1) that within certain not very wide ranges of temperature the rule of van't Hoff applies to the digestive processes in living cold-blooded vertebrates, the average of eight valid coefficients being 2.62. (2) The range of temperature within which the speed of digestion is doubled with a rise of 10° is different for the different classes of vertebrates; this range being smallest or most restricted in the two types of Amphibia used. (3) The five coefficients which in the table have been designated with the reference figure 1 reveal influences of the temperature used, which are quite apart from their effect on the speed of chemical reactions: those numbers which are greater than 3.00 indicating that the lower temperature of the two temperatures compared exercises a destructive or inhibitive action on the digestive secretions; whereas numbers smaller than 2.00 indicate that the higher temperature of the two temperatures compared likewise inhibits or destroys ferment action.

[11] These coefficients are obtained as the quotients resulting from $\frac{\text{Rate at } Tn}{\text{Rate at } T \times \frac{x}{10}}$. Tn being the higher temperature, T the lower temperature, and x the difference between these two.

THE RELATION OF IONS TO CONTRACTILE PROCESSES. — IV. THE INFLUENCE OF VARIOUS ELECTROLYTES IN RESTORING MUSCULAR CONTRACTILITY AFTER ITS LOSS IN SOLUTIONS OF SUGAR AND OF MAGNESIUM CHLORIDE.

By RALPH S. LILLIE.

[*From the Marine Biological Laboratory, Woods Hole.*]

IT is well known that all varieties of muscle suffer gradual loss of irritability when transferred from the normal medium or its equivalent physiological salt solution to pure isotonic solutions of sugar or other indifferent non-electrolyte. The action of these substances is not toxic — dilution of the normal medium with its volume or more of sugar solution does not materially affect the properties of the tissue; the essential condition of the change is the withdrawal of certain electrolytes; when these are restored, irritability returns. Anæsthetics produce a similar temporary and reversible suspension of function, and the basis of the effect is in all probability the same in both cases. Muscular contractility in the intact organism thus depends on the presence of electrolytes in the external medium; and the researches of J. Loeb and Overton, with others, have shown that sodium salts, usually in association with small quantities of potassium and calcium, have a quite specific and peculiar relation to this property.

Other contractile tissues differ from muscle in their relation to the electrolytes of the medium. Many cilia continue their activity in media practically free from salt (fresh-water Protozoa, Vermes, Mollusca); the contractile fibrils or myonemata in the ectosarc of many ciliate Protozoa (*e. g.*, Stentor) and the stalk of Vorticella seem also independent of salts in the external medium. In marine animals the cilia show a more evident dependence on the salts and are gradually checked in isotonic sugar solutions (*e. g.*, Arenicola). The above relation of sodium salts to contractility thus appears characteristic of muscle but not of contractile tissues in general.

Overton[1] has made an extensive study of the action of different salts in restoring contractility to frog's muscle after prolonged immersion in isotonic sugar solutions. He finds sodium salts to be the most effective; of the other alkali metals lithium and to a less degree cæsium (and ammonium) partly exhibit this power, but not rubidium or potassium; the alkali earth chlorides also show it, but to a still more limited degree. The specific relation of sodium ions to irritability is thus a striking peculiarity of this form of contractile tissue. What is the ground of this specificity?

Hoeber[2] has answered this question in essentially the following manner: in solutions containing sodium salts the plasma membrane preserves its normal permeability, but not in solutions in which sodium is replaced by other metals. Both Overton and Hoeber refer the toxic action of potassium salts to an alteration of this normal permeability. Assuming with Bernstein[3] and Brünings[4] that the normal potential difference between exterior and interior of the resting cell, as shown by the demarcation current, depends on the peculiar permeability of the plasma membrane (free penetrability to certain cations, but not to anions), Hoeber has investigated the influence of ions on the permeability of muscle by studying the alterations which the various salts induce in the demarcation current. He finds in general that muscular irritability is restored only by those salts in whose solutions the normal external positivity is found, *i. e.*, in which, on the membrane theory, the plasma membrane shows its peculiar differential permeability in relation to the two classes of ions. The salts affect permeability by their action on the colloids of the plasma membrane; sodium salts affect these colloids in such a manner as to impart that peculiar consistency to which the normal permeability corresponds. The characteristic electrical surface polarization, on which the possibility of stimulation depends, is thus restored in favorable solutions of sodium salts. Potassium salts, on the other hand, induce abnormal increase in permeability; of this the local negativity resulting from their action is the sign; the loss of irritability and eventual toxicity are due to

[1] OVERTON: Archiv für die gesammte Physiologie, 1902, xcii, p. 346; *Ibid.*, 1904, cv, p. 176.

[2] HOEBER: Physikalische Chemie der Zelle und der Gewebe, chapters 8, 10. *Cf.* also, Archiv für die gesammte Physiologie, 1907, cxx, p. 492.

[3] BERNSTEIN: Archiv für die gesammte Physiologie, 1902, xcii, p. 521.

[4] BRÜNINGS: *Ibid.*, 1903, xcviii, p. 241; 1903, c, p. 367.

the abolition of the normal permeability. Hence such salts cannot restore irritability.

It is essentially because stimulation is associated with a negative variation similar to that resulting from the local action of potassium salts or from other injurious influences — many of which can be shown to increase permeability — that a temporary increase in permeability is assumed to be an essential feature of the stimulation process. Salts thus affect irritability by influencing the condition of the plasma membrane and so determining the readiness with which the permeability change of stimulation is effected. The all-importance of specific external electrolytes to such a tissue as muscle is therefore evident, since its normal physiological action depends on the preservation of a condition of permeability which only sodium salts — typically in association with smaller quantities of calcium and potassium salts — can give.

In a recent paper [5] I have attempted to show that this temporary increase in permeability — which Overton, Bernstein, Brünings, and Hoeber agree in regarding as a fundamental feature of the process — is in itself sufficient to account for the increased liberation of energy which is the essential consequence of stimulation. It is assumed that increased permeability of the plasma membrane will facilitate the escape of metabolic products from the cell; and since the increased energy production following stimulation is associated with an increased loss of carbon dioxide,[6] the hypothesis is suggested that it is the increased rate of escape of this oxidation product from the tissue that determines the increased rate of oxidation and so of energy production in the tissue at this time. Rapid removal of the reaction products from a chemical reaction promotes, and their accumulation checks, the progress of the reaction; these corollaries of the law of mass action must apply to the energy-yielding oxidative reactions in living cells. The rate of oxidation and so of energy production in muscle must then have a functional dependence on the rate of escape of the final oxidation products — mainly carbon dioxide; this rate must be profoundly influenced if not altogether determined by the degree of permeability of the plasma membrane. Increased permeability thus means increased evolution of carbon dioxide, hence increased oxidation and energy

[5] LILLIE, R.: This journal, 1909, xxiv, p. 14.

[6] Not yet satisfactorily demonstrated for nerve, where, however, the energy production is admittedly very slight.

production within the tissue; this, on the present hypothesis, is the condition during stimulation. Conversely, decreased surface permeability means decreased loss of carbon dioxide and hence retarded oxidation and energy production, — in a word, inhibition. The plasma membrane thus constitutes, by virtue of its varying permeability, perhaps the chief means of regulating the velocity of oxidative and no doubt other metabolic processes within the cell.

The exact conditions of the high velocity of these processes in the living cell are not understood; apparently favorable combinations of catalyzers and co-ferments exist, and the structural conditions in the tissue favor their action; under these conditions the progress of reactions to equilibria will be rapid; and disturbances of equilibria, as by the above changes in permeability, will have correspondingly marked and rapid effects. The promptitude and the energy of the response to stimulation may conceivably thus be explained. Just why reaction velocities are so high in living cells constitutes of course a separate problem.

It will probably be agreed that increased energy production is the essential and primary change following stimulation. The question as to the exact means by which the transformed chemical energy is converted into the mechanical energy of contraction is a distinct problem for which various solutions have been suggested. The evidence, in my opinion, favors some form of the general view expressed by various physiologists (d'Arsonval, Bernstein, Imbert, J. Loeb, and others) that the energy of muscular contraction is transformed surface energy. Perhaps the most distinctive feature of colloidal systems is their large potential surface energy,[7] and presumably a portion of this appears as free mechanical energy in contraction. Increase in the surface tension of the colloidal particles forming the fibrillæ would involve increased coherence of the contiguous particles in the fibril and consequently shortening. Increase of surface tension is a change such as would lead in a simple colloidal solution to coalescence of particles and eventually to coagulation; such a change may therefore be called coagulative, and it is possible that a reversible coagulative change of this kind, under the influence of hydrogen ions freed in oxidation, may be the immediate condition of the contraction.[8] Bernstein's proof that

[7] *Cf.* Wolfgang Ostwald's article in Oppenheimer's Handbuch der Biochemie, Bd. i, p. 839.

[8] Lillie, R.: *Loc. cit.*; also This journal, 1908, xxii, p. 75.

the temperature coefficient of contractile energy, with a stimulus of given intensity, is *negative,* supports strongly the general view that muscular energy is transformed surface energy;[9] and the only surface of area sufficient to account for the quantity of energy transformed is the united surface of the colloidal particles forming the contractile elements.

Alterations in the permeability of the plasma membrane involving stimulation or the reverse may be variously induced. In the preceding paper I have considered the stimulating and inhibiting action of electrolytes and fat solvents from this point of view. The present paper describes the results of a study of the action of different electrolytes and combinations of electrolytes in restoring contractility to the musculature of Arenicola larvæ after its removal by the action (1) of sugar solutions and (2) of various electrolyte solutions, chiefly magnesium chloride.

EXPERIMENTAL.

I. Restoration of Contraction after Treatment with Dextrose Solutions.

In pure solutions of non-electrolytes muscular contractions gradually disappear; ciliary movement is also retarded and eventually ceases, but may continue for some hours before its final disappearance. The following is typical of the action of isotonic dextrose solutions:

June 8, 1908. — Arenicola larvæ were collected in watch glasses by heliotropism in the usual manner and placed in *m*-dextrose solution (Kahlbaum's dextrose in flat solid cakes) at 10.35 A. M.; after the larvæ had settled the solution was changed to remove all trace of sea water. Observation at five-minute intervals showed the following:

10.40. A. M. Larvæ swim slowly and collect in clumps; cilia are much slower than normal; larvæ are becoming stiff, but still show sluggish muscular contractions.

10.45 A. M. Cilia continue slowly; larvæ are rigid, but show some slight muscular contraction; when the larvæ are distributed uniformly through the solution by a pipette, the ciliary action causes them to collect at the bottom of the watch glass in groups or clumps. The larval body shows slight shrinkage from the cuticle.

[9] Bernstein: Archiv für die gesammte Physiologie, 1908, cxxii, p. 129.

10.50 A. M. Slow ciliary movement; larvæ are quite rigid, no muscular contractions are seen. There is now distinct shrinkage from the cuticle.

11.00 A. M. Condition as at 10.50. Slow ciliary movement continues; no muscular contractions are seen.

In dextrose solutions ciliary movement may continue for two to three hours, but muscular contractions are always found to have completely or almost completely ceased after twenty to thirty minutes. Some variation has been found in the action of different solutions of dextrose; Kahlbaum's dextrose is not quite free from chlorides, and a part of the variation may be due to this circumstance. Thus, while the above record is typical, I have found in a number of experiments occasional muscular contractions after an hour or even more in the dextrose solution. Such movements are slight and close observation is required to detect them. A number of careful comparisons were made between dextrose solutions and solutions of Kahlbaum's crystallized cane sugar, which appears absolutely free from electrolytes. Thus in three separate determinations with *m*-cane-sugar solutions all ciliary movement was found to have ceased in a little over an hour (a trace of movement remained in one experiment after one hour and seventeen minutes); in dextrose solutions some ciliary movement nearly always remains after two hours. Muscular contractions disappear in cane-sugar solutions as in dextrose; in the above three experiments careful search showed feeble contractions in a few larvæ after thirty, forty-five, and fifteen [10] minutes respectively. Solutions of these two non-electrolytes have thus closely similar action; but in dextrose solutions complete disappearance of contractions usually requires somewhat longer. This difference may be due to the slight electrolyte content of the dextrose solutions. In the following experiments with salts I have used dextrose solutions to deprive the muscles of contractility; these are more satisfactory than cane-sugar solutions on account of their relatively slight viscosity and low specific gravity, — which is less than that of the larvæ, while with cane-sugar the contrary is the case.

Action of pure solutions of various sodium salts. — In the experiments about to be described I have studied the action of a series of salts in restoring contractility to larvæ after its removal by dextrose solutions. Kahlbaum's salts were used in practically all cases.

[10] No observation between fifteen minutes and forty-three minutes in this case. After forty-three minutes prolonged observation showed no trace of contraction.

Muscular contractions, having at first an almost normal character, invariably return after transfer from sugar solutions to pure solutions of sodium salts. The injurious action of the pure salt solution quickly becomes evident, and contractions, at first energetic, become weaker and soon cease, within an interval which varies considerably with the different salts according to the nature of the anion. Pure solutions of sodium salts are also highly injurious to cilia, most of which, as a rule, are arrested and in large part disintegrated within the first few seconds after transfer; a few may remain active for some time longer; this effect also shows significant variations according to the nature of the anion, and is more rapid (*e. g.*) with bromide or iodide than with acetate, sulphate, or tartrate.

The following record will illustrate:

TABLE I.

June 11, 1908. — Larvæ were transferred from sea water to *m*-dextrose solution at 2.35 P. M.; this solution was changed for fresh at 2.45. The following solutions of sodium salts in *m*/2 concentrations were added to portions of larvæ in watch glasses at the times indicated (the action of KCl, NH_4Cl, and LiCl was also studied in this series; these salts will be considered later). The immediate effect of each salt was observed under the microscope with about 60 diameters' magnification. The intensity of the immediate stimulating action is indicated by the promptitude and vigor of the initial contraction or shortening which immediately follows the contact of the solution; this varies in a highly characteristic manner with the nature of the anion; the character of this initial contraction is therefore indicated in each case.

1. *m*/2 NaCl. 3.17. Muscular contractions begin at once with a moderate initial contraction. Cilia are partly disintegrated at once, but many continue slowly. By 3.20 muscular contractions are very feeble; by 3.25 none are seen and most cilia have ceased. At 3.49 there is no muscular movement and cilia have practically ceased.
2. *m*/2 NaBr. 3.26. Well-marked initial contraction; arrest and disintegration of cilia are more complete than with NaCl; a few cilia remain feebly active. By 3.28 muscular contractions have almost ceased. A trace of ciliary movement remains at 3.47; no movement is seen later.
3. *m*/2 NaI. 3.30. Marked initial contraction; feeble muscular movements during the succeeding relaxation; cilia nearly all cease and liquefy at once. By 3.32 muscular contractions have practically ceased — a trace of movement is seen. At 3.44 a trace of ciliary movement persists; no muscular movement. No movement seen later.
4. *m*/2 $NaNO_3$. 3.38. Moderate initial contraction; muscular contractions somewhat slight and cease soon; cilia mostly cease at once. At 3.40 a few contractions at intervals; a little ciliary movement. At 3.43 a few contractions. At 3.50 no muscular movement; trace of ciliary. No movement later.
5. *m*/2 $NaClO_3$. 3.41. Action very similar to *m*/2 $NaNO_3$; a few contractions at 3.52; none seen later. A faint trace of ciliary movement remains after one hour.
6. *m*/2 $NaCOOCH_3$. 3.53. Well-marked initial contraction; cilia continue movement in most larvæ. At 3.58 contractions occur at intervals; cilia slowly active in a large

proportion. At 4.31 and 4.44 cilia are still largely active, but no muscular movement is seen.

7. $m/2$ Na_2SO_4. 3.54.5. Initial contraction, followed by well-marked muscular movement; cilia continue in a large proportion. At 4.00 only a few muscular contractions are seen; fair ciliary movement remains. At 4.45 a few feeble contractions are seen; ciliary movement has almost ceased.
8. $m/2$ $Na_2S_2O_3$. 3.56.5. Immediate effect as in $m/2$ Na_2SO_4. At 4.10 occasional contractions are seen; most cilia have ceased. At 4.27 a few contractions are seen, and a little ciliary movement. No movement at 4.48.
9. $m/2$ $Na_2C_4H_4O_6$. 4.02. Slow muscular movements at first. At 4.07 a few contractions occur at intervals; cilia continue in a fair proportion. At 4.24 there is considerable slow ciliary movement; no contractions are seen. At 4.50 a very few contractions are seen; a little ciliary movement remains.
10. $m/2$ Na_3 citrate. 4.03. Muscular contractions are slight and cease soon; cilia continue for a short time. At 4.05 a few occasional contractions are seen; cilia have almost ceased. At 4.22 a trace of ciliary movement remains; no muscular contraction. No movement seen later.
11. $m/2$ Na_2HPO_4. 4.10. Contractions begin somewhat feebly; cilia continue for some little time. At 4.20 occasional contractions are seen; considerable ciliary movement remains. At 4.54 no movement is seen.
12. $m/2$ $Na_4Fe(CN)_6$. 4.12. Contractions appear slowly; cilia continue in a fair proportion of larvæ. At 4.18 a few feeble contractions are seen; cilia have almost ceased at 4.19. No movement seen later.

Four other series of experiments with sodium salts gave a similar general result. A certain definite relation of the anions to ciliary movement appears quite uniformly. In solutions of sodium acetate particularly, also in those of sulphate, tartrate, and phosphate, there is less immediate destruction of cilia than in chloride, bromide, iodide, and nitrate, and the average duration of the movement is distinctly greater. If the relative action of the salts is measured by the average maximal duration of ciliary movement in their respective solutions, the toxicity of the anions is seen to increase in essentially this order: $COOCH_3 < SO_4 < C_4H_4O_6 < HPO_4 < Cl < NO_3$ (and ClO_3) $< Br < I$.[11] Since this is also the general order of increasing action of anions in affecting the aggregation state of hydrophilous colloids, it appears evident that the immediate destructive action is dependent on alteration (presumably increased dispersion) of the tissue colloids. Similar relations appear, although less clearly, for muscular contraction. The results of these experiments may be briefly indicated as follows: In Table II after each salt is given the latest interval (after placing in the solution) at which any trace of ciliary or of muscular contraction was

[11] Hoeber finds essentially similar conditions for the cilia of the frog's œsophagus. *Cf.* Biochemische Zeitschrift, 1909, xvii, p. 518.

TABLE II.

The results of five separate series of experiments with $m/2$ solutions of the following salts are tabulated. Each vertical column gives the results of a single series. Ciliary movement usually outlasts muscular contraction; the latest interval at which either form of activity was observed is given for each solution. The + mark indicates considerable activity at the time of the latest observation.

Salt		Series.				
		1	2	3	4	5
NaCl	*cil.*	32 m.	14 m.	63 m.	20 m.	2 h. 23 m.
	musc.	3m. + (< 8 m)	14 m.	2.5 m. + (< 17 m.)	< 20 m.	29 m.
NaBr	*cil.*	21 m.	12 m.	16 m.	14 m.	....
	musc.	2 m.	13 m.	< 16 m.	< 14 m.	....
NaI	*cil.*	14 m.	1.5 m.	< 2 m.	< 2 m.	....
	musc.	2 m.	9.5 m.	13 m.	2 m+ (< 9 m.)	....
$NaNO_3$	*cil.*	12 m.	7 m.	58 m.	9 m.	....
	musc.	5 m.	7 m.	< 11.5 m.	2 m. + (< 9 m.)	
$NaClO_3$	*cil.*	59 m.	....	....	< 11 m.	....
	musc.	11 m.	....	....	11 m.	....
$NaCOOCH_3$	*cil.*	51 m.+	2 h. 34m.	1 h. 45 m.+	1 h. 33 m.+	2 h. 18 m.+
	musc.	5 m.+	41 m.	< 16 m.	8 m.	24 m.
Na_2SO_4	*cil.*	50 m.	....	....	1 h. 32 m.	2 h. 17.m.
	musc.	50 m.	....	....	63 m.	46 m.
$Na_2S_2O_3$	*cil.*	30 m.	13 m.	45 m.	*ca.* 6 m.	....
	musc.	30 m.	13 m.	< 14 m.	6 m.+ (< 23 m.)	....
$Na_2C_4H_4O_6$	*cil.*	48 m.	< 12 m.	47 m.	1 h. 22 m.	2 h. 14 m
	musc.	48 m.	12 m.	< 13 m.	56 m.	45 m.
Na_3 citrate	*cil.*	19 m.	....	....	2 m. (< 4 m.)	....
	musc.	2 m.	....	....	< 4 m.	....
Na_2HPO_4	*cil.*	10 m.+	< 12 m.	47 m.	....	43 m.
	musc.	10 m.	40 m.	< 13 m.	....	21 m.
$Na_4Fe(CN)_6$	*cil.*	7 m.	....	....	....	....
	musc.	6 m.	....	....	....	....

definitely observed. Observation cannot be continuous in such experiments and registration is impracticable: hence the maximal duration of the respective movements is only approximately indicated; the approximation is sufficiently close, however, to admit no doubt of the existence of the above constant differences between the different salts.

The above-described differences for the various salts will appear clearly, especially for ciliary movement, on inspection of the above table. The action of solutions belonging to the same series should be compared; the cilia of different lots of larvæ show unequal degrees of resistance to the destructive action of the solutions: those of Series 5 and 3, for example, were more than usually resistant. With muscle the differences appear less decided than with cilia; the difficulty of determining the maximal duration of movement is greater on account of the intermittence of the contractions. In sulphate, tartrate, acetate, and phosphate contractions typically last longer than in bromide, iodide, and nitrate, and chloride is usually intermediate. The same general order of toxicity is thus seen as for cilia.

In their immediate stimulating action on muscle the salts show similar differences. The initial contraction increases in energy from chloride to bromide and from bromide to iodide, and is as a rule relatively slight with the less toxic salts. A parallelism thus exists between toxicity and stimulating power, indicating that stimulation and toxic action depend on alteration of the same structural elements of the tissue. On the present theory the plasma membrane is the primary structure affected in both instances. A vigorous stimulating action, such as that shown by sodium iodide, implies a rapid and pronounced increase in the permeability of this membrane; this implies a more far reaching charge in the colloidal aggregation state than that produced by a more weakly acting salt. Such a change, however, if carried too far cannot fail to have a disintegrative or destructive action. Hence those salts which stimulate must powerfully tend to be the most toxic, mainly because of the rapidity with which they induce abnormal or pathological increase of permeability. This effect of pure sodium salts can be largely counteracted by the addition of a little calcium or other favorable cation, which checks both the stimulating and the toxic action.[12]

[12] *Cf.* my preceding paper, *Loc. cit.*, p. 24.

In interpreting the physiological action of salts we must regard changes in permeability as of fundamental importance. In the preceding paper it was shown that the stimulating action of pure solutions of sodium and other salts is associated with a marked increase in permeability, and the stimulation was referred directly to this latter change. The influence of sugar solutions in repressing irritability and contraction without initial stimulation receives a satisfactory explanation on the assumption that withdrawal of the normal salts from the medium renders the plasma membranes of the irritable elements *less* permeable than normally. Hence the normal transfer or interchange of material across the plasma membrane is retarded or prevented; escape of carbon dioxide and along with this the energy-yielding oxidative processes are checked. A state of decreased activity and irritability, corresponding to anæsthesia, is thus induced. The evidence that permeability is actually decreased in sugar solutions is indirect at present. The resemblance between the action of such solutions and those of magnesium chloride favors this interpretation; both solutions remove contractility without stimulating and without causing loss of pigment, and recovery on subsequent transfer to sea water is prompt and complete.[12] On the other hand, solutions which remove contractility only after a strong initial stimulation and loss of pigment (pure NaCl, KCl, etc.) have a markedly toxic action, and restoration of contraction in sea water is delayed and imperfect (see later, p. 488). It is evident that loss of contraction in sugar or magnesium chloride solutions is due to a change of a very different kind from that induced by pure sodium or potassium chloride.[13] The prompt and perfect recovery in sea water indicates that the properties and physico-chemical constitution of the tissue have remained essentially unaltered during the stay in the former solutions; the complete reversibility of the change is thus explained. During *increased* permeability, on the contrary, there is necessarily a loss of material from the tissue, — this effect is partly visible in the characteristic loss of pigment, — and such a change if extreme must lead to injurious alteration of chemical constitution or structure. Hence the relative irreversibility of changes involving marked and prolonged increase of permeability. If permeability is altered in sugar

[12] *Cf.* preceding paper, p. 25.

[13] *Cf.* HOEBER: Physikalische Chemie der Zelle und der Gewebe, 2te Auflage, p. 294.

solutions, it is certainly not in the direction of increase, and all the analogies indicate a decrease.

We assume therefore that in sugar solutions the muscle cells are temporarily rendered less permeable than normal. The above electrolytes, by their direct action on the colloids of the plasma membrane, increase the permeability of the latter and so restore irritability; contractions accordingly result, which are the more vigorous the more pronounced the increase in permeability, — hence the relation of the stimulating effect to the character of the ions. If the increase in permeability is abnormally great, toxic effects follow and the contractions soon cease.

The above relation of the anions of the sodium salts to the stimulating action recalls Mathews' hypothesis that anions are the stimulating ions, while cations in general have the reverse action.[14] This view implies that in stimulation the aggregation state of the colloids is altered in a definite manner; whether the change is in the direction of coagulation or of an increased dispersion must depend on the nature of the colloids concerned in the stimulation process. These, however, are probably negative, so that increased dispersion would correspond to stimulation. It is possible that increased colloidal dispersion may be especially favorable to stimulation in certain irritable tissues, such as nerve, to which Mathews' conclusions apply more particularly; but the rule is not a universal one, as certain of the following experiments show; stimulation (*e. g.*) may be due directly to the action of hydrogen ions (see p. 476). We must conclude that the essential change in stimulation is neither coagulation nor increased dispersion of the plasma membrane colloids, but simply a temporary increase in permeability; apparently this may result from either change provided the alteration in colloidal consistency is sufficient.

Action of pure solutions of alkali and alkali earth chlorides. — Pure solutions of salts of the other alkali metals also produce contractions differing characteristically from those shown in solutions of sodium salts. Several series of experiments were performed with chlorides of the alkali and alkali earth metals in $m/2$, $m/4$, and $m/8$ concentrations.

In general, the following results have appeared: Solutions of lithium chloride, and to a less degree of cæsium chloride, resemble sodium chloride in their general action on muscle, producing rela-

[14] Mathews: This journal, 1904, xi, p. 455.

tively slight initial contractions followed by muscular movements which last for several minutes; potassium and rubidium chlorides, on the other hand, cause a strong initial shortening followed by feeble contractions which last only for the brief period occupied by the relaxation (from a few seconds to a minute in *m*/2 solutions); ammonium chloride resembles potassium rather than sodium chloride. Of the alkali earth chlorides, that of magnesium has no stimulating action — in its solutions larvæ remain extended and motionless; calcium chloride produces feeble movements which last for some time; in strontium chloride solutions contractions are also feeble, and considerable shortening results; in barium chloride solutions shortening is more decided and contractions cease much sooner.

The following record describes a typical series of experiments:

TABLE III

June 12, 1908. — Larvæ were placed in *m*-dextrose solution at 10.10 A. M., and transferred to *m*/2 solutions of the following salts at the times designated.

1. *m*/2 KCl. 11.03. Immediate shortening results, followed in a few seconds by a gradual relaxation with feeble bending movements. No contractions are seen after the relaxation is complete (about 1 m.). Cilia continue vibrating for three to four hours; have ceased by 4.25 P. M.
2. *m*/2 RbCl. 11.05.5. Effect is like KCl: there is a marked initial contraction followed by feeble contractions which cease in about one minute. Cilia continue for about three hours; have ceased by 4.25.
3. *m*/2 CsCl. 11.08. Initial contraction is relatively slight as compared with KCl or RbCl, and muscular contractions last for some time; a little muscular movement at 11.15. Cilia continue for three to four hours.
4. *m*/2 NH_4Cl. 11.16. Moderate muscular contractions appear at once, and last for less than two minutes. Cilia have almost ceased by 11.31; no movement at 11.53.
5. *m*/2 LiCl. 11.19. Slight initial contraction; muscular movements are well marked at first; have practically ceased in three minutes. Most cilia cease at once; a trace of ciliary movement remains at 11.33.
6. *m*/2 NaCl. 11.26. Slight initial shortening; muscular contractions are well marked and continue for three or four minutes; have almost ceased by 11.29. No contractions at 11.38. Cilia are slowed from the first; a little movement remains at 11.38, none at 12.00.
7. *m*/2 $MgCl_2$. 12.01. No initial contraction; a few doubtful muscular movements at first contact of solution; thereafter none seen. Cilia continue actively; have almost ceased at 2.05.
8. *m*/2 $CaCl_2$. 12.04. Feeble muscular contractions appear and last some time. Cilia are slowed and partly disintegrated; cease within eight minutes. Larvæ gradually shorten; feeble contractions continue at intervals; these have ceased by 2.06.
9. *m*/2 $SrCl_2$. 12.09. Muscular contractions are slight and sluggish; larvæ gradually shorten; a little contraction at 12.21; none at 12.47. Cilia cease almost immediately (< 1 m.).
10. *m*/2 $BaCl_2$. 12.16. Marked muscular shortening which persists; larvæ show no definite contractions afterward, remaining shortened and motionless. Slow ciliary movement remains in a fair proportion at 12.22; none at 12.47.

In less concentrated solutions of the above salts (*m*/4 and *m*/8) similar effects are seen, but the contractions tend to last longer. The results of all of last summer's experiments with pure solutions of the above salts are summarized in Table IV:

In this table the times indicate as before approximately the maximal duration of both ciliary and muscular movement in the respective solutions. Each vertical column represents a single series of experiments in which larvæ were transferred at intervals from the dextrose solution to the salt solution. The length of the stay in the non-electrolyte solution has varied somewhat — within the range of about thirty-five minutes to an hour and a half, — so that the corresponding figures for different series are not strictly comparable; in Series 10 and 11 in particular ciliary movement had largely ceased in the dextrose solution before transfer; hence there was less revival than in Series 9 with the same salts in the same concentrations.

To preserve an approximate isotonicity the *m*/4 and *m*/8 solutions were prepared by diluting the *m*/2 salt solution with *m*-dextrose solution.

The characteristic action of the alkali chlorides is as follows: Potassium and rubidium chlorides produce at the moment of contact with the solution temporary but pronounced contractions which cease very soon; ammonium chloride has a less powerful initial stimulating action and sustains contractions somewhat longer; while in sodium, lithium, and cæsium chlorides the immediate stimulating action is still less pronounced and contractions last for a considerable time — typically longer with sodium and cæsium than with lithium. The influence on cilia is also characteristic and different from that on muscle. Rubidium, potassium, and cæsium chlorides all sustain ciliary movement for prolonged periods (some hours), though cæsium appears somewhat less favorable than the other two; in ammonium chloride cilia are active at first but cease soon; while in sodium and lithium chloride they largely undergo disintegration shortly after contact with the solution; although a few may remain slowly active, especially in sodium chloride, for some time longer. The alkali earth chlorides show the following constant peculiarities of action. All produce muscular contractions except magnesium chloride; in solutions of this salt the larvæ remain stiff and extended although the cilia remain active; the characteristic anæsthetic action is thus shown. With calcium chloride sluggish muscular contractions, lasting some time, are typical; in strontium chloride similar contractions are seen, but they cease much sooner.

Cilia also cease sooner in strontium than in calcium chloride, and last longest in magnesium chloride. Barium is rapidly destructive to both ciliary and muscular movement, and after the initial muscular contraction no further movement is seen in solutions of its chloride.

It is noteworthy that muscular contractions in sodium, lithium, and cæsium chlorides may show at first an almost normal character; the toxicity of the pure solution prevents a more complete recovery and contractions cease before many minutes. Addition of a little calcium to pure solutions of sodium or lithium chloride renders the solution much more favorable, and contractions may then last for several hours.[15] When the difference in the conditions of experiment and in the character of the organisms is considered, it is remarkable how close an agreement the above results show with those of Overton [16] on vertebrate muscle. The partial recovery of irritability with calcium and strontium salts (not with Mg in Arenicola) and the peculiar resemblance of cæsium to lithium and sodium are seen in Arenicola also. The inference seems clear that the contractile elements are similarly affected by electrolytes in both classes of organisms; a fundamental similarity in physico-chemical constitution is thus indicated. The order of increasing favorability of the alkali cations for Arenicola muscle, as indicated by the degree to which normal contractions return in solutions of the respective chlorides, is essentially: K and Rb $< NH_4 <$ Li $<$ Cs $<$ Na; this, according to Hoeber,[17] is practically identical with the order of decreasing effectiveness in the precipitation of lecithin and egg-albumin by the above chlorides. Since the plasma membrane appears to be essentially a mixture of protein and lipoid material, the view is confirmed that the stimulating action of salts is due to their altering the aggregation state of the colloids composing this structure and so increasing its permeability. The characteristic order of relative action in the case of the sodium salts, as described above (p. 466), also supports this conclusion.

On the present view, any solution, in order to stimulate, must so alter the plasma membrane as to increase its permeability, at least temporarily. It was shown in the preceding paper that when normal larvæ are transferred from sea water to pure solutions of most of

[15] *Cf.* the experiments with magnesium chloride larvæ below, p. 485.

[16] Overton: *Loc. cit.*

[17] Hoeber: Beiträge zur chemischen Physiologie und Pathologie, 1907, xi, p. 35.

TABLE IV.

NUMBER OF SERIES AND CONCENTRATION OF SALT.

Salt.		1 ($m/2$)	2 ($m/2$)	3 ($m/2$)	4 ($m/2$)	5 ($m/2$)
NaCl	cil.	20 m.	2 h. 33 m.	32 m.	34 m.	7 m.
	musc.	< 20 m.	29 m.	3 m.+ (< 19 m.)	3 m.+ (< 12 m.)	7 m.
LiCl	cil.	7 m.	7 m.	8 m. (23 m.)	14 m.	12 m.
	musc.	< 2 m.	3 m.	< 8 m	< 3 m.	< 3 m.
CsCl	cil.	...	...	...	5 h. 17 m.	1 h. 2 m.
	musc.	...	...	...	1 h. 2 m.	13 m.
KCl	cil.	2 h. 16 m.	2 h. 34 m.	1 h. 52 m.	2 h. 57 m.	1 h. 6 m.
	musc.	< 1 m.	< 1 m.	< 1 m.	< 1 m.	< 1 m.
RbCl	cil.	...	...	...	2 h. 6 m.	1 h. 3 m.+
	musc.	...	...	...	< 1 m.	< 1 m.
NH_4Cl	cil.	3 m.+	34.5 m.	13 m.	15 m.	8 m.
	musc.	3 m.	2.5 m.	about 2 m.	< 2 m.	about 2 m.
$MgCl_2$	cil.	...	...	...	2 h. 4 m.	36.5 m.
	musc.	...	...	...	0	0
$CaCl_2$	cil.	...	...	...	about 4 m.	16 m. (< 33 m.)
	musc.	...	...	...	42 m.+ (< 2 h.)	33 m.
$SrCl_2$	cil.	...	...	...	1 m.	3 m.
	musc.	...	...	...	12 m.	12 m.
$BaCl_2$	cil.	...	...	...	6 m.	7.5 m.
	musc.	...	...	...	< ½ m.	< ½ m.

the above salts, strong contractions result and evidence of increased permeability is seen in a loss of pigment from the cells. Magnesium chloride, however, does not produce these effects; it appears in fact to decrease rather than to increase permeability. This exceptional behavior explains why this salt does not restore contractility to larvæ from sugar solutions. We may infer that an electrolyte must increase permeability in order to stimulate; and since the

TABLE IV (*Continued*).

NUMBER OF SERIES AND CONCENTRATION OF SALT.

6 (*m*/4)	7 (*m*/4)	8 (*m*/4)	9 (*m*/8)	10 (*m*/8)	11 (*m*/8)
21 m.	14 m. (< 55 m.)	....	14 m. (< 41 m.)	none seen	< 2 m.
13 m.+ (< 21 m.)	6 m. (< 14 m.)	....	41 m.	5 m. (< 15 m.)	10 m.
6.5 m.	2 m. (< 4 m.)	....	< 3.5 m.	< 2 m.	< 1 m.
2.5 m.	2 m. (< 4 m.)	....	6.5 m.	8.5 m.	4 m. (< 8 m.)
25 m.+	5 h. 44 m.	....	1 h. 28 m. (< $3\frac{3}{4}$ h.)	5 m.	?
13 m. (< 25 m.)	4 m. (< 11 m.)	....	55 m.	17 m.	29 m.
35 m.++	5 h. 52 m.	....	about 22 h.	3 h.	50 m.+
< 1 m.	< 1 m.	....	3 m.	3.5 m.	2 m.
28 m.++	5 h. 45 m.	....	about 22 h.	3 h.	42 m.+
< 2 m.	< 1 m.	....	3 m.	4 m.	2 m.
31 m.	26.5 m.	....	29 m. (< 1 h.)	6 m. (< 17 m.)	4.5 m.+
< 2 m.	about 3 m.	....	6 m.	2 m. (< 6 m.)	4.5 m.
....	45 m.+ (< 3 h.)	1 h. 6 m.	....	none seen	13 m.+
....	0	0	....	0	0
....	45 m.	28 m. (< 1 h.)	....	none seen	13 m.+
....	3 h. 47 m.	1 h. 3 m.+ (< 3.5 h.)	....	27 m.+ (< $3\frac{3}{4}$ h.)	13 m.+
....	23 m.	8 m. (< 19 m.)	....	12.5 m.	< 3.5 m.
....	23 m.	55 m.	....	12.5 m. (< 24 m.)	< 3.5 m.
....	7 m.	2.5 m.	....	none seen	
....	< $\frac{1}{2}$ m.	< $\frac{1}{2}$ m.	....	< $\frac{1}{2}$ m.	

above salts show wide differences in their action on colloids, that the means by which this increase is produced is of secondary importance.

The ease with which an irritable tissue is stimulated indicates that the normal resting condition of the plasma membrane is an unstable one and very readily altered in the direction of increased permeability. A variety of external changes of condition may thus

act as stimuli. To the resting state a certain definite state of aggregation of the colloids must be supposed to correspond; alteration of this state, if carried sufficiently far in the direction either of coagulation or of increased dispersion, should therefore increase permeability and cause stimulation. Either acid or alkali ought thus to stimulate under appropriate conditions, although hydrogen and hydroxyl ions have in general opposite action on the aggregation state of colloids. The action of these electrolytes on larvæ from sugar solutions has therefore been studied in some detail.

Action of acid and alkali. — In the following experiments the larvæ, after a stay of known duration in pure *m*-dextrose solution, were transferred to *m*-dextrose containing acid (H_2SO_4, HCl, $HCOOCH_3$) and alkali (NaOH) in the concentrations named.

The following table gives the record of a typical series of experiments with hydrochloric acid.

TABLE V.

June 15, 1908. — Larvæ were transferred from sea water to *m*-dextrose solution at 2.50 P. M.; this solution was changed for fresh at 3.10. Thence they were brought at the times indicated into the following series of *m*-dextrose solutions containing hydrochloric acid in the concentrations named. The results were as follows:

1. *n*/400 HCl. 3.16. Larvæ shorten markedly and show slight contractions for the first few seconds; no further movement is seen. Cilia cease at once. Larvæ remain permanently shortened and stick to the glass.
2. *n*/800 HCl. 3.18. The action is like that of Solution 1. Ciliary movement lasts for a few seconds only.
3. *n*/1600 HCl. 3.21. Both ciliary and muscular movements last longer than in Solutions 1 and 2, but cease within two minutes. Larvæ remain shortened and stick to the glass.
4. *n*/3200 HCl. 3.24. Muscular contractions begin after a latent interval of some seconds; they are well marked and typical and last several minutes; in a few larvæ cilia also remain active after five minutes. The permanent shortening and adhesion to glass are less than in Solution 3.
5. *n*/6400 HCl. 3.30.5. Ciliary movement continues. Larvæ shorten somewhat at first, but definite muscular movements do not appear for almost a minute; contractions are then well marked and continue for some time; a few slight movements are seen at 3.39. At 4.11 a trace of ciliary movement remains, but no muscular. There is slight permanent contraction.
6. *n*/12800 HCl. 3.39. Practically no muscular movement is seen till 3.40, when well-marked contractions begin and last some time; at 4.12 there are still contractions at intervals. No definite contractions are seen at 4.23 or afterwards. A little ciliary movement remains at 4.50.
7. *n*/25600 HCl. 3.44. Cilia continue actively, but no muscular contractions are seen for about two minutes, when feeble movements of the setæ begin; at 3.47 contractions are well marked though feeble. Slight contractions continue at intervals for about forty minutes. A few cilia remain active after one hour.

A repetition of the above series gave essentially the same results. Similar series of experiments were performed with H_2SO_4 (concentrations ranging from *n*/50 to *n*/25600) and with acetic acid (three series with concentrations as above from *n*/400 to *n*/25600).

It is evident that there is a well-marked though temporary return of irritability and normal contraction under the influence of acid. In the stronger solutions (*n*/400 — *n*/1600) the contractions are immediate, but irritability is soon destroyed. In the weaker solutions they appear more gradually, are more nearly normal in character, and last longer.

One uniform effect, found in all of the solutions of the above series (except the most dilute), is a permanent shortening or increase of muscular tone in the acid solutions. This tonic contraction occurs at once as soon as the larvæ are brought into the solution, although definite contractions of the bending and squirming type may not appear for an interval (Solutions 5 and 6 above). To show the extent of this shortening and its variation in solutions of different acid content, measurements with the ocular micrometer were made of the larvæ after about forty-five minutes in the several solutions of the above series. Arenicola larvæ at the swarming stage are of very uniform size (about 0.3 to 0.32 mm.), the extremes varying from the average by not more than 10 per cent, while the great majority show a much smaller range of variation. The larvæ measured were selected at random from the large number contained in each watch-glass. (See Table VI.)

The degree of tonic shortening thus shows a definite relation to the concentration of the acid. It is pronounced in the higher concentrations, and is distinct in the strong acid down to *n*/6400 and in the weak to *n*/3200. If, as the present theory assumes, the plasma membrane is permeable to hydrogen ions, it is clear that increasing the hydrogen ion concentration in the outer medium must have the effect of increasing it also within the cell; the inference therefore seems justified that the degree of shortening is a function of the hydrogen ion concentration within the muscle cell. If the conditions of shortening in acid solutions are similar to those of normal contraction, the general view which ascribes shortening in the latter process to increased hydrogen ion concentration within the contractile elements receives confirmation.

In the interpretation of the above stimulating action of acid the existence of several independently varying factors must be recog-

nized. It has already been pointed out that there is a certain delay in the appearance of active contractions in the more dilute solutions (from $n/3200$ HCl on); the increase in tonic contraction is immediate, but the definite bending or squirming movements do not appear until after a certain interval or latent period, which is the more prolonged the weaker the acid solution. A consistent explanation of

TABLE VI.

The measurements give the average length of the larvæ in millimetres after about forty-five minutes in the solution.

1. $n/400$ HCl. Larvæ are coagulated, and distintegration has begun.
2. $n/800$ HCl. The same.
3. $n/1600$ HCl. 0.27 mm. (average of 16 larvæ).
4. $n/3200$ HCl. 0.272 mm. (average of 17).
5. $n/6400$ HCl. 0.295 mm. (average of 20).
6. $n/12800$ HCl. 0.306 mm. (average of 21).
7. $n/25600$ HCl. 0.307 mm. (average of 20).

The control larvæ, in neutral dextrose solution for about an hour, showed an average length of 0.318 mm. (average of 20). Similar measurements with the acetic acid solutions showed a similar result. The measurements for two series (A and B) are given. The number of measurements in each case is given in brackets.

	Series A[1]	Series B[2]
1. $n/200$ $HCOOCH_3$.	0.254 mm. (4)	. . . .
2. $n/400$ $HCOOCH_3$.	0.246 mm. (5)	0.243 mm. (4).
3. $n/800$ $HCOOCH_3$.	0.257 mm. (5)	0.247 mm. (6)
4. $n/1600$ $HCOOCH_3$.	0.28 mm. (5)	0.265 mm. (7).
5. $n/3200$ $HCOOCH_3$.	0.291 mm. (6)	0.278 mm. (8).
6. $n/6400$ $HCOOCH_3$.	0.313 mm. (9)	0.282 mm. (7).
7. $n/12800$ $HCOOCH_3$	0.311 mm. (11)	0.288 mm. (13)
8. $n/25600$ $HCOOCH_3$	0.30 mm. (12)	0.288 mm. (10).

[1] Control, in neutral sugar solution, 0.3 mm. (14).

[2] Control (neutral *m*-dextrose), 0.286 mm. (14). Larvæ are younger and somewhat shorter than in Series A.

these phenomena may be based on the membrane theory as follows: Since the plasma membrane is readily permeable to hydrogen ions, increase in the external concentration of these latter must at once have a depolarizing influence;[18] hence the internal concentration of hydrogen ions undergoes an immediate and corresponding rise, producing increase of muscular tone. The acid at the same time affects, by its coagulative action, the colloids of the membrane, and

[18] The state of physiological polarization (outer surface of cell positive) is assumed to be due to a partial separation of hydrogen ions from the corresponding anions at the membrane on account of the latter's impermeability to anions.

gradually increases the latter's permeability; when this has been increased to a certain critical degree, stimulation follows; this effect will naturally be the more rapid the more concentrated the solution of acid. During a certain period after the permeability has reached a favorable condition for stimulation irritability will remain; but with the progressive alteration of the membrane conditions become less favorable, and eventually irritability is lost, presumably when the permeability has been increased beyond a certain critical degree; this stage will naturally be reached more gradually in the less concentrated solutions, hence contractions last longer in these.

Action of alkali. — The action of slightly alkaline dextrose solutions is different from that of acid and is also highly characteristic. Little or no evident action is seen in solutions of *n*/100 NaOH and weaker. In higher concentrations (*n*/25, *n*/50 NaOH) the most conspicuous immediate effect is a distinct straightening and elongation of the larval body due apparently to decrease of the normal muscular tone, and accompanied by somewhat slight bending movements which last intermittently for ten to twenty minutes. In *n*/50 NaOH the elongation is less and the contractions are slighter than in *n*/25; and in *n*/100, *n*/200, and *n*/400 NaOH contractions are either very weak or entirely absent, and elongation is inconspicuous. Ciliary movement is arrested within ten minutes in *n*/25 NaOH; in lower concentrations little effect is seen. The following measurements of larvæ, taken within ten to fifteen minutes after transfer from neutral to alkaline dextrose solutions, will illustrate the characteristic change in muscular tone.

1. *n*/25 NaOH 0.323 mm. (average of 32 larvæ).
2. *n*/50 NaOH 0.311 mm. (average of 37 larvæ).
3. *n*/100 NaOH 0.317 mm. (average of 30 larvæ).

In neutral *m*-dextrose the average of 22 larvæ was 0.31 mm. There is thus an average increase in length of about 4 to 5 per cent in *n*/25 NaOH. In certain individuals the elongation is decidedly more pronounced than this.

Alkali thus has relatively slight stimulating action as compared with acid, and in the higher concentrations used above induces distinct lengthening. The effect appears contrary in its general nature to that of acid, and suggests that the hydroxyl ions alter permeability in the inverse sense. Decreased permeability, as I have

indicated in the preceding paper, is a condition of inhibition; and inhibition is known to be associated with decrease of tone in the cardiac and skeletal muscle of vertebrates. The slight muscular bendings shown by Arenicola larvæ in the stronger solutions may be supposed to result from inequalities in the action of the alkali at different regions of the muscle cells. It is noteworthy that in *m/2* $MgCl_2$, which almost certainly decreases permeability in these larvæ, the animals tend to remain straight and extended, though without undergoing noticeable elongation. I have not yet made comparative measurements of the length of larvæ in magnesium chloride and other anæsthetizing media. It may be that this effect is peculiar to Arenicola larvæ and not of any general significance, since slight alkalinization of the medium is usually observed to favor rather than to inhibit muscular contraction — in vertebrate muscle at least. Bethe, however, has found weak alkalinization of the sea water to check, and weak acidulation to stimulate, the rhythmical contractions of the medusa Rhizostoma.[19] These conditions show a certain resemblance to those above described. Further comparative observations are needed on the action of dilute acid and alkali on contractile tissues.

II. Restoration of Contraction after Treatment with Isotonic Magnesium Chloride Solution.

In *m/2* $MgCl_2$ solutions muscular contraction is rapidly and completely lost, and the larvæ remain extended, rigid, and non-contractile during their stay in the solution. The loss of muscular contractility is more rapid than in isotonic sugar solutions; within a minute contractions are always found to have completely ceased; the cilia, however, continue their activity at a diminished rate, and the larvæ are slowly propelled through the solution and collect at the bottom in the typical groups or clumps.

Recovery of contractions occurs, as with larvæ from sugar solutions, on transfer to appropriate salt solutions. This recovery is prompt and complete in sea water, and in sodium chloride solutions containing a little calcium. In pure solutions of sodium salts, however, only few and comparatively slight contractions appear;[20] *m/2*

[19] Bethe: Archiv für die gesammte Physiologie, 1909, cxxvii, p. 219.

[20] The addition of a trace of calcium chloride makes a surprising difference (see below, p. 485). In the experiments cited in a former paper where pure sodium

sodium chloride often produces no apparent effect after $m/2$ $MgCl_2$ — a striking difference from its action after sugar solution. Similarly $m/2$ potassium chloride produces much less energetic contractions after magnesium chloride than after sugar (*cf.* p. 483).

It is thus evident that a given solution, *e. g.*, $m/2$ NaCl, varies in its action on the contractile tissues according to the medium to which they were previously exposed. Larvæ brought from sea water to pure sodium chloride solution contract strongly and lose pigment; transfer to the same solution from m-dextrose produces decidedly less energetic contractions; and after $m/2$ magnesium chloride little or no movement results. These facts indicate that the degree of impermeability resulting from the action of magnesium chloride is greater than that produced by sugar solution; hence the increase of permeability requisite for stimulation is less readily induced after treatment with this salt. The state of irritability of larvæ kept in the above three media thus decreases in the order: sea water $>$ sugar solution $>$ magnesium chloride solution. The immediate toxic action of a given salt on larvæ from these media — as shown, for instance, in the destruction of cilia — also shows this order of decrease.

Action of pure solutions of sodium salts. — Apart from this difference in the intensity of the immediate stimulating effect, the action of sodium salts is essentially similar to that described above for larvæ from sugar solution, and similar differences of action between different salts are seen. The following series of experiments will illustrate:

TABLE VII.

June 25, 1908. — In each experiment larvæ were transferred from sea water to $m/2$ $MgCl_2$, where they remained exactly seven minutes; thence they were transferred to the solution of the experiment. In each case the time of placing in the $m/2$ $MgCl_2$ is indicated in parentheses.

1. $m/2$ NaCl (10.10 to $m/2$ $MgCl_2$), 10.17. No muscular contractions are seen. Cilia remain active for some hours (influence of the previous exposure to $MgCl_2$).
2. $m/2$ NaBr (10.15 to $m/2$ $MgCl_2$), 10.22. A few slight contractions are seen at first; no contractions are seen at 10.23, 10.26, and 10.36. Cilia remain active.
3. $m/2$ NaI (10.20 to $m/2$ $MgCl_5$), 10.27. No contractions are seen at first; within ten to fifteen minutes well-marked contractions appear at intervals in most larvæ. Cilia remain active after four hours.

chloride solutions are described as producing well-marked contractions in larvæ from $m/2$ $MgCl_2$, a little calcium seems to have been present in the salts used. Kahlbaum's sodium and magnesium chlorides appear to be practically free from this element.

4. $m/2$ $NaNO_3$ (10.25 to $m/2$ $MgCl_2$), 10.32. Slight contractions appear in a few within two minutes. No contractions are seen later. Cilia remain active.
5. $m/2$ $NaCOOCH_3$ (10.30 to $m/2$ $MgCl_2$), 10.37. No muscular contractions are seen. Cilia remain active for hours.
6. $m/2$ $NaClO_3$ (10.35 to $m/2$ $MgCl_2$), 10.42. Almost no contraction results; one or two slight movements are seen at first. Cilia remain active.
7. $m/2$ Na_2SO_4 (10.40 to $m/2$ $MgCl_2$), 10.47. A few slight contractions are seen at 10.51. Cilia cease sooner than in the above solutions; have largely ceased within an hour.
8. $m/2$ $Na_2C_4H_4O_6$ (10.45 to $m/2$ $MgCl_2$), 10.52. Contractions appear in a fair proportion of larvæ within two to three minutes. Cilia cease comparatively soon, as in Na_2SO_4.
9. $m/2$ Na_2HPO_4 (10.50 to $m/2$ $MgCl_2$), 10.57. Well-marked contractions appear in a good proportion of larvæ; effect greater than in any of the preceding solutions. Cilia cease comparatively soon (about one hour).
10. $m/2$ Na_3 citrate (10.55 to $m/2$ $MgCl_2$), 11.02. No contractions are seen. Cilia cease in less than twenty minutes.

In a second precisely similar series, with four minutes' exposure to $m/2$ $MgCl_2$, essentially the same effects were seen, although the differences between the individual salts were somewhat less distinct. Return of contractions in pure solutions of sodium salts is thus, in general, relatively slight as compared with that seen after sugar solutions. The salts show similar though less decided differences in the intensity of their stimulating action. The following is the order of increasing intensity of action for the monovalent anions: $COOCH_3$ and $Cl < NO_3$, ClO_3 and $Br < I$. Tartrate and sulphate cause slight, and phosphate well-marked, return of contractions; possibly their action as precipitants of calcium is a factor in the effect, — this, however, I have not definitely determined as yet. The differences in toxicity show corresponding relations, as shown by the following experiments: After about four hours in the above solutions a number of larvæ were transferred from each solution to fresh sea water. There was distinct recovery of contractility in those from acetate and sulphate, and to a less degree from tartrate, but not from the others. In a third series in which larvæ, after a variable exposure to magnesium chloride, were brought into $m/2$ solutions of NaCl, NaBr, NaI, $NaNO_3$, Na_2SO_4 and Na-citrate, a similar result appeared: on transfer to sea water, after about two and a half hours in these solutions, well-marked and vigorous contractions returned in the larvæ from chloride and sulphate, but from no others. Tartrate, sulphate, acetate, and chloride belong to those anions whose action on colloids, according to Hofmeister and Pauli, is relatively slight; the salts with anions of more pronounced action — Br, NO_3, ClO_3, I — exceed the others both in immediate stimu-

lating power and in toxicity. The basis of these characteristic differences in action has been discussed above (p. 466 *seq.*); in the present experiments the plasma membrane, having been rendered relatively impermeable by the action of the magnesium, apparently does not undergo such prompt and ready alteration of permeability as before, hence the stimulating effect is less marked and the differences between the salts appear less sharply defined.

Action of pure solutions of alkali and alkali earth chlorides. — Pure isotonic solutions of the other alkali chlorides, with the exception of potassium (and possibly rubidium, which I have not yet tried), also produce little if any contraction in larvæ from magnesium chloride. Even potassium chloride causes only slight contractions. Calcium and strontium chlorides, on the other hand, have well-marked action. The following series of experiments will illustrate:

TABLE VIII.

June 23, 1908. — In this series the larvæ remained in $m/2$ $MgCl_2$ for five minutes, thence were transferred to the respective solutions.

1. $m/2$ KCl (10.52 to $m/2$ $MgCl_2$), 10.57. Slight muscular contractions appear, lasting for about a minute. Cilia continue actively.
2. $m/2$ NH_4Cl (11.01 to $m/2$ $MgCl_2$), 11.06. No contractions are seen. Cilia have ceased by 11.47.
3. $m/2$ LiCl (11.09 to $m/2$ $MgCl_2$), 11.14. No contractions are seen. Cilia remain slowly active at 11.47.
4. $m/2$ NaCl (11.17 to $m/2$ $MgCl_2$), 11.22. No contractions are seen. Cilia remain slowly active at 11.50.
5. $m/2$ $CaCl_2$ (11.30 to $m/2$ $MgCl_2$), 11.35. Well-marked contractions appear at once, which continue slowly at 11.52. Cilia remain active in a fair proportion of larvæ at 11.52.
6. $m/2$ $SrCl_2$ (11.38 to $m/2$ $MgCl_2$), 11.43. Vigorous initial shortening results, followed by slight contractions which diminish and cease in about ten minutes. Cilia cease within ten minutes.

A second similar series with variable and longer exposure to $m/2$ $MgCl_2$ yielded a practically identical result.

The susceptibility to stimulation by pure solutions of alkali chlorides has thus been greatly diminished as compared with that seen after sugar solutions. Since the typical strong initial contraction with its associated loss of pigment is absent in these solutions after the treatment with magnesium chloride, it is evident that the latter salt has increased the resistance to the change of permeability which the alkali chlorides normally induce. That this is the case is also shown by the marked decrease in the toxic action of these salts resulting from brief exposure to magnesium chloride solutions (see below, pp. 489–490).

On the other hand, the contractions produced by the alkali earth chlorides do not appear to be decreased by previous treatment with magnesium chloride. Calcium chloride in *m*/2 solution produces little or no immediate contraction in larvæ from sea water;[21] but in larvæ from magnesium chloride solutions well-marked contractions at once appear, as described in Table VIII. Calcium appears thus to antagonize the action of magnesium; the striking effects produced by the addition of a little calcium to solutions of sodium and lithium chlorides (see below, p. 485) are no doubt due partly to this as well as to a simple antitoxic action. Strontium has a somewhat similar action to calcium, but is decidedly more toxic. Why calcium should have this antagonistic action to magnesium remains unexplained at present. The physiological contrast between these metals appears to be widespread, especially in relation to muscular contraction (reviving action of calcium on the heart beat, etc.).

Action of acid and alkali in low concentrations. — After treatment with magnesium chloride larvæ are also much less readily affected by acid and alkali than after sugar solutions. Thus, in one series of experiments larvæ were transferred from *m*/2 $MgCl_2$ to a series of *m*-dextrose solutions containing hydrochloric acid in the concentrations *n*/200, *n*/400, *n*/800, *n*/1600, *n*/3200, and *n*/6400; in no solution was any contraction seen.[22] A similar negative result appeared in solutions of sodium hydrate (concentrations from *n*/25 to *n*/1600). Experiments were also tried with pure *m*/2 sodium chloride solutions, plus HCl and NaOH from *n*/200 to *n*/6400 as before. In this case the action of the acid is added to that of the salt (which has a slight action, as already seen), and some effect was seen; slight contractions appeared in acid of *n*/1600 to *n*/6400 concentration; alkali, on the contrary, had little or no demonstrable effect, even in the higher concentrations. These results resemble those found after dextrose solutions (*cf.* p. 476), where also acid produces contractions (more vigorous than after magnesium chloride), while alkali has no such action except in relatively high concentrations (*n*/25 and *n*/50). On the present interpretation acid in those concentrations would appear to increase, and alkali to decrease, permeability; this inference is confirmed by the contrast in the toxic effects of the two sets of solutions. Thus larvæ, after one and a half hours in a series of six solutions of

[21] *Cf.* the preceding paper; also below, p. 487.

[22] Acid in *m*/2 $MgCl_2$ solutions (HCl *n*/100 to *n*/3200) also produces no contractions.

m/2 NaCl + HCl (between *n*/200 and *n*/6400, as above), were brought into fresh sea water; no contractions resulted; the animals had an opaque coagulated appearance and were evidently dead. On the contrary, larvæ from the corresponding alkaline solutions recovered and showed vigorous contractions in all cases except in *n*/200 and *n*/400 NaOH. Alkali and acid in low concentrations thus appear to have opposite influences on the permeability of the plasma membrane in these organisms (see above, p. 479).

Action of mixtures of two salts. — Addition of a little potassium chloride to a pure *m*/2 sodium chloride solution appears somewhat favorable to the production of contractions in larvæ from magnesium chloride solutions. In a series of six solutions in which KCl was added to *m*/2 NaCl in the proportions *m*/50, *m*/100, *m*/200, *m*/400, *m*/800, and *m*/1600, slight contractions appeared in every case. These effects, however, were not decidedly different from those found with pure *m*/2 NaCl in which occasionally slight contractions are seen, especially if the previous stay in the *m*/2 $MgCl_2$ has been brief.

On the other hand, the effects following the addition of a little calcium chloride to pure sodium chloride solutions are most pronounced and highly characteristic. The importance of calcium to muscular contractions, and its power of counteracting the anæsthetic action of magnesium, are clearly shown by the following experiments:

June 27, 1908. — Larvæ after complete loss of muscular contractility in *m*/2 $MgCl_2$ were transferred to the following solutions: (1) *m*/2 NaCl, and (2–11) a series of ten solutions of (about) *m*/2 NaCl containing $CaCl_2$ in concentrations ranging from *m*/50 (24 volumes *m*/2 NaCl + 1 volume *m*/2 $CaCl_2$), *m*/100, *m*/200, etc., in regular series to *m*/51200. In pure *m*/2 NaCl no contractions were seen; in solutions containing $CaCl_2$ in concentrations from *m*/50 to *m*/800 vigorous squirming and bending movements began instantly, and slight contractions remained for three or more hours. In the other solutions similar effects were seen, the contractions growing progressively less pronounced as the calcium content diminished; in *m*/6400 and *m*/12800 $CaCl_2$ there was a certain delay in the appearance of the contractions, and these were comparatively slight; while in *m*/25600 $CaCl_2$ only a few contractions were seen, and in *m*/51200 $CaCl_2$ practically none.

The favorable influence of the calcium chloride is thus distinctly perceptible in concentrations so low as *m*/25600. Strontium chloride

has a somewhat similar though less favorable action: a series of experiments with *m*/2 NaCl containing $SrCl_2$ in concentrations from *m*/50 to *m*/3200 showed vigorous contractions in all for some minutes after the transfer from *m*/2 $MgCl_2$, and slower movements continued for some time (about one hour) afterwards. In similar experiments with sodium and barium chlorides the antitoxic action was obscured by the marked toxicity of the latter salt; in all the solutions — relatively gradually in *m*/1600 and *m*/3200 $BaCl_2$ — the larvæ slowly underwent permanent shortening and all movement ceased within a few minutes; there were no active bending contractions as with calcium or strontium. Barium thus shows in its physiological action a decided contrast to calcium and strontium.

Transfer from *m*/2 $MgCl_2$ to mixtures of lithium and calcium chlorides ($CaCl_2$ from *m*/10 to *m*/160, and from *m*/50 to *m*/800) is also followed by well-marked contractions, more sluggish than in the corresponding sodium chloride solutions and ceasing sooner. No contractions appear in pure *m*/2 lithium chloride. The optimum concentration of calcium appears higher with lithium than with sodium chloride; contractions lasted longest in a mixture of four volumes *m*/2 $LiCl_2$ + one volume *m*/2 $CaCl_2$, where a few contractions remained after more than an hour. Contractions tend to be sluggish in lithium solutions, and to cease soon, even in the presence of a favorable proportion of calcium; lithium is thus at best an imperfect substitute for sodium.

Addition of calcium to pure solutions of potassium chloride does not counteract the toxicity of the latter salt, but the contractions which follow the transfer from magnesium chloride are decidedly more vigorous and last distinctly longer (three to four minutes) than in the pure *m*/2 KCl. The specific action of the calcium is thus apparent, although even in its presence the potassium quickly destroys all muscular contractility. In the experiments thus summarized $CaCl_2$ was added to *m*/2 KCl in the concentrations *m*/50, *m*/100, etc., to *m*/1600, and, in another series, *m*/400, *m*/600, *m*/800, *m*/1200, *m*/1600, and *m*/2100.

III. Mode of Action of Different Pure Salt Solutions.

In my preceding paper attention was directed to the contrast between the immediate action of pure solutions of alkali chlorides on

the one hand, and of magnesium and calcium chlorides on the other. Larvæ transferred from sea water to the former group of solutions undergo instant shortening with loss of pigment, followed by relaxation and cessation of movement; while in magnesium and calcium chloride solutions the contractions cease by degrees, without muscular shortening and without loss of pigment. On retransfer to sea water after more or less prolonged stay in the respective solutions contractions are found to return gradually and imperfectly in the case of the former group of salts, and promptly and completely in the latter.

This contrast between the two groups of salts is to be referred to the difference in their immediate influence on the permeability of the plasma membrane. Marked and prolonged increase of permeability involves toxic action, since one of its consequences must be a partial disorganization of the cell through loss of essential constituents. Decreased permeability, on the other hand, simply arrests stimulation and its associated metabolic processes by retarding or preventing the escape of metabolic products, especially carbon dioxide. Arrest of activity in a living cell can thus be induced by two essentially opposite means,[23] — one injurious, the other not.

This difference of action is further indicated by the following experiments. To confirm the view that the toxic action of the first group of salts is not due primarily to their entrance into the interior of the cell, but to an alteration of surface permeability, the following experiments were performed. Larvæ were transferred to pure $m/2$ LiCl, KCl, and NaCl (1) directly from sea water, and (2) from magnesium chloride solution in which they had remained for several minutes. After a certain stay in the alkali chloride solution they were brought again into sea water. The alkali chlorides, as already seen, have little immediate action on magnesium chloride larvæ; *i. e.*, on the present view, the permeability remains, for some time at least, practically the same as in the magnesium solution. Such larvæ should therefore show prompt and complete recovery on return to sea water, in contrast to those which were placed in the alkali chloride solution directly from sea water and which, as already pointed out, undergo immediate marked increase in permeability and recover contractility slowly and imperfectly on return

[23] *Cf.* HOEBER: Physikalische Chemie der Zelle und der Gewebe, 2te Auflage, 1906, pp. 293–294.

to the normal medium. These expectations were completely realized, as the experiments of Table X, p. 490, will illustrate.

The following experiments (Table IX) show the differences in promptitude and completeness of recovery between larvæ exposed

TABLE IX

Stay in Solution.		Effect of Transfer to Sea Water.
1. *m*/2 LiCl.	54 m.	No immediate result; larvæ remain motionless after forty-five minutes. At two and one-half hours after transfer contractions are well marked.
	1 h. 52 m.	No contractions are seen for a long time. At one and one-half hours after transfer larvæ show slight contractions at intervals.
	4 h. 20 m.	At one and one-half hours a few slight contractions. At two and one-half hours well-marked contractions at intervals.
2. *m*/2 NaCl.	54 m.	No immediate effect. At twenty-three minutes a few faint contractions are seen. After two and one-half hours contractions are well marked.
	1 h. 50 m.	No contractions are seen for a long time. After one and one-half hours slight contractions occur at infrequent intervals.
	4 h. 17 m.	No contractions are seen at one and one-half hours. Slight movement has returned after about two and one-half hours.
3. *m*/2 KCl.	53 m.	No contractions at twenty minutes. At thirty-seven minutes slight jerky contractions are seen in a good proportion. At two and one-half hours contractions are more active than after NaCl.
	1 h. 45 m.	No contractions at twenty minutes. Contractions are well marked after one hour thirty-seven minutes.
	4 h. 12 m.	No contraction at fifty-eight minutes after transfer. At one- and one-half hours a few slight contractions; next day most larvæ show well-marked contractions, better than after NaCl or LiCl.
4. *m*/2 $MgCl_2$.	52 m.	Vigorous contractions at once. After thirty minutes, movement appears practically normal.
	1 h. 42 m.	Immediate return of vigorous contractions.
	4 h. 10 m.	Immediate contractions.
5. *m*/2 $CaCl_2$.	52 m.	Immediate vigorous contractions.
	1 h. 40 m.	Active contractions return almost immediately.
	4 h. 5 m.	Well-marked contractions return after an interval of a few seconds.
6. *m*/2 $SrCl_2$.	50 m.	No contractions at first or at forty-five minutes after transfer. At two hours and twenty minutes only a few slight infrequent contractions are seen. Practically no return of contractility.
7. *m*/2 $BaCl_2$.	54 m.	No return of contractions.
8. *m*/2 $MnCl_2$.	52 m.	No movements at thirty-three minutes after transfer. At two hours and eleven minutes larvæ show jerky contractions at intervals; after five hours contractions are vigorous and frequent.
	3 h. 30 m.	No movement is seen for more than two hours. Next day a large proportion of larvæ show good contractions.

for approximately equal periods to solutions of various salts and then returned to sea water. Larvæ were brought from sea water into *m*/2 solutions of the following salts: LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$, and $MnCl_2$. The immediate effect of the alkali chlorides and of strontium and barium chloride is to produce a strong initial contraction with loss of pigment, followed by weaker movements which cease within a few minutes (more rapidly in $BaCl_2$ and KCl), while in magnesium and calcium chlorides contractions cease by degrees without the initial contraction or evident increase of permeability. Manganese chloride has an immediate action very similar to that of magnesium chloride; there is no initial contraction, and muscular movements cease in a few seconds; ciliary action continues for an hour or more. Larvæ were transferred from each of these solutions to sea water after varying intervals of time with the results indicated in Table IX.

The return of muscular contractions in sea water is thus imperfect and delayed after exposure to lithium, sodium, or potassium chloride, and the more so the longer the stay in the solution. Potassium appears the least toxic of these three salts (see below, Table X, p. 490). On the other hand, after several hours in *m*/2 $MgCl_2$ vigorous contractions return at once; the same is true for *m*/2 $CaCl_2$, although its action is somewhat more toxic. Strontium and barium chloride are both decidedly toxic, particularly the latter. Manganese chloride, however, resembles the second of the above two groups of salts; although more toxic than magnesium, it appears to differ from the latter chiefly in the less ready reversibility of its action. This corresponds to the difference between the reversibility of the precipitating action of alkali earth and of heavy metal cations on colloidal solutions.[24]

If the larvæ have previously been treated with magnesium chloride, the toxic action of the alkali chlorides is much less pronounced and contractions return promptly on transfer to sea water. The following experiments will illustrate (see Table X).

The protective influence of previous treatment with magnesium chloride appears clearly from these experiments. By this treatment the plasma membrane seems to have been given a consistency which is afterwards altered only slightly and gradually by the alkali chloride. That the latter salt eventually changes the permeability of the

[24] *Cf.* Pauli: Beiträge zur chemischen Physiologie und Pathologie, 1905, vi, p. 233.

membrane is, however, clearly indicated by the results of another series of experiments; larvæ treated for twelve minutes with *m/2* $MgCl_2$ and then left for twelve minutes in *m/2* KCl showed instant recovery in sea water, but after two hours in the *m/2* KCl and return

TABLE X

July 3, 1908. — Larvæ were placed in *m*/2 $MgCl_2$ at 3.20 P. M. After an interval portions were transferred to *m*/2 LiCl, NaCl, and KCl, and thence, after a certain interval, to sea water. The effects following transfer to sea water were compared with those found in larvæ treated for the same length of time with these solutions but without previous exposure to *m*/2 $MgCl_2$.

The exposures to *m*/2 $MgCl_2$, preceding the transfer to *m*/2 LiCl, NaCl, and KCl, were, respectively, 13.5, 17.5, and 24 minutes.

Immediate action of salt solution and length of exposure.	Action of sea water on larvæ exposed to *m*/2 alkali chloride for period given in first column.	
	A. On larvæ placed in solution after treatment with *m*/2 $MgCl_2$.	B. On larvæ transferred to solution directly from sea water.
1. *m*/2 LiCl. 21.5 m. Marked contractions in larvæ from sea water; none in larvæ from *m*/2 $MgCl_2$.	In *m*/2 $MgCl_2$ for 13.5 m. Immediate vigorous contractions in practically all. Larvæ are still largely active after 19 h.	No contractions are seen in sea water for almost an hour, when feeble twitches begin. Next day only a little movement remains — much less than in Experiment 1 A.
2. *m*/2 NaCl. 24.5 m. Marked contractions in larvæ from sea water; none after $MgCl_2$.	In *m*/2 $MgCl_2$ for 17.5 m. Immediate active and vigorous contractions. Next day, after 19 h., larvæ show vigorous and apparently normal movement.	No contractions appear in sea water for the first 20 m. Slight movements begin in about ½ h. Next day (19 h.) most larvæ are disintegrated and dead; a little intermittent movement remains. Marked contrast to 2 A.
3. *m*/2 KCl. 24 m. Marked contraction after sea water, as usual; none seen after *m*/2 $MgCl_2$.	In *m*/2 $MgCl_2$ for 24 m. Vigorous contractions appear within ½ m. Next day all larvæ are active and apparently normal.	No movement appears in sea water for about 10 m. Contractions are well marked after ½ h. Next day all larvæ are living and active.

to sea water there was a delay of over fifteen minutes in the return of contractions. Recovery proved almost complete, nevertheless, while larvæ left for about the same time in *m/2* KCl, but without previous treatment with *m/2* $MgCl_2$, showed only partial and imperfect revival of contractions. The potassium chloride during the two hours of its action had evidently produced considerable alteration in the contractile tissues, in spite of the previous decrease of permeability

by the magnesium salt. The marked and sudden increase of permeability in pure alkali chloride solutions, which otherwise results and to which a large part of the toxic action is to be ascribed, is however prevented by previous exposure to magnesium chloride.

Summary.

1. Arenicola larvæ lose muscular contractility gradually in pure isotonic solutions of non-electrolytes (dextrose, cane-sugar), more rapidly in solutions of magnesium salts. The effect resembles anæsthesia, and contractility is readily restored on return to sea water or to various electrolyte solutions.

2. Pure solutions of sodium salts produce well-marked contractions in larvæ from isotonic sugar solutions, but few or none in those from *m*/2 $MgCl_2$. The addition of a little calcium chloride to sodium chloride solutions greatly increases the ability of the latter to restore normal contractility; calcium shows perceptible action in dilutions so low as *m*/25600.

3. In the intensity of their immediate stimulating power sodium salts show a definite relation to the character of the anions; the order of increasing toxicity is, in general, also that of increasing stimulating power. This order is essentially as follows: $COOCH_3$ $SO_4 < C_4H_4O_6 < HPO_4 < Cl < NO_3$ and $ClO_3 < Br < I$. This is also the characteristic order of relative action on colloids.

4. Of the alkali chlorides, sodium, lithium, and cæsium restore approximately normal contractions, for a short time, in larvæ from sugar solution. Rubidium and potassium have a more powerful immediate stimulating action, but destroy contractility more rapidly. The order of increasing favorable action is: K and $Rb < NH_4 < Li < Cs < Na$. This order is also the order of decreasing action in the precipitation of lecithin and egg albumin by these salts.

5. Of pure solutions of the alkali earth chlorides magnesium produces no contractions; calcium and strontium produce slight contractions lasting some time; barium causes marked shortening and quickly destroys contractility. The order of increasing toxicity is $Mg < Ca < Sr < Ba$.

6. Weak solutions of acid (HCl, H_2SO_4, $HCOOCH_3$) in concentrations from *n*/3200 to *n*/12800 restore, for a short time, almost normal contractions in larvæ from sugar solutions. Alkali has little or no such action in concentrations of *n*/50 and lower. Acid and

alkali have opposite influence on muscular tone, the former increasing and the latter decreasing the tonic contraction. Larvæ previously treated with *m*/2 $MgCl_2$ are unaffected by acid and alkali in these concentrations.

7. A given salt solution (*e. g.*, *m*/2 NaCl, KCl) produces different effects according to the medium in which the larvæ remained before being brought into the solution. Larvæ transferred from sea water to *m*/2 NaCl undergo marked stimulation and increase in permeability; larvæ from sugar solution show less energetic contractions; and from *m*/2 $MgCl_2$ few or none. In the latter case the toxic action of the pure *m*/2 NaCl (etc.) is also greatly diminished.

8. The action of electrolytes in altering the permeability of the plasma membranes, and the relation of these changes of permeability to the irritability and to the energy production of the tissue (stimulation, inhibition, anæsthetization) are discussed throughout the paper.

THE ABSORPTION OF FATS STAINED WITH SUDAN III.

By LAFAYETTE B. MENDEL.[1]

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

IN a recent note in this journal Whitehead[2] has attempted to test the question as to whether fats are absorbed in a state of emulsion by feeding butter stained with Sudan III to a half-grown cat and subsequently examining the lacteals for the dye. The animal was killed four and a half hours after the ingestion of the fat and the intestines were observed. "It was noticed that, while the mucous membrane was stained quite red, the lacteals in the mesentery were white." Microscopic evidence having demonstrated that fat had actually been absorbed in great abundance from the gut, the absence of visible dye beyond the intestinal lumen was taken as proof that the fat "had not been absorbed as such, but had been taken up by the villi in water-soluble forms of its constituents. The presumption is that the fatty acids enter the villi in soaps." The last statement seemed to be justified from the fact that oleic acid, in distinction from soaps, readily dissolves Sudan III; hence the butter fat could not have traversed the absorbing intestinal wall in the form of its constituent fatty acids without transporting some of the dye.

We were quite unprepared for this conclusion, which is at variance with our own experience as well as diverse facts noted in the literature of the subject. It is true that the water-soluble Sudan III dissolves with great readiness in glacial acetic, lactic, oleic, palmitic, and stearic acids, but not in glycerol, pure soaps (sodium and potassium palmitate), or soap solutions. Yet the evidence has rapidly accumulated in recent years to indicate that free fatty acids, which are freely soluble in the bile, are readily absorbed as such from the

[1] The experiments were carried out with the assistance of Miss A. F. BLOOD and Mr. G. A. BAITSELL.

[2] WHITEHEAD: This journal, 1909, xxiv, p. 294.

alimentary tract.[3] Furthermore, it has been observed that the adipose tissue of animals fed with Sudan III may become deeply tinted with the pigment which does not appear elsewhere in the body in quantities evident to the unaided eye. Are we to assume in these cases that the dye has entered the circulation through some channel other than the lacteals and the thoracic duct, either in suspension or otherwise than dissolved in fat or fatty acid?

The attempt to solve the problem of the form in which fats are absorbed, by feeding stained fats, is not new. Hofbauer[4] made use of alkanna red, which resembles Sudan III in its solubilities. He too reasoned that if fats were hydrolyzed prior to absorption and transformed into water-soluble compounds, the dye would be precipitated in the lumen of the gut and the chyle fat would be colorless. The actual experiments showed, however, that the chyle fat was stained red. Since other amorphous particles are not absorbed as such, Hofbauer assumed that deposited alkanna likewise was not; and he drew the conclusion that the fat is absorbed in emulsion. Hofbauer overlooked the rôle of the fatty acids in transporting the coloring matter he used. His error of interpretation has been pointed out by Friedenthal.[5]

The experiment of Whitehead was entirely analogous to those of Hofbauer; yet the American investigator failed to discover the stained fat in the lacteals. Correct as his conclusions regarding fat digestion prior to absorption are in the light of our present knowledge from other sources, his actual observations are not in accord with the numerous published experiments with alkanna nor our own with Sudan III. *The dye readily passes into the lacteal channels when it is fed with fats.* A few protocols will suffice to indicate the methods of investigation employed.

I. A small dog was fed 900 c.c. of milk containing 1 gm. of Sudan III. (Gruebler's). Four hours later the animal received a hypodermic injection of 2 gm. of chloral hydrate and was then anæsthetized with A. C. E. mixture. Lymph was collected from a cannula placed in the thoracic duct. The flow was normal, ranging from 25 c.c. to 15 c.c. per hour.

[3] For example, *cf.* PLANT: This journal, 1908, xxiii, p. 73.

[4] HOFBAUER: Archiv für die gesammte Physiologie, 1900, lxxxi, p. 263.

[5] FRIEDENTHAL: Centralblatt für Physiologie, 1900, xiv, p. 258; also in his Arbeiten aus dem Gebiet der experimentellen Physiologie, 1908, p. 188. *Cf.* also PFLÜGER: Archiv für die gesammte Physiologie, 1900, lxxxi, p. 375.

The lymph itself was of a bright pink color and opaque, as is characteristic of "digestion lymph." Microscopically it showed the presence of numerous lymphocytes filled with fat globules. Fifty-five c.c. of the fluid were dried and extracted with ether, whereby both fat and Sudan pigment were easily removed. At the end of three hours, *i. e.* nine hours after the beginning of the feeding, the dog was killed by bleeding and the intestines and mesentery inspected at the same time. The small lymph vessels arising from the intestine were distinctly pink in tinge, precisely as the thoracic duct appeared *in situ*, plainly differentiated from the surrounding connective tissue by its unique color.

The small intestines still contained some of the pigment in the upper half of the tract. The larger lymphatic glands, pancreas Aselli, and in some spots the Peyer's patches, were tinged pink. The passage of the dye into the lymphatics was placed beyond doubt. The cervical lymph vessels were unstained.

II. In another small dog the same results were noted. The animal died from an overdose of anæsthetic four and one-quarter hours after being fed a litre of milk containing 1 gm. of Sudan III. A post-mortem lymph flow (4.1, 2.5, 1.2, 0.2 c.c. in successive ten-minute periods) was observed, and the bright pink lymph contained Sudan as in the previous case. The lymphatics of the mesentery examined two hours after death of the animal were barely visible. The pancreas Aselli was distinctly pink.

III. A large cat was anæsthetized with ether and C. E., four and one-quarter hours after a meal of 400 c.c. of milk containing 0.5 gm. Sudan III. The lymph collected from a cannula in the thoracic duct flowed freely (3.6 c.c. in ten minutes) and was opaque and pink. The dye was removed readily by extracting the dried lymph with ether. The cat was killed five and one-fourth hours after feeding and the intestines and mesentery examined. The pink color was distinct in the larger lymphatic vessels coming from the upper gut. It was noted that the lower part (ileum) contained no color and the lymph vessels arising therefrom were not tinted. The pancreas Aselli was stained pink in its headward portion in correspondence with the preceding. The urine was free from the pigment.

IV. To duplicate Whitehead's experiment more closely and meet the objection that in our experiments much of the dye was suspended in the milk rather than dissolved in the fat droplets, a cat was fed meat mixed with 30 gm. of butter fat stained deep red with Sudan III. Five and a half hours later the animal was anæsthetized with ether and blood was removed from the carotid artery. The serum from the defibrinated, centrifugated blood was opaque with fat droplets and colored bright pink. The Sudan pigment could be recovered by extraction with ether. The thoracic duct, the lymphatics of the mesentery throughout the entire

length of the small intestine, the pancreas Aselli, and the cistern of the chyle were all bright pink. No lymph was collected for examination.

V. The experiment with stained butter has been repeated on a dog also, with the same outcome. In one experiment similar to III, the lymph was colorless and transparent. Investigation showed that although the stomach was well filled with the stained milk, none had passed into the intestine. The failure of absorption was thus explained.

In seeking for a possible explanation of Whitehead's failure to detect the pigment in the lacteal vessels in his experiment, we noted (as mentioned above) that only those lymphatics which arise from seats of actual absorption are stained visibly to the naked eye. Furthermore, if the mesentery is examined *after death* these vessels are likely to be in part collapsed and empty so that they fail to disclose the characteristic stain. The collection of the contents of the lymphatics and extraction of the pigment with ether removes all doubt. Like Whitehead, we can recommend experiments such as those quoted, for demonstration purposes.

Summary.

When fat stained with water-insoluble dyes, like Sudan III, is fed, *the pigments readily pass into the lymphatic vessels* and thereby reach the blood stream. Since these compounds are soluble in free fatty acids as well as in neutral fats, their presence in the lymph cannot be taken as evidence either for or against the possibility of the digestion of fats prior to their absorption.

INDEX TO VOL. XXIV.

ABSORPTION of fat, 294, 493.
Acapnia and shock, 66.

BENEDICT, F. G. An apparatus for studying the respiratory exchange, 345.
BENEDICT, F. G. See CARPENTER and BENEDICT, 187, 203.
Blood, coagulation of, 406.
Blood pressure, affected by smoking, 104.
Bone ash, effect on metabolism and digestion, 297.
BRUCE, J. W., J. R. MILLER, and D. R. HOOKER. The effect of smoking upon the blood pressures and upon the volume of the hand, 104.

CARPENTER, T. M., and F. G. BENEDICT. Mercurial poisoning of men in a respiration chamber, 187.
CARPENTER, T. M., and F. G. BENEDICT. Preliminary observations on metabolism during fever, 203.
Centre of gravity, determination in men, 286.
Coagulation, in muscle, 1.
——, of blood, 406.
——, related to heat-shortening, 178.
Contractility, related to ions, 459.
Contractions, rhythmical, in Gonionemus, 117.
COOK, F. C. The effects of chloride, sulphate, nitrate, and nitrite radicles of some common bases on the frog's heart, 263.
Coronary vessels, innervation of, 391.
Creatinin output in man, 45.

DESICCATION, an improved method, 297.
Digestion, rate in cold-blooded vertebrates, 447.

ENZYMES, relation of secretion to blood supply, 234.
ERLANGER, J. Can functional union be reestablished between the mammalian auricles and ventricles after the destruction of a segment of the auriculo-ventricular bundle? 375.
EVERINGHAM, S. See LEE and EVERINGHAM, 384.

FARADIC stimulation, the measurement of "make" shocks, 269.
Fat absorption, 294, 493.
Fatigue, of spinal cord, 384.
Fever, metabolism in, 203.

HART, E. B., E. V. MCCOLLUM, and G. C. HUMPHREY. The rôle of the ash constituents of wheat bran in the metabolism of herbivora, 86.
Heart, auriculo-ventricular bundle, 375.
——, innervation of coronary vessels, 391.
——, relation of inorganic salts to contractility, 263.
——, relation of vagus action to temperature, 341.
HENDERSON, Y. Acapnia and shock.—III. Shock after laparotomy; its prevention, production, and relief, 66.
HOOKER, D. R. See BRUCE, MILLER, and HOOKER, 104.
HUMPHREY, G. C. See HART, MCCOLLUM, and HUMPHREY, 86.
Hydrolysis, of crystallized albumin from hen's egg, 252.
——, of muscle of scallop, 161.
——, of ox muscle, 437.
——, of vitellin, 153.

INDUCTORIUM, measurement of "make" shocks, 269.

JONES, D. B. See OSBORNE, JONES, and LEAVENWORTH, 252.
JONES, D. B. See OSBORNE and JONES, 153, 161, 437.

KRISTELLER, L. See LEVENE and KRISTELLER, 45.

LEAVENWORTH, C. S. See OSBORNE, JONES, and LEAVENWORTH, 252.
LEE, F. S., and S. EVERINGHAM. Pseudofatigue of the spinal cord, 384.
LEVENE, P. A., and L. KRISTELLER. Factors regulating the creatinine output in man, 45.
LILLIE, R. S. On the connection between changes of permeability and stimulation, and on the significance of changes in permeability to carbon dioxide, 14.
LILLIE, R. S. The relation of ions to contractile processes. — IV. The influence of various electrolytes in restoring muscular contractility after its loss in solutions of sugar and of magnesium chloride, 459.
LOTHROP, A. P. The effects of bone ash in the diet on the gastro-intestinal conditions of dogs, 297.
LOVETT, R. W. See REYNOLDS and LOVETT, 286.
LYON, E. P. On rheotropism. — II. Rheotropism of fish blind in one eye, 244.

McCOLLUM, E. V. See HART, McCOLLUM, and HUMPHREY, 86.
MARTIN, E. G. A quantitative study of faradic stimulation. — III. The measurement of "make" shocks, 269.
MEIGS, E. B. Concerning the supposed connection between protein coagulation and the heat shortening of animal tissues, 177.
MEIGS, E. B. Heat coagulation in smooth muscle; a comparison of the effects of heat on smooth and striated muscle, 1.
MENDEL, L. B. The absorption of fats stained with Sudan III, 493.
MENDEL, L. B., and H. G. WELLS. Experimental studies on the physiology of the molluscs, — fourth paper, 170.
Mercurial poisoning of men in a respiration chamber, 187.
Metabolism, in fever, 203.
——, rôle of wheat bran, 86.
MILLER, J. R. See BRUCE, MILLER, and HOOKER, 104.
Molluscs, chemical physiology of, 170.
Muscle, coagulation in, 1.
——, contractility restored by various electrolytes, 451.
——, heat shortening, 178.

OSBORNE, T. B., and D. B. JONES. Hydrolysis in ox muscle, 437.
OSBORNE, T. B., and D. B. JONES. Hydrolysis of the muscle of scallop (Pectens irradians), 161.
OSBORNE, T. B., and D. B. JONES. Hydrolysis of vitellin from the hen's egg, 153.
OSBORNE, T. B., D. B. JONES, and C. S. LEAVENWORTH. Hydrolysis of crystallized albumin from hen's egg, 252.

PERMEABILITY, relation to CO_2, 14.
PIKE, F. H. Studies in the physiology of the central nervous system. — The general phenomena of spinal shock, 124.

RESPIRATION chamber, poisoning in, 187.
Respiratory exchange, method for study of, 345.
RETTGER, L. J. The coagulation of blood, 406.
REYNOLDS, E., and R. W. LOVETT. A method of determining the position of the centre of gravity in its relation to certain bony landmarks in the erect position, 286.
Rheotropism of fish blind in one eye, 244.
Rhythmical contractions in Gonionemus, 117.
RIDDLE, O. The rate of digestion in cold-blooded vertebrates. — The influence of season and temperature, 447.
Rigor caloris, 178.
RYAN, J. G. The variations in the enzyme concentration with the variation in the blood supply to the secreting gland, 234.

SECRETION, enzyme concentration related to blood supply, 234.
SHACKELL, L. F. An improved method of desiccation, with some applications to biological problems, 325.
Shock, 124.
—— and acapnia, 66.

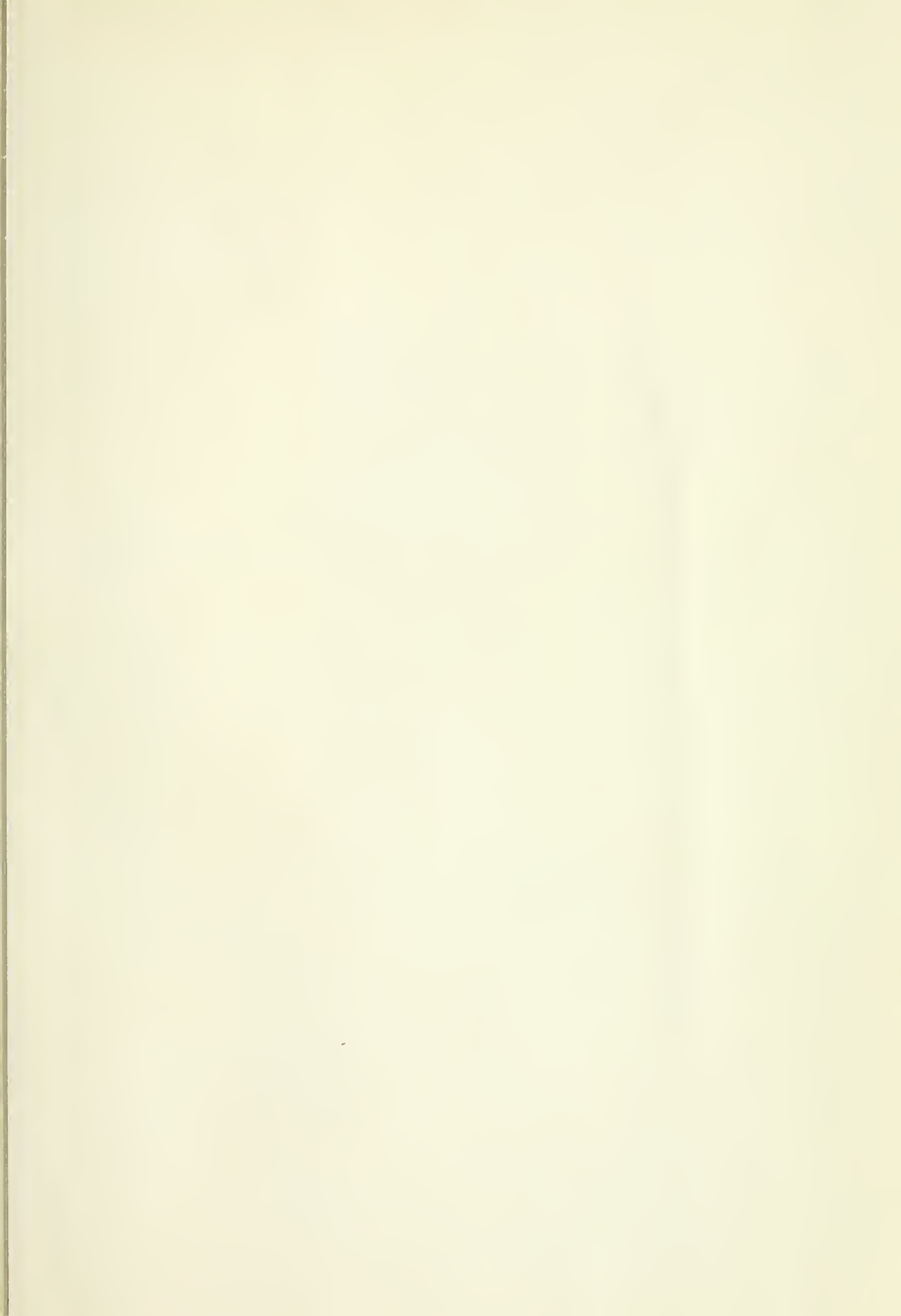
Smoking, effect on blood pressure, 104.
Spinal cord, pseudo-fatigue of, 384.
STEWART, G. N. The influence of the temperature of the heart on the activity of the vagus in the tortoise, 341.
Stimulation, relation to permeability, 14.

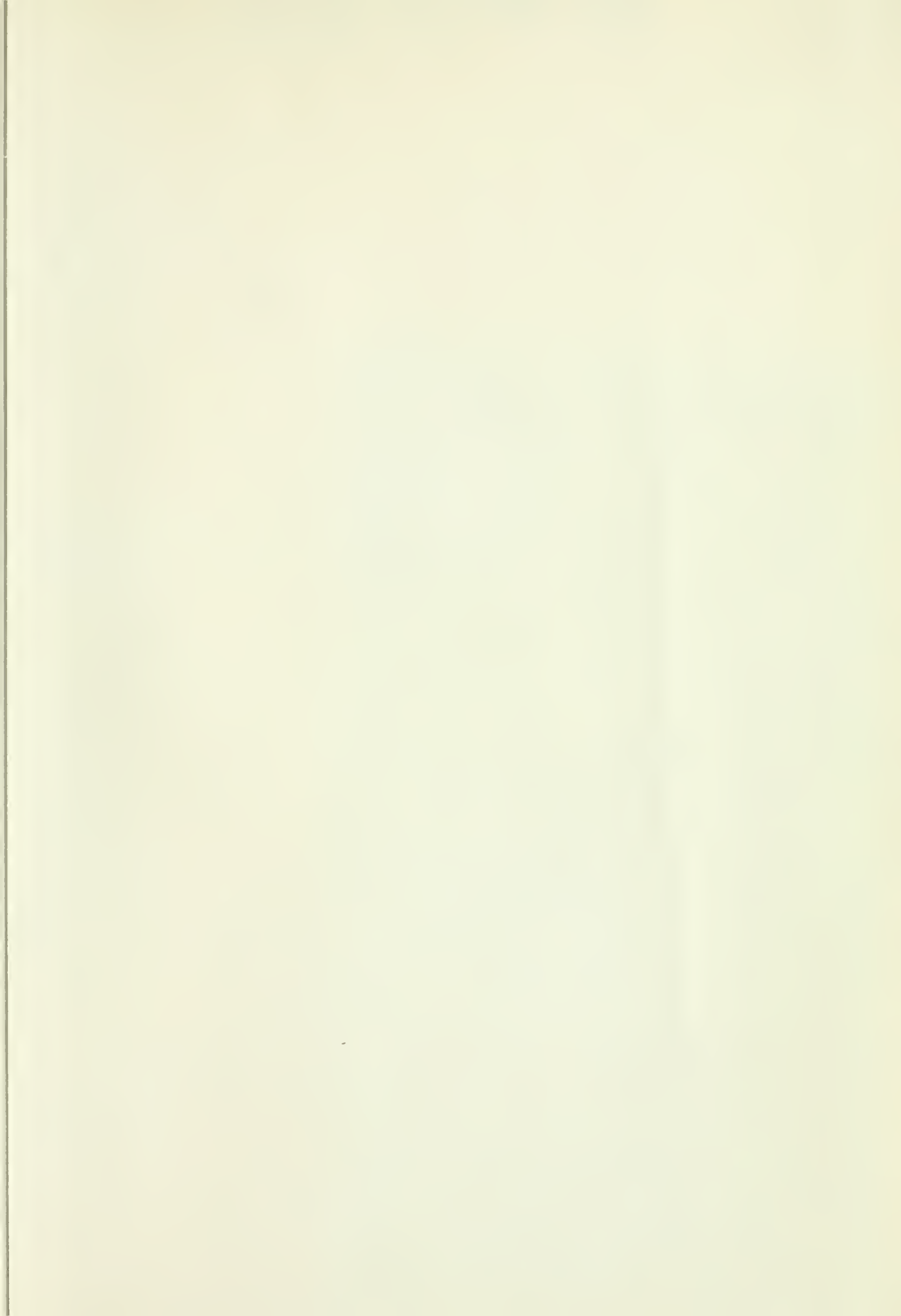
TERRY, O. P. The production by hydrogen peroxide of rhythmical contractions in the marginless bell of Gonionemus, 117.

VAGUS, effect of temperature upon, 341.

WELLS, H. G. See MENDELL and WELLS, 170.
Wheat bran, rôle of ash constituents in metabolism, 86.
WHITEHEAD, R. H. A note on the absorption of fat, 294.
WIGGERS, C. J. The innervation of the coronary vessels, 391

665 -4





BINDING SECT. MAR 4 1966

QP American Journal of Physiology
1
A5
v.24
cop.2

Biological
& Medical
Serials

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY

STORAGE