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NO. I.

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THE MECHANICAL EFFECTS PRODUCED BY THE CONTRACTION OF INDIVIDUAL MUSCLES OF THE THIGH OF THE FROG.

BY WARREN P. LOMBARD AND F. M. ABBOTT.

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

INTRODUCTION.

MANY years ago one of the writers of this paper attempted, under the guidance of Professor Carl Ludwig, to determine the method of spread of reflex processes in the spinal cord of the frog.<sup>1</sup> In the course of this work he was compelled to recognize that many of the movements of the hind limb of the frog which have the appearance of being the result of finely adjusted nervous co-ordinations, are really due to the mechanical conditions under which the muscles act on the bones. The lesson was forced home, that before we can arrive at reliable conclusions as to the method of action of the central nervous mechanisms concerned in reflex actions, we must obtain a clear picture of the mechanics of the limb itself.

In a recent paper<sup>2</sup> it has been shown that not only the effectiveness of the contraction of a given muscle alters with the gain and loss of leverage which comes as the bones have their position changed, but that the character of the movement which a given muscle may produce may be greatly altered, indeed in some cases reversed, by a change in the relative positions of the bones on which the muscle in

<sup>1</sup> Die räumliche und zeitliche Aufeinanderfolge reflectorisch contrahirter muskeln. Archiv für Anatomie und Physiologie, 1885, p. 408.

<sup>2</sup> "The tendon action and leverage of two-joint muscles of the hind leg of the frog with special reference to the spring movement." Contributions to Medical Research, dedicated to Victor Clarence Vaughan, University of Michigan, June 1903, p. 280.

question acts. Thus a muscle which in one position of a bone may act as a flexor, in another position of the bone may act as an extensor, and a muscle which in one position of a bone may carry it dorsally, in another position may carry it ventrally. In the same paper the remarkable action of the two joint muscles was pointed out, and it was shown how muscles which act as antagonists in certain positions of the bones may in other positions act as synergists. It is evident that we can form no estimate of the part played by the central nervous system in co-ordinated movements of locomotion, for example, until we have ascertained in how far these movements are determined by the purely mechanical conditions existing in the limbs.

Just as it is unsafe to draw any conclusions concerning central co-ordination before the mechanics of the limbs have been exhaustively studied, so it is unsafe to draw conclusions concerning the combined action of the muscles until the method of action of the separate muscles has been ascertained. Nor can even this be done profitably until it has been found in how far the character of the movements of the bones is determined by the structure of the joint surfaces and ligaments, for these largely decide not only the limits but the directions in which the bones shall move under the influence of muscular contractions.

In the course of the experiments reported in this paper, the action of the structures of the joints to determine the direction and to limit the extent of the movements of the bones was examined with considerable care, but was not made the subject of exhaustive investigation. It is believed, however, that sufficient work was done in this direction to permit us to recognize how far the motion which results from the contraction of a muscle is caused by the action of the muscle itself, as distinguished from the passive influence exerted by the structures of the joint.

This research does not deal with the action of muscles under physiological conditions, but with the mechanical effects of the shortening of individual muscles.

All of the infinite number of positions which the bones entering into the hip and knee joints can take with respect to each other can be reached by appropriate muscular action. It would be, however, impossible to determine experimentally the exact method of action of each of the muscles which takes part in placing the leg in each of these innumerable positions, or to ascertain what would be the action of each of the muscles when the leg occupied each of these positions.

In this research the attempt was made to find out what kinds of movements could be caused by contraction of the muscles of the thigh when the bones entering into the hip and knee joints occupied certain definite positions. In the case of the muscles which act on the hip, for example, when the pelvis was fixed and the thigh was free to move, under each of the following conditions, namely, —

*a.* When pelvis and femur were placed horizontally, and the femur was without rotation and was flexed, half extended, and extended.

*b.* When the pelvis was horizontal, and the femur had been carried either ventrally or dorsally out of the horizontal plane a desired number of degrees, without rotation, and was flexed, half extended, and extended.

*c.* When the pelvis had been tilted dorsally or ventrally a desired number of degrees, and the femur which was horizontal and without rotation, was flexed, half extended, and extended.

*d.* The effect of rotation of the femur about its long axis was also noted in the above positions of the bones.

The effect on the pelvis when the femur was fixed and the pelvis was free to move was also studied.

A similar plan of work was carried out in studying the action of the muscles of the thigh on the knee joint.

When the ability of a given muscle to flex and extend the thigh or lower leg, to carry it dorsally and ventrally, to rotate it dorsally and ventrally, and to rotate the pelvis about the transverse axis, has been ascertained, when the bones occupy the above positions, a fairly accurate picture of the mechanical action of the muscle, when acting alone, is obtained. Under normal conditions muscles never act independently, all ordinary movements being the resultant of the synchronous action of many muscles. In some few cases the effect of the combined action of two or more muscles was studied, but in general this research was limited to studying the action of individual muscles when acting alone.

**Terms used to describe the muscles and the movements which they produce.** — One of the greatest difficulties which we encountered at the beginning of our work was to settle upon suitable terms for describing the direction, character, and extent of the movements produced by the muscles, and before we enter upon a description of our experiments it will be necessary for us to define the terms which we employ.

Gaupp states, in his admirable revision of Ecker's and Wiederheim's "*Anatomie des Frosches*,"<sup>1</sup> his reason for the choice of the

<sup>1</sup> GAUPP: Braunschweig, 1896, pp. viii and ix.

names which he has applied to the muscles. His point of view is well chosen, and his nomenclature will be followed in this paper. Short names and names the same as those given to homologous human muscles are much more convenient to the physiologist than long compounds giving the origin and insertion, or purporting to describe the function, of the muscles. Indeed the function of the muscles is so imperfectly understood that the last method is very undesirable.

Although Gaupp will be followed in the naming of the muscles, we shall have to depart somewhat from the terminology which he employs for the description of the movements which they produce. It is unfortunate that we cannot merely adopt the terms made use of by the human anatomist. The body of the frog is propelled with its long axis approximately horizontal, and that of man with its long axis nearly vertical. The body of the frog is supported by the hind limbs only when the animal is squatting and during the early part of the leap, *i. e.*, when the legs are considerably flexed; the body of man has to be supported by the legs not only in the above positions, but when the legs are extended, by standing and ordinary movements of locomotion. This difference in the direction of the movement of the body and the way it is supported, is associated with a difference in the position of the limbs with respect to the trunk. The structure of the hip joint and the attachment of the muscles surrounding it, in the case of the frog, is such that the thigh and consequently the whole leg when in the extended position, compared with the human leg, is rotated outward through about a quarter of a circle. As Gaupp states,<sup>1</sup> "By extended leg, *i. e.*, put as far back as possible in the direction of the prolongation of the long axis of the body, the surface of the lower leg which corresponds with the front surface by man faces laterally; the back of the lower leg in the median direction; the lateral side dorsally; and the median side ventrally."

As a result of this difference in the position of the leg with respect to the trunk, movements of the leg, which, from the consideration of the muscles involved, may be classed as the same for frog and man, take place in different directions. For example, flexion of the hind leg both of the frog and man causes adjacent segments of the limb to approach, and extension to separate, but occurs in general in the case of the frog in a horizontal plane, and in the case of man in a vertical plane. Abduction and adduction carry the human leg in the lat-

<sup>1</sup> GAUPP: Braunschweig, 1896, p. 189.

eral and the median directions, but corresponding movements carry the leg of the frog dorsally and ventrally. Rotation of the leg of the frog, in the sense which is termed external rotation by man, turns the leg dorsally, and internal rotation turns it ventrally.

It is not easy to choose satisfactory terms to describe the movements of the hind leg of the frog, and the terms employed in this paper were decided upon only after the character of the movements had been carefully studied, and many attempts had been made to describe them with accuracy. In giving these definitions, unless it be otherwise stated, the body of the frog will be considered to be placed horizontally, with the dorsum upwards.

**The initial position.**—In order to state clearly the position which the bones hold at a given time, and the movements which they undergo as a result of the action of the muscles, it is necessary to establish for each of the bones three definite axes and planes. This must be done arbitrarily. The femur, for example, has a double S-shaped curvature, and the peculiar relation of the heads of the bone to the shaft is such that there are no definite points on the bone which can be taken to fix these axes. It was decided, therefore, to place the bones in some special position, and then establish a long, a vertical, and a horizontal axis for each of them. The position first chosen was the resting, semi-extended position produced by the elastic tension of the soft parts. This position, however, was found to vary somewhat in different frogs and under different conditions, and it was finally found best to place the leg in a position which differs but little from this, and to which we have given the name "initial position." In this position the pelvis-leg preparation lies, dorsum upwards, with the iliac wings, the femur, os cruris, and tarsus horizontal, and the femur forming a right angle with the long axis of the pelvis, the os cruris a right angle with the femur, and the tarsus a right angle with the os cruris.

In the initial position the following axes may be established for the pelvis and the bones of the leg. These axes are not to be considered as axes about which the bones rotate, but as axes which can be referred to in describing the movements of the bones.

*The axes of the pelvis.*—The *long axis* passes through a point midway between the centres of the sacro-iliac joints, and a point midway between the centres of the acetabula. In all of our measurements, however, the *long axis of the iliac wing* was used. This is represented by a line passing through the centre of the proximal end and the

centre of the point of origin of the glutæus magnus on the superior process of the wing ilium. The *transverse axis* passes through the centres of the acetabula, cuts the long axis at a right angle, and is parallel to a line connecting the centres of the sacro-iliac joints. The *sagittal axis* passes through the point of intersection of the other two axes, and is perpendicular to them.

*The axes of the femur.* — The *long axis* of the femur passes through the centre of the proximal end, and the centre of the distal end of the shaft of the bone. The *transverse axis* passes through the centre of the shaft of the femur at its distal end, lies in the horizontal plane, and forms a right angle with the long axis. The *sagittal axis* passes through the point of intersection of the other two axes, and forms a right angle with each of them. It has been found possible, by means of a special arrangement described under "Method of Work," to bore a hole through the shaft of the femur in the direction of the sagittal axis and to insert a slender needle in this hole. The needle then represents the sagittal axis of the femur, and is of the greatest assistance in the determination of the amount that the femur is rotated about its long axis.

*The axes of the os cruris.* — These axes are established in the same manner as those of the femur.

*The axes of the tarsus.* — The *long axis* passes through the centres of the extremities of the bone. The *sagittal axis* passes through the distal extremity of the bone in the direction of the long diameter of the end of the bone, and makes a right angle with the long axis. In the initial position it is not vertical, but it is inclined about  $30^\circ$ ; that is, the bone can be considered to be rotated ventrally  $30^\circ$ , clockwise, as seen from the distal end of the bone. The *transverse axis* passes through the centre of the distal extremity of the bone, and forms a right angle with the other two axes.

**The frontal, transverse, and sagittal planes of the pelvis, femur, os cruris, and tarsus.** — The frontal plane passes through the long and transverse axes of the bone in question; the transverse plane passes through the transverse and sagittal axes; and the sagittal plane passes through the sagittal and longitudinal axes.

**Flexion and extension.** — Gaupp uses the terms flexion and extension when speaking of the ordinary bending and straightening of the limb at the knee, but describes similar movements at the hip as abduction (for flexion) and adduction (for extension).

When the leg is thrust out or drawn up to the body in swimming,

the thigh and lower leg move caudad and cephalad in practically the same plane. This being so, it does not seem advisable to describe the movements of the thigh as adduction and abduction, and movements of the crus of a similar type as extension and flexion. Moreover, the term adduction does not serve to describe the whole course of the thigh as it is thrust away from the trunk; it first leaves, and then approaches, the long axis of the body, is first abducted and later adducted. The terms abduction and adduction should not be used for any of the movements of the thigh of the frog; they are not necessary and are apt to cause confusion.

Gad says: <sup>1</sup> "The drawing up of the thigh to the position ready for the spring, because the thigh is rotated outwards so far that the knee moves in the transverse plane, resembles abduction in man, but is to be regarded as flexion because carried out by muscles homologous to the flexors in man." The point is well taken, but the movement is more complex than Gad describes it. In taking the squatting position, the limb does not simply move in what he describes as the transverse plane; during the last part of the movement it is carried ventrally, so as to bring the thigh somewhat under as well as side of the belly. Gaupp describes this oblique movement, in which the knee is carried cephalad and ventrally at the same time, as ventral-flexion, and the reverse movement, which straightens the limb, as extension. This usage is found to be very inconvenient when one attempts to give a detailed statement of the action of individual muscles.

We have found it best to describe movements of the thigh which cause it to approach the pelvis, and movements of the crus which cause it to approach the thigh, as flexion, and movements which cause these parts to separate as extension. We use these terms regardless of the plane in which the movements occur. If, in the course of flexion or extension at the hip, the thigh is carried out of the horizontal plane, this fact can be indicated by stating that it is carried ventrally or dorsally at the same time that it is flexed or extended. In general it is more convenient to speak of the more distal portion of the limb as being flexed or extended with respect to the more proximal, *e. g.*, to speak of the thigh as being flexed on the pelvis. As a matter of fact the same effect is produced in a joint regardless of which of the two parts is fixed and which moves with respect to the other.

**Over-flexion.** — In the case of man flexion of the knee is limited by

<sup>1</sup> GAD: Verhandlungen der physikalischen medicinischen Gesellschaft in Würzburg, N. F., 1884, xviii, p. 171.

the lower leg coming in contact with the thigh or sooner. In the case of the frog this limitation does not exist, because the lower leg can be carried dorsally and rotated ventrally sufficiently to enable it to pass by the dorsal side of the thigh. This is made possible by the curvature of the lower end of the femur, the form of the joint surfaces, the method of attachment of the ligaments, and the way the muscles are arranged on the femur and os cruris near the knee. Flexion movements continued until the lower leg passes by and begins to separate from the thigh, according to the definition just given, might be termed extension; as, however, this position is reached by a continuation of flexion, we shall speak of that part of the flexion movement which carries the lower leg past the thigh as over-flexion.

Movements of the foot which bend and straighten the limb at the ankle are described by Gaupp as dorsal flexion and plantar flexion. It is not very satisfactory to speak of a straightening of the limb at the knee as extension and a straightening of the limb at the ankle as flexion, nor is it consistent to use the same term, flexion, for two antagonistic types of movement. Since the terms extension and flexion are to be used in the case of the hip and knee, it seems best to employ these same terms for similar movements at the ankle.

In the case of the movements of the toes, however, one has to speak of plantar and dorsal flexion, since the toes can pass from the straightened, extended position to one which is more bent by moving in the direction of either the dorsum or the plantar surface of the foot.

**To be carried dorsally or ventrally.** — If rotation occurs in a joint of a type to carry the free end of the bone out of the horizontal plane in the dorsal direction, the bone is said to be carried dorsally, and if the free end is carried out of the horizontal plane in the ventral direction, the bone is said to be carried ventrally. If the pelvis is fixed and the thigh is free to move, the thigh may be carried dorsally by rotating about an axis which passes horizontally through the head of the femur and which is perpendicular to its long axis. Similarly the pelvis might be carried dorsally or ventrally when the femur is fixed, by rotating about the horizontal transverse axis of the pelvis. We shall have frequent occasion to refer to this latter type of movement, and shall speak of the pelvis as being tilted dorsally and ventrally, so as to become inclined to the horizontal plane.

When the femur is fixed with its frontal plane horizontal, the os cruris, in flexing, may be carried past the femur and become over-flexed. In making this movement the os cruris is carried dorsally.

Under normal conditions this same movement is made when the thigh itself has been carried dorsally out of the horizontal plane, and consequently the movement of the crus does not take place in the dorsal direction: nevertheless, inasmuch as the os cruris makes the same movement with respect to the frontal plane of the femur, the same term will be used to describe it.

**Ventral and dorsal rotation.** — These terms imply rotation about the long axis of the bone. Ventral rotation is employed in the same sense as inward rotation by man, *i. e.*, clockwise, as seen from the distal end of the bone; and dorsal rotation is used in the same sense as outward rotation by man, *i. e.*, contra-clockwise.

**Names of the surfaces of the limbs.** — Quain states that, from the standpoint of the morphologist, the dorsum of the foot and the anterior surface of the lower leg of man belong to the extensor surface of the limb, and the sole of the foot and the back of the lower leg to the flexor surface, so that the muscles on the front of the lower leg should be called extensors and the muscles on the back of the lower leg flexors. Nevertheless it is customary to term the raising of the foot on the lower leg flexion, and its depression, extension.<sup>1</sup> In the very next sentence he proceeds to say: "The gastrocnemius acts both as a flexor of the knee and extensor of the ankle joint," *i. e.*, although on the flexor surface it is both flexor and extensor. As a matter of fact, each of the many two-joint muscles of the hind leg, with one exception, is a flexor with respect to one, and an extensor with respect to the other of the joints upon which it acts. Not all of the one-joint muscles, even, have a constant flexing and extending action.

When the hind leg of the frog is drawn up to the sitting position, it is folded upon itself, each successive link in the chain of bones, as far as the toes, moving in the opposite direction to the preceding one. Physiologically the leg has no flexor or extensor surface; it does not coil up, but folds together. We shall follow Gaupp in referring to the surfaces of the leg as the *dorsal, ventral, lateral, and median* surfaces, by which are meant those which look in these directions when the frog lies on its belly with extended legs. This is convenient in spite of the fact that when the leg has been drawn forward to the sitting position, the rotation taking place at the hip, knee, and ankle, causes the directions which these surfaces face to be completely changed; *e. g.*, the lateral surfaces of the thigh and tarsus and the median surface of the lower leg come to look nearly dorsally. We

<sup>1</sup> QUAIN: *Anatomy*, 1892, ii, p. 273.

shall avoid the terms flexor and extensor surface of the leg. It is often convenient, however, to refer to the *flexor* and *extensor sides* of the *hip*, *knee*, and *ankle joints*, as Hering does, meaning the sides over which the tendons of the muscles which bend or straighten these joints pass; and we shall adopt this usage.

The body of a frog usually holds a more or less horizontal, and that of a man a more or less vertical, position. There are, consequently, a number of terms which are ordinarily employed to describe the movements of the body as a whole or of its members, which, unless defined, are apt to cause confusion.

*Forwards* and *backwards* refer to movements towards or away from the direction in which the animal usually progresses. In the case of the frog a forward movement is *cephalad*, and a backward movement is *caudad*.

*Up* and *down*, away from and towards the earth, as applied to the movements of the frog, mean in the ventral and dorsal directions respectively.

*Inwards* and *outwards*, *median* and *lateral*, signify respectively towards and away from the sagittal plane of the trunk.

*Proximal* and *distal* mean towards and away from the trunk.

#### A METHOD OF PICTURING THE CHARACTER AND EXTENT OF THE MOVEMENTS OF THE PELVIS, FEMUR, AND OS CRURIS.

It is so difficult to form a clear mental picture of the movements of the hind limb of the frog that, although we have just defined the terms which we use, a little repetition may perhaps be pardoned.

The positions of the bones entering into a joint can be readily described by the use of the following three pairs of terms: flexion and extension, carried dorsally and carried ventrally, rotated dorsally and rotated ventrally. The simplest picture of the way in which we use these terms can be obtained as follows. Think of a book partly opened and standing on its edge, and of the two bones entering into a given joint, as lying, dorsal surface upwards, across the middle of the opposite pages, that is, as horizontal, and the joint in question at the point where the pages meet. Opening the book can be said to extend, and closing the book to flex, the joint. If one page is fixed and the other is moved, the bone on the moving page can be said to flex with respect to the other when the book is closed, and extend with respect to the other when the book is opened. The amount of flexion

and extension can be stated in degrees by stating the angle made by the pages. The book could be said either to be opened  $60^\circ$  or closed  $120^\circ$ . We have found it best to describe the angle in the sense of the amount that the book is opened, *i. e.*, that the bones are extended, and for our purposes it has been sufficient to state this angle as  $10^\circ$ ,  $20^\circ$ ,  $30^\circ$ , etc.

Instead of the two bones lying directly across the middle of the pages, one of them might lie diagonally across the page, with its free end pointing either towards the top or towards the bottom of the page. If a bone is placed so that its free end points upwards, we speak of it as having been carried dorsally out of the horizontal plane, and if its free end points downwards, as having been carried ventrally out of the horizontal plane. The terms dorsal and ventral are used because in practice we work with the dorsum of the frog looking upwards. The amount that the bone has been carried dorsally could be measured by the angle which it would make with the horizontal plane, and could be stated in degrees, and the amount it had been carried ventrally could be similarly described.

Evidently a bone which lies diagonally across the page, pointing towards the upper corner, for example, that is carried dorsally, can be extended or flexed with respect to the other bone by opening or closing the book, and its position with respect to the other bone can be readily described by stating the two angles, *e. g.*, carried dorsally  $45^\circ$  and extended  $110^\circ$ . Both of the bones might have their positions changed with respect to the horizontal plane; one might point up the page and the other down the page, for example, and their relative positions could be stated by describing the angles which they make with the horizontal plane, and the amount the book was opened, *i. e.*, the amount the joint was extended. In practice, however, it is more convenient to consider the more cephalad of the two bones as horizontal, and to speak of the other as carried dorsally or ventrally with respect to it.

To get a mental picture of the meaning of the terms "rotated dorsally and ventrally," think first of the bones as lying, with the dorsal side upwards, horizontally across the middle of the two pages, the book being more or less open, and the distal end of the bone to be rotated as projecting slightly beyond the edge of the page. Then think of a needle put into the end of the bone in such a position that it shall be parallel with the edge of the page, *i. e.*, vertical. We will

say that in this position the bone is without rotation. Now imagine the bone to be turned round, while still in the same position on the page, so that the needle shall make an angle with the page. The size of the angle determines the amount of the rotation, and if the rotation has carried the needle in the direction that the hands of a clock move, when the observer is looking towards the edge of the page, the end of the bone, we can speak of the rotation as ventral, and if the needle has moved in the opposite direction, *i. e.*, counter-clockwise, we can speak of the rotation as dorsal. In case the bone, instead of lying across the middle of the page had pointed towards the upper corner the needle would be no longer vertical, but the amount of rotation could be estimated the same as before, by sighting along the bone and noting the angle which the needle made with the plane of the page.

In the above description we have thought of the book as standing vertically on its lower edge, and the dorsum of the bone as directed upwards. Of course the positions of the two bones would be the same with respect to each other, in case the book were lifted up and given some other position, for the surfaces and long axes of the bones would hold the same relation to each other, regardless of the positions which they might hold in space. If the position of the book were altered, a bone lying across the middle of the page might be no longer horizontal, but to avoid confusion we should still have to describe its position as before, and when it was carried dorsally, for example, still have to speak of it as being carried dorsally out of the horizontal plane.

In brief, the amount that a bone is extended with respect to another can be expressed by stating the angle made by the projection of the long axis of the free bone on the horizontal plane with the sagittal plane of the bone which is regarded fixed.

The amount that a bone has been carried dorsally or ventrally can be expressed by stating the angle which the projection of the long axis of the bone on the vertical plane passing through that axis makes with the horizontal plane.

The amount that a bone is rotated about its long axis can be expressed by stating the angle which the projection of the sagittal axis of the bone on the transverse plane makes with the vertical plane passing through the long axis of the bone.

METHOD OF WORK AND APPARATUS.

Our experiments were made chiefly with the leopard frog, but the dissections shown in Plates I and II were made on the bull frog. These two forms do not show any marked differences in the structure of the knee joint and in the method of attachment of the muscles.

A freshly prepared pelvis-limb, pelvis-thigh, thigh-leg, or leg-foot preparation was used in our experiments. A frog was rapidly decapitated; the pelvis and hind limbs were separated from the trunk by cutting away the abdominal muscles and viscera, and severing the spinal column just above the last vertebra; finally, the skin was removed. The pelvis-limb preparation thus obtained was used in certain experiments, but in most of them all superfluous parts were cut away, for it was soon found that the weight of distant parts interfered with the action of the more delicate muscles, or through the action of gravity introduced movements which did not belong to them. For example, when only the one-joint muscles of the hip were to be studied, the lower leg was cut away at the knee, and the large two-joint muscles of the thigh were removed.

Under normal conditions a muscle when acting shortens by contracting, and thus causes its points of attachment to approach. The ideal method would be to cause contraction of the muscle by electrical excitation of the muscle itself, or, better, its nerve. The study of the action of a muscle requires a long time, however, for it must be made to act repeatedly and under a great variety of conditions. Since the physiological contraction power of the isolated muscle is lost at the end of a few tetani, it was found necessary in the early experiments to secure a shortening of the muscle, or rather an approach of its points of attachment, in another way. This was done, when the muscles acting on the hip were to be studied, by fastening a thread to the central end of a cut tendon or muscle, passing this thread through a fine hole, drilled with the aid of a dental engine in the bone of the pelvis at the middle of the point of attachment of the tendon or the muscle fibres, and pulling on this thread by hand or by a rubber band or spring. In case the attachment of the muscle was a broad one, it was found necessary to split the muscle longitudinally for a short distance, to tie a thread to each of the halves, and to pass these threads through two holes in the pelvis, bored at points corresponding with the outer borders of the attachment of the muscle. By pulling on these threads, first separately and then together, an

approximately correct picture of the action of the muscle was obtained.

In all cases the results thus observed were verified by repeatedly observing the effect of direct electrical excitation of the muscle, and in some cases the effect of indirect excitation through its nerve. When the muscle was electrically excited, the feeblest effective tetanizing induction current was used, the key being closed only long enough to reveal the effect of the contraction. The electrodes were of fine, flexible copper wire, so that the movement should not be checked. When electrical excitation was employed, great care was taken to avoid the effects of spread of current. In some cases the neighboring muscles were insulated by strips of rubber dam.

In most cases all the muscles which might influence the result were dissected off, unless it was thought that their removal would alter the direction of the strain of the muscle being studied, and those which were left were cut in halves, so as to do away with the effect of their contraction. The removal of superfluous masses of muscle had the advantage that it made the parts to be moved much lighter, did away with the elastic tension of antagonists, and made it possible to use much feebler exciting currents than would otherwise be required. Many of the muscles which cross the hip joint help to keep the head of the femur in the socket, and care was taken when they were cut away to see that the head of the bone kept its proper position when the muscle which was being studied contracted.

**The suspension method.**—To successfully study the mechanical action of individual muscles of the leg of the frog, three conditions must be provided: one of the bones entering the joint must be fixed, the other must move with the utmost freedom, and the effects of gravity must be eliminated. These conditions were obtained by the following method. In studying the action of muscles on the hip, for example, the pelvis was fastened firmly in a clamp with the iliac wings either parallel with the earth or tilted dorsally any desired number of degrees. The thigh, leg, and foot were suspended horizontally from hooks which passed through the fascia over the knee, ankle, and one of the toes, the hooks being fastened to flexible threads which passed over freely moving pulleys to weights which just counterbalanced the weights of the parts supported. To insure freedom of movement of the parts, the threads to the pulleys were about 50 cm. long, and the pulleys themselves were supported by threads about a metre long. The counterbalancing weights were

little cornucopias of paper filled to the required amount with shot. By this arrangement the effects of gravity were largely overcome, and a given muscle was permitted to show not only its power to flex and extend, but to move the thigh ventrally or dorsally, and to rotate it about its long axis.

**Method of inserting the needle used to study rotation.** — In order to determine the amount of rotation of the femur, for example, about its long axis, it was found best to insert a needle into the distal end of the shaft, in such a position that the needle should be vertical when the femur was horizontal and was without rotation, *i. e.*, in the initial position, and that the needle should not interfere with the action of the muscles. To do this the bone had to be fastened securely, and a hole had to be drilled in it with a fine drill. A special apparatus was needed for this purpose. This apparatus consisted of three pieces, — a horizontal shelf on which the preparation was placed, a standard carrying a small clamp to hold the femur, and a drill press. The device for clamping the femur was as follows. A standard with a worm gear attachment, which permitted it to be rotated in a vertical plane, carried a clamp with a worm gear which allowed a rod which it supported to be rotated in a horizontal plane. A bone clamp projected downwards at a right angle from this horizontal rod, and in practice with the assistance of a plumb line was made vertical. This bone clamp consisted of two parts, — a rod which was clamped to the horizontal rod and which had at its lower extremity a socket and set screw, and the bone clamp proper on the end of a rod which fitted the socket, and which could be raised or lowered or rotated without changing its position with respect to the vertical plane.

The drill press consisted of a *Basilischer Universalstativ*, a stand which had surrounding its vertical rod a metal sleeve which could be raised or lowered by means of a worm screw, and which had attached to it at a right angle a two-joint arm, at the end of which the drill-holder of a dental engine was clamped vertically. This improvised drill press permitted the drill to be raised or lowered with accuracy, and to be given any desired vertical position.

When the hole was to be bored, the preparation was placed on the horizontal shelf and given the "initial position." In this position the femur formed a right angle with the long axis of the pelvis, the os cruris a right angle with the femur, and the tarsus a right angle with the os cruris, and the bones were levelled up, so that they should be

horizontal, by means of little pieces of cork placed beneath them. It was assumed that in this position the femur was without rotation. The reason for this assumption is that this is about the position which is given to the bones by the elastic tension of the soft parts when the muscles are at rest, and the frog is floating with the hind limb in its normal semi-extended position. The standard carrying the bone clamp was then moved to the preparation; the clamp was carefully adjusted to the shaft of the bone, and made fast. The clamped bone was then brought beneath the drill by moving the standard supporting it, and the hole was bored. Finally, a delicate needle was inserted into the hole to represent the sagittal axis of the bone.

THE RELATION WHICH CERTAIN MOVEMENTS OF THE FEMUR BEAR TO CERTAIN MOVEMENTS OF THE PELVIS, AND THEIR EFFECT ON THE ACTION OF MUSCLES.

One is likely to misunderstand the effect of various positions of the femur and pelvis on the movements produced by the contraction of muscles, unless he first observes the relation which certain movements of the femur bear to certain movements of the pelvis, and the relative positions of the bones which result.

I. The simplest case is when pelvis (iliac wings) and femur are horizontal and the pelvis is fixed. If the femur rotates about an axis which passes vertically through the head of the bone, it is flexed if the femur approaches the pelvis and extended if the femur leaves the pelvis; if the femur rotates about an axis which passes horizontally through the head of the bone and at a right angle to its long axis, the femur is carried either ventrally or dorsally out of the horizontal plane; if the femur rotates about the long axis, it rotates ventrally if it rotates clockwise and dorsally if it rotates contra-clockwise as seen from the distal end.

A more complicated case is when the femur is rotated about an axis which passes obliquely through the head of the bone. It then makes movements of two different types at the same time: it moves either dorsally or ventrally out of the horizontal plane and at the same time either flexes or extends.

A still more complicated case is when the femur rotates about an axis passing obliquely through the head of the bone, and at the same time rotates about its long axis, in which case all three types of movement occur simultaneously. This last case is a common one, and may

be still further complicated by the direction of the oblique axis being changed while the movement is in progress.

II. Not only is it possible for the femur to move and change its position with respect to the pelvis, but the pelvis may move and change its position with respect to the femur.

The simplest case is when pelvis (iliac wings) and femur are horizontal, and the femur is fixed. If the pelvis rotates about an axis which passes vertically through the head of the femur, or if the pelvis rotates about an axis which passes horizontally through the head of the femur and perpendicular to its long axis, the same effects are produced in the hip joint and on the relative positions of the points of origin and insertion of the muscles, as when the pelvis is fixed and the femur is free to move and rotates about these axes.

Rotation of the pelvis about its transverse axis may have the same effect or a very different effect from rotating the femur about its long axis, the effect depending on the position which the femur holds to the pelvis at the time the rotation occurs.

*A.* If the femur and pelvis are horizontal, and the femur is extended  $90^\circ$ , so that the sagittal plane of the femur and the transverse plane of the pelvis are in the same vertical plane, the femur is to be considered to be without rotation and the pelvis without inclination. In this case rotation of the femur about its long axis and rotation of the pelvis about its transverse axis produce like rotation effects in the hip joint, for these two axes coincide, the one being a prolongation of the other. Tilting the pelvis dorsally is the same as rotating the femur ventrally, and tilting the pelvis ventrally is the same as rotating the femur dorsally. Although this correspondence exists, the effects on the action of the muscles surrounding the hip joint are quite different. The diameter of the femur is so small that rotation of the bone about its long axis, although it may alter the tension of the rotation muscles, has little effect to alter the direction of the strain of the muscles. On the other hand, rotation of the pelvis on its transverse axis, *i. e.*, tilting it dorsally or ventrally out of the horizontal plane, may so change the relation of the points of origin of muscles on the pelvis to the points of insertion on the femur as to entirely alter the action of the muscles; for example, a muscle which in one position of the pelvis is a flexor of the femur, in another position may become an extensor, or one which in one position carries the femur ventrally, in another position may carry it dorsally.

*B.* If the femur and pelvis were horizontal, and the femur could be

flexed or extended until the sagittal planes of femur and pelvis were parallel, the femur would be without rotation and the pelvis without inclination. In this case rotation of the femur about its long axis and rotation of the pelvis about its transverse axis would not produce like rotation effects in the hip joint, for these two axes would not coincide, but the long axis of the femur would be perpendicular to the transverse axis of the pelvis.

(a) Tilting the pelvis dorsally when the femur was flexed, would be the same as carrying the flexed femur ventrally; and tilting the pelvis ventrally, the same as carrying the femur dorsally.

(b) Tilting the pelvis dorsally when the femur was extended, would be the same as carrying the extended femur dorsally; and tilting the pelvis ventrally, the same as carrying the femur ventrally.

C. If the femur and pelvis are horizontal, and the femur is flexed or extended incompletely, so that the sagittal plane of the femur makes an angle of less or of more than  $90^\circ$  with the sagittal plane of the pelvis, and the sagittal plane of the femur is vertical, the femur is without rotation and the pelvis without inclination. In this case rotation of the femur about its long axis and rotation of the pelvis about its transverse axis do not produce like rotation effects in the hip joint, because the axes of rotation are different.

(a) Tilting the pelvis dorsally when the femur is flexed, is the same as carrying the flexed femur ventrally and rotating it ventrally; and tilting the pelvis ventrally, the same as carrying the flexed femur dorsally and rotating it dorsally.

(b) Tilting the pelvis dorsally when the femur is extended, is the same as carrying the extended femur dorsally and rotating it ventrally; and tilting the pelvis ventrally, the same as carrying the extended femur ventrally and rotating it dorsally.

It is evident, from what has been said, that rotation of the pelvis about its transverse axis not only must influence the movement of the frog through altering the position of the trunk with respect to the earth, and consequently the direction in which it will be propelled by the extension of the thighs, but will have a marked effect on the action of the muscles, the character of this influence depending largely on the amount of extension of the thighs with respect to the pelvis at the time that the tilting of the pelvis takes place. Some of these effects on the action of the muscles will be brought out later in the description of the action of the individual muscles.

ACTION OF MUSCLES OF THE THIGH.

All the muscles acting on the hip joint arise from the pelvis. From the standpoint of their action they can be divided into two classes: (a) those inserted on the femur, which are one-joint muscles and act only on the hip joint; (b) those inserted on the os cruris, which are two-joint muscles and act on both the hip and knee joints. The long two-joint muscles form the superficial layer surrounding the thigh on all sides. The short one-joint muscles, most of which are covered by the two-joint muscles, are divided by Gaupp into surface, middle, and deep layers. The surface layer lies on the lateral and dorsal surfaces of the hip; the middle layer surrounds the whole inner surface of the joint from the anterior almost to the posterior spine of the pelvis: the deep layer consists of only one muscle, which lies close to the capsule of the joint, everywhere concentric with the middle layer, only extending somewhat further dorsally. The following is the list, as given by Gaupp:

I. Long muscles of the thigh.

a. Muscles of the lateral (forward) surface.

Triceps. — Caput anticum, the cruralis.

Caput medium, the tensor fasciæ latæ.

Caput posticum, the glutæus magnus.

b. Muscles of the medio-ventral surface.

Sartorius.

Adductor longus.

Adductor magnus.

Gracilis major.

Gracilis minor.

c. Muscles on the medio-dorsal surface.

Ilio-fibularis.

Semimembranosus.

Semitendinosus.

II. Short muscles of the thigh.

a. Surface layer.

Iliacus internus.

Iliacus externus.

Ilio-femoralis.

Pyriformis.

b. Middle layer.

Pectineus.

Obturator externus.

Quadrator femoris.

Gemellus.

c. Deep layer.

Obturator internus.

The positions of the points of origin of these muscles with respect to the acetabulum are shown in Fig. 1; the points of insertion of the long muscles at the knee are shown in the dissections given in Plates I and II.

#### HOW MOVEMENTS OF THE HIP JOINT ARE INFLUENCED BY THE PLACE OF ORIGIN AND OF INSERTION OF THE MUSCLES.

The position of the point of origin of a muscle on the pelvis, with respect to the acetabulum and the head of the femur, goes far to determine the action of the muscle, although sometimes the place of insertion on the femur modifies the movement, this being especially the case with the rotators. If one thinks of the pelvis, seen from the lateral side, as projected on the sagittal plane, one can plot a map of the positions of the points of origin of the muscles with respect to the centre of the acetabulum. Fig. 1 shows such a map. The cephalo-caudad axis, CC, has been drawn through the centre of the acetabulum, parallel to the long axis of the wing of the ilium (that is, a line drawn through the centre of its proximal end and through the centre of the point of origin of the glutæus magnus on the superior process). The dorso-ventral axis, DV, has been drawn through the centre of the acetabulum at right angles to the cephalo-caudad axis. Lines have been drawn also from the centre of the acetabulum, through the centre of the place of origin of each of the muscles; and these indicate in a general way the direction in which the femur could be supposed to move, cephalad, caudad, dorsad, and ventrad, when any one of the muscles in question shortened by contracting. A circle having its centre in the centre of the acetabulum has been divided in degrees, and this permits one to state in degrees how far dorsal or ventral to the cephalo-caudad axis and how far cephalad or caudad to the dorso-ventral axis the point of origin of any given muscle lies. For example, the origin of the glutæus magnus lies  $72^{\circ}$  dorsal and  $18^{\circ}$  cephalad; the ventral head of the semitendinosus lies  $30^{\circ}$  ventral and  $60^{\circ}$  caudad.

The principal action of the muscles which have their origin

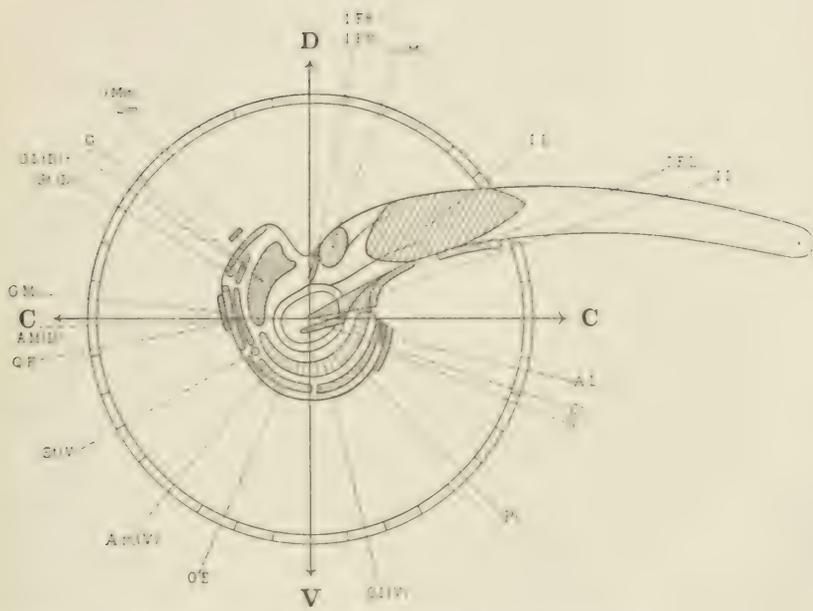


FIGURE 1.—The points of origin of the muscles of the thigh with respect to the acetabulum.



cephalad to the acetabulum is to flex the femur, and of those the origin of which is caudad to the acetabulum is to extend the femur. The chief action of muscles the points of origin of which are ventral and dorsal, is respectively to carry the femur ventrally and dorsally. Such muscles may also act to tilt the pelvis dorsally or ventrally. Certain muscles may rotate the femur dorsally or ventrally, these effects being largely determined by the method of attachment on the femur.

#### ACTION OF MUSCLES TO FLEX AND EXTEND THE FEMUR.

**Pelvis and femur horizontal.**—Muscles the points of origin of which lie on the pelvis  $30^\circ$  or more cephalad to the dorso-ventral axis of the map, act to flex the femur. Such muscles are the cruralis, adductor longus, sartorius, pectineus, iliacus externus, tensor fasciæ latæ, iliacus internus, and glutæus magnus.

Muscles the points of origin of which lie on the pelvis  $30^\circ$  or more caudad to the dorso-ventral axis, except the ventral head of the adductor magnus, extend the femur. Such muscles are the semiten-dinosus (both ventral and dorsal heads), quadratus femoris, adductor magnus (dorsal head), gemellus, semimembranosus, and gracilis minor.

A muscle the point of origin of which lies directly dorsal to the acetabulum acts to carry the femur dorsally when the long axis of the femur forms a right angle with the long axis of the pelvis; but if the femur be flexed, the line of strain of the contracting muscle is carried to the flexor side of the axis for flexion and extension, and the muscle acquires the power to flex as well as to carry dorsally; and if the femur is extended, the muscle for a similar reason acquires the power to extend the femur as well as to carry dorsally. Gad<sup>1</sup> has already attracted attention to this reversal of the action of the vastus internus (glutæus magnus) and the "adductors" (muscles the points of origin of which lie ventral to the acetabulum). In speaking of the ilio-psoas Gad says: "It distinguishes itself in this respect from other muscles which, like the vastus internus, flex the already flexed thigh further, but from a given extension on act to extend. I think that the adductors also show such a reversal of the method of action. The explanation is that these muscles lie

<sup>1</sup> GAD: Verhandlungen der physikalischen medicinischen Gesellschaft in Würzburg, N. F., 1884, xviii, p. 172.

### EXPLANATION OF PLATE ONE.

PLATE I.—Dissection of right knee of bull frog. *A. L.*, adductor longus; *E. B.*, extensor brevis; *E. B'*., tendon of extensor brevis; *E. L. L.*, external lateral ligament; *G.*, gastrocnemius (plantaris longus); *G'*., extra tendon of gastrocnemius; *G. M.*, gracilis magnus; *G. Min.*, gracilis minor; *I. Fb.*, ilio-fibularis (biceps); *I. L. L.*, internal lateral ligament; *P.*, peroneus; *P'*., tendon of peroneus; *S.*, sartorius; *Sm.*, semimembranosus; *St.*, semitendinosus; *T.*, triceps; *T. A.*, tibialis anticus longus; *T. A'*., tendon of tibialis anticus longus.

FIGURE 1.—Map of points of origin of muscles of right thigh of leopard frog, on the lateral side of pelvis. *C. C.*, cephalo-caudad axis; *D. V.*, dorso-ventral axis; *A. L.*, adductor longus; *Cr.*, cruralis; *S.*, sartorius; *Pl.*, pectineus; *O. I. (V)*, obturator internus, ventral head; *O. E.*, obturator externus; *A. M. (V)*, adductor magnus, ventral head; *St. (V)*, semitendinosus, ventral head; *Q. F.*, quadrator femoris; *A. M. (D.)*, adductor magnus, dorsal head; *G. M.*, gracilis magnus; *St. (D.)*, semitendinosus, dorsal head; *O. I. (D.)*, obturator internus, dorsal head; *G.*, gemellus; *Sm.*, semimembranosus; *G. Min.*, gracilis minor; *I. Fb.*, ilio-fibularis; *I. Fm.*, ilio-femoralis; *Gl. M.*, glutæus magnus; *I. E.*, iliacus externus; *T. F. L.*, tensor fasciæ latæ; *I. I.*, iliacus internus.





outside of the plane of rotation of the os femoris, and are so inserted above that the result of their pull comes to lie now on the flexor, now on the extensor, side of the sagittally directed axis of rotation." Such muscles are the following:

**Adductor magnus** (ventral head) —  
flexes femur if extended less than  $45^{\circ}$ .  
extends femur if extended more than  $45^{\circ}$ .

**Obturator externus** —  
flexes femur if extended less than  $70^{\circ}$ .  
extends femur if extended more than  $70^{\circ}$ .

**Ilio-femoralis** —  
flexes femur if extended less than  $80^{\circ}$ .  
extends femur if extended more than  $80^{\circ}$ .

**Ilio-fibularis** —  
flexes femur if extended less than  $90^{\circ}$ .  
extends femur if extended more than  $90^{\circ}$ .

The obturator internus also shows a reversal of its action, but behaves differently from the rest of these muscles, because the relation of the tendon of insertion to the head of the femur so changes, as the thigh is flexed and extended, that it flexes when the femur is extended more than  $120^{\circ}$ , and extends when it is extended less than  $120^{\circ}$ .

**Pelvis tilted dorsally  $40^{\circ}$ , the femur being horizontal.** — Not only may the action of certain muscles be reversed, from the standpoint of flexion and extension during the progress of extension of the femur, when the pelvis and femur are horizontal, but a further change in their action may take place when, the femur remaining horizontal, the pelvis is tilted dorsally, as occurs when the frog takes the sitting position. The dead point for the obturator externus is changed from  $70^{\circ}$  to  $110^{\circ}$  extension, and for the ventral head of the adductor magnus, from  $45^{\circ}$  to  $90^{\circ}$  extension. The ilio-fibularis and ilio-femoralis, which acted to flex when the pelvis was horizontal and the femur extended not more than  $80^{\circ}$ , become pure extensors when the pelvis is tilted dorsally. Finally, the glutæus magnus, which is a pure flexor when the pelvis is horizontal, becomes a pure extensor when the pelvis is tilted dorsally  $40^{\circ}$ , as in the sitting position.

The explanation offered by Gad — "that the muscles lie outside of the plane of rotation of the os femoris, and are so inserted above that the result of their pull comes to lie now on the flexor, and now on the extensor side of the sagittally directed axis of rotation" — is correct in

part, but examination of the map shows that the character of the reversal of the action of certain muscles is not wholly explained by the position of the points of origin with respect to the axis of rotation. The method of insertion on the femur and os cruris also plays its part in causing the line of strain to be transferred from the one to the other side of the axis of rotation. The fact that the ilio-fibularis, ilio-femoralis, and obturator externus are inserted to the median side of the long axis of the femur, and the glutæus magnus somewhat to the dorsal side, helps to explain the divergence in the action of these muscles.

#### ACTION OF MUSCLES TO CARRY THE FEMUR DORSALLY AND VENTRALLY.

Muscles the points of origin of which lie on the pelvis  $20^{\circ}$  or more dorsal to the cephalo-caudad axis of the map, act to carry the femur dorsally. Such muscles are semitendinosus (dorsal head), gemellus, semimembranosus, ilio-fibularis, ilio-femoralis, pyriformis, glutæus magnus, iliacus internus, tensor fasciæ latæ.

Muscles the points of origin of which lie on the pelvis  $20^{\circ}$  or more ventral to the cephalo-caudad axis of the map, act to carry the femur ventrally. Such muscles are: adductor longus, sartorius, pectineus, obturator externus, adductor magnus (ventral head), semitendinosus (ventral head).

Muscles the points of origin of which lie on the pelvis less than  $20^{\circ}$  dorsal or ventral to the cephalo-caudad axis, show a reversal of their action. If the femur has been carried out of the horizontal plane ventrally, they tend to carry it dorsally; or if it has been carried out of the horizontal plane dorsally, they tend to carry it ventrally. Such muscles are: quadratus femoris, adductor magnus (dorsal head), gracilis major, and iliacus internus. The cruralis, being attached over the capsule, shows little effect to carry the femur either dorsally or ventrally, and the obturator internus, because of its extensive origin is omitted from the list. The adductor magnus (dorsal head), the origin of which lies caudad and to both sides of the cephalo-caudad axis, shows a peculiar action; it carries the femur dorsally in case it has been flexed and carried dorsally, or extended and carried ventrally, and it carries it ventrally if it has been flexed and carried ventrally, or extended and carried dorsally. The quadratus femoris would probably do the same.

Just as in the case of muscles which are flexors and extensors, so with those that carry the femur dorsally and ventrally, the action may be markedly changed by tilting the pelvis dorsally.

#### ACTION OF MUSCLES TO ROTATE THE FEMUR.

Of course the method of insertion of the muscle on the femur is of importance in determining its action. A muscle may run parallel with the femur from origin to insertion, in which case the femur will move towards the side on which the muscle lies. When the femur rotates about its long axis, the radius of movement is so short that the displacement is small, and the power to flex, extend, carry ventrally, and carry dorsally are not altered much. On the other hand, if a muscle in passing from its point of origin on the pelvis to its insertion on the femur winds round the shaft of the femur, it acquires the power to rotate the femur about its long axis. Muscles which rotate the femur ventrally, *i. e.*, clockwise to one looking at the distal end of the bone, wind round the bone from the point of origin contra-clockwise; and those that rotate the femur dorsally, *i. e.*, contra-clockwise, wind round it clockwise. The most important ventral rotator is the iliacus externus, and the most important dorsal rotator is the obturator internus. Other muscles which rotate ventrally are: sartorius, ilio-fibularis, ilio-femoralis, gracilis magnus, semimembranosus (when the femur is rotated dorsally), and iliacus internus; and one which rotates dorsally is the dorsal head of the adductor magnus.

The power of a muscle to rotate the femur may be increased or decreased by tilting the pelvis, because a muscle which winds from its point of origin round the shaft of the bone will be made to do so either more or less by changing the position of the point of origin with respect to the shaft of the bone.

#### ACTION OF MUSCLES TO TILT THE PELVIS DORSALLY OR VENTRALLY.

Muscles having their points of origin on the pelvis dorsal to the cephalo-caudad axis tilt the pelvis dorsally if the femur be extended, and ventrally if it be flexed, and the effect is the stronger the more dorsal the origin of the muscle. Muscles which have their points of origin on the pelvis ventral to the cephalo-caudad axis, tilt the pelvis dorsally if the femur be flexed and ventrally if it be extended, and the effect is the stronger the more ventral the point of origin.

When the femur is flexed, the muscles which tilt the pelvis dorsally are: sartorius, pectineus, obturator internus, obturator externus, adductor magnus (ventral head), semitendinosus (ventral head). Those that tilt the pelvis ventrally are: semitendinosus (dorsal head), gemellus, semimembranosus, ilio-fibularis, ilio-femoralis, glutæus magnus, iliacus externus, tensor fasciæ latæ, and iliacus internus. These two lists would have to be reversed when the femur is extended. The quadratus femoris, adductor magnus (dorsal head), gracilis major, cruralis, and adductor longus have their points of origin so near the cephalo-caudad axis that they have but little action to tilt the pelvis.

#### EFFECT OF STRUCTURE OF KNEE JOINT ON THE ACTION OF MUSCLES.

The effect of the muscles on the knee joint is largely determined by the character of the joint surfaces in apposition at the time the contraction occurs, and on the action of the ligaments to limit and guide the movements. The amount that the knee is extended at the time that the muscles contract has therefore a great influence on the resulting movement. The peculiar character of the knee joint of the frog deserves a careful description, but only a few points concerning it can be brought out here (see Plate II, Fig. 1). It is only the more median portions of the condyles which form the rotation surfaces, the lateral part of the head of the femur serving for the attachment of the tendons of the peroneus, tibialis anticus longus, and extensor brevis muscles, and the lateral part of the head of the os cruris carrying the grooves in which the tendons of these muscles play. The middle portion of the joint is occupied by the strong ligaments which bind the bones together. In addition to the internal ligaments, there is a strong ligament on the dorsal and another on the ventral side of the joint which binds together the condyles. These ligaments ("the lateral ligaments") slide over the smooth surfaces of the condyles as the joint is flexed and extended, and the condylar surfaces are so formed that the ligaments are always kept tense, and keep the rotation surfaces in close apposition.

The structure of the joint is such that it can be considered to have two distinct rotation surfaces, the one formed by the extremities, the other by the median surfaces of the heads of the bones. The change from the one to the other of these surfaces comes when the joint has been flexed, to about  $110^{\circ}$  extension. Up to this point the move-

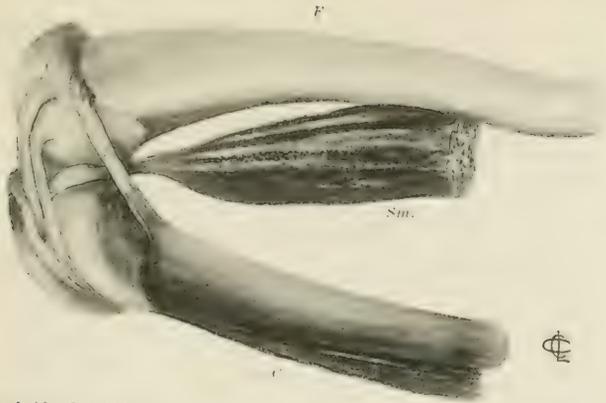
ment is one of simple flexion, and beyond this point, in the direction of flexion, a carrying of the os cruris dorsally with respect to the femur and a ventral rotation of the os cruris become possible in addition to the flexion. The proximal portion of the os cruris may be described as consisting of a dorsal (lateral, Gaupp) and a ventral (median, Gaupp) condyle, and between these a central portion which serves for the attachment of the internal ligaments of the joint. Looked at from the dorsal side, the dorsal condyle has the appearance of a disk, with rounded border, growing out of the shaft of the bone. By extended knee the part of the disk most proximal to the shaft of the bone is opposed to the rotation surface of the corresponding condyle of the femur, and when the knee is overflexed, the part of the disk nearest the median surface of the shaft of the os cruris is in contact with the median surface of the dorsal condyle of the femur. Indeed, the overflexion is checked by the ligaments only when the median surface of the shaft of the os cruris almost touches the median surface of the shaft of the femur. In the process of flexion the border of the disk of the dorsal condyle slides on the surface of the femur in such a way as to give the effect of rolling upon it, but the motion is really more of a sliding than of a rolling character. As the crus passes from flexion to overflexion, the os cruris rotates ventrally and moves dorsally, a twisting movement being seen at the place of contact of the rotation surfaces of the dorsal condyles.

The lateral part of the ventral condyle has the same disklike form as the dorsal condyle, and the rounded border is continued as a gentle curve on to the most proximal part of the disk. Beyond this, toward the median side, the curvature becomes much more rapid, and on the inner side of this part there is a distinctly flattened surface sloping inward to form the ventral side of the intercondylar fossa. When the joint is extended, the proximal surface of the ventral condyle is in contact with the most distal part of the ventral portion of the head of the femur, and slides and rolls on this as the joint flexes to about  $110^{\circ}$ , but beyond this point new surfaces come in contact. The middle portion of the head of the femur projects somewhat beyond the rest, fitting into the intercondylar fossa of the os cruris, and the flattened inner surface of the ventral condyle of the os cruris slides on this portion of the head of the femur, which is here slightly flattened to correspond. It is the flattening of these two surfaces and their direction which makes it possible for the os cruris to be carried dorsally and rotated ventrally as it passes into overflexion. The movement of these

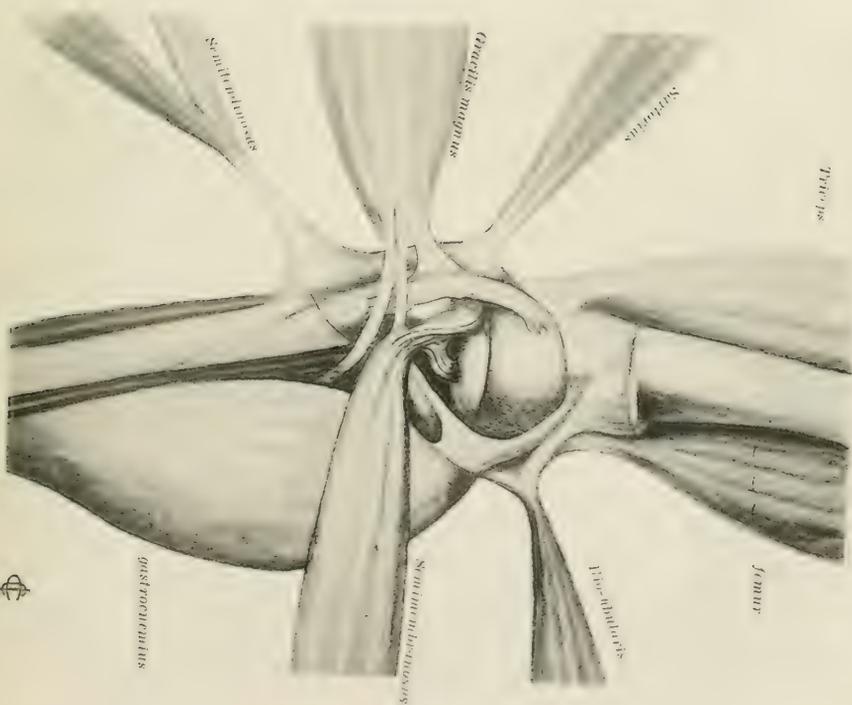
#### EXPLANATION OF PLATE TWO.

PLATE II, FIGURE 1.—Dissection of ventral side of right knee of bull frog. *F*, femur; *C*, os cruris; *Sm.*, semimembranosus muscle. The knee is almost completely flexed, and the rotation surfaces of the condyles of the os cruris are in contact with the median surface of the head of the femur. If the os cruris had been flexed 20° further, the median surface of the head of the femur would have come to bear in the intercondylar fossa, on the median side of the head of the os cruris. The dissection shows the relation of the tendon of the semimembranosus to the joint, and explains the action of the muscle to over-flex the os cruris on the femur.

FIGURE 2.—Dissection of ventral side of right knee of bull frog, showing the way the tendons of the muscles of the thigh enter the joint.

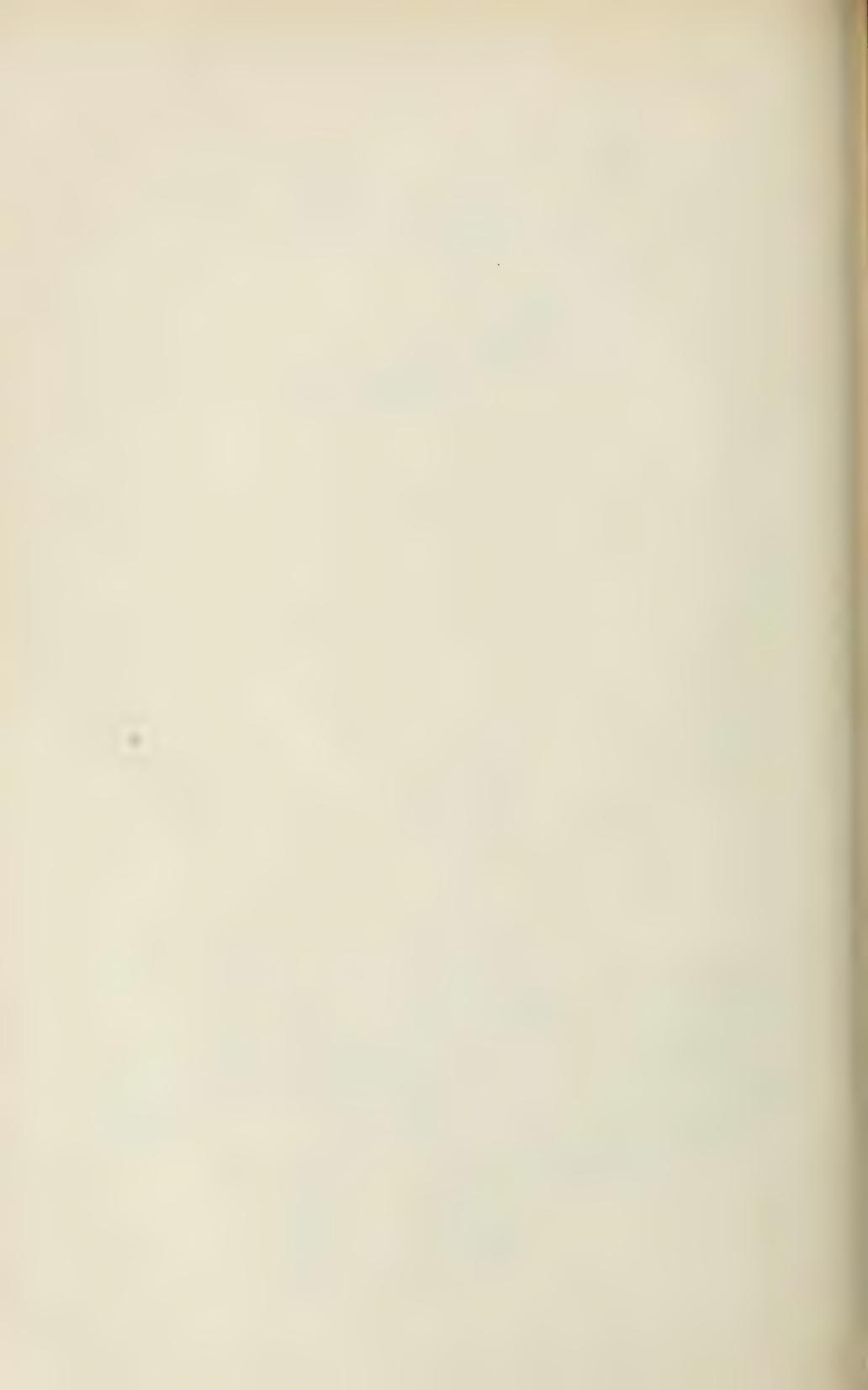


Ventral side of right leg.



LOMBARD AND ABBOTT.

VENTRAL SIDE. RIGHT LEG OF FROG.



two surfaces on each other is distinctly a sliding motion. The os cruris when completely flexed can be carried dorsally about 45°. The ventral rotation does not commence until overflexion has commenced, and is the result of the form of the surfaces on the median sides of the two bones, and the action of the lateral ligaments of the joint. The dorsal condyle of the crus acts as if pivoted on the dorsal condyle of the femur, and as the os cruris is carried dorsally and is rotated ventrally, its ventral condyle describes a circle about its dorsal condyle.

#### ACTION OF INDIVIDUAL MUSCLES.

In the following description of the action of the muscles of the thighs they have been arranged, for convenience of reference, alphabetically. The position of the points of origin of the muscles of the thigh on the pelvis is shown in Fig. 1, and the relation of the tendons of the long muscles to the knee joint is shown in Plate I and Plate II.

**Adductor longus.**— This muscle resembles in form the sartorius, by which all of it but the lateral border is covered. It arises from the outer surface of the ilium just caudad to the anterior spine of the pelvis, and beneath the line of insertion of the sartorius. The line of origin lies cephalad and slightly ventral to the acetabulum and the head of the femur, and from this point the muscle runs along the ventral side of the thigh, crossing from the lateral to the median side, to be inserted by a tendon common to it and the ventral head of the adductor magnus on the epicondylus medialis. This is a feeble muscle which acts to flex the thigh and to a less extent to carry it ventrally.

**Adductor magnus.**— This is a powerful muscle lying on the median and ventral sides of the thigh. It is seen in a triangular space between the sartorius and gracilis muscles in the proximal part of the thigh, and is covered by them in the distal portion. It arises from the ischium and the adjacent part of the remanens cartilage by three heads, and is inserted on the distal end of the femur, where it almost completely surrounds the bone. The muscle as a whole acts to extend the thigh on the pelvis, to carry the thigh ventrally, and to rotate it dorsally.

The *caput ventrale* arises from the border of the remanens cartilage and the neighboring part of the ischium close to the symphysis pubis, the line of origin being more ventral than caudad to the acetabulum and the head of the femur. The muscle fibres extend to

the distal end of the femur, where they are inserted just proximal to the epiphysis, in part on the median and to a lesser extent on the ventral surface of the shaft of the bone, and in part along with the fibres of the adductor longus on a tendon which is attached to the epicondylus medialis. In the distal part of its course the fibres of this head are closely related to those of the accessory and dorsal heads, and where they are inserted on the median surface of the bone they form a common muscle. The ventral head lies for the most part along the medio-ventral surface of the femur, but the fibres shift their place with respect to the shaft of the femur as it is carried from the flexed to the extended position, lying when it is flexed somewhat more to the latero-ventral, and when it is extended to the medio-ventral side of the bone. When the pelvis is tilted dorsally, the origin of this head is carried cephalad, and the proximal part of the muscle lies still more to the latero-ventral side of the flexed femur.

Electrical excitation shows that the ventral head of the muscle, when pelvis and femur are in the horizontal plane, has above all the power to carry the thigh ventrally. In addition to this it can extend the thigh if it is already extended  $45^\circ$ ; but if the amount of extension is less than  $45^\circ$ , this head acts to flex the thigh. When the pelvis is tilted dorsally, the thigh being in the horizontal plane, the origin of the ventral head is carried somewhat more cephalad with respect to the head of the femur, and this head acts as a flexor when the thigh is extended  $90^\circ$  or less; when the extension is more than this, the thigh is extended. The power of this head of the muscle to carry the thigh ventrally is so great, in all positions of the limb, that its extensor and especially its flexor action are well seen only when the ventral movement is prevented, for example, in the case of a suspended preparation, by holding the thread supporting the knee, or by holding a smooth needle against the limb. In case the flexed femur is carried ventrally before the ventral head of the muscle is excited, the femur is seen to rotate dorsally, and to describe a segment of a cone as it passes from the flexed and ventral to the extended horizontal position. These effects are not due directly to the action of the muscle, but to the passive action of joint surfaces and the ligamentum ventrale.

The *caput dorsale* arises in sequence to the ventral head from the border of the ischium, and lies directly caudad to the acetabulum and the head of the femur. The fibres which have the more ventral origin are inserted near the distal end on the median side of the

shaft of the bone, and those having the more dorsal origin wind round the end of the shaft and are inserted on the dorsal, dorso-lateral, and lateral surfaces, the insertion on the lateral surface including the lower third of the bone. Thus the ventral part of the head of the muscle lies along the median, and the dorsal part along the median and dorsal surfaces of the shaft of the femur, some of the distal portion winding over the dorso-lateral surface of the shaft well on to the lateral side.

When both pelvis and femur are in the horizontal plane, this head of the muscle acts as a powerful extensor. In addition to this it rotates the thigh dorsally, this action being most marked when the thigh is flexed and the pelvis tilted dorsally. This latter action is important, because by it the proximal end of the *os cruris* is kept beneath the distal end of the femur, and the trunk is thrown forwards and upwards by the extending thighs, instead of the legs being driven out sideways. This head of the muscle shows little tendency to carry the thigh either dorsally or ventrally when the pelvis and thigh are horizontal, but if in the flexed position the femur is carried slightly dorsally, this head will carry it dorsally, and if the femur be carried slightly ventrally, it will carry it ventrally; if in the extended position the femur has been carried dorsally, this head will carry it ventrally as far as the horizontal plane, and if the femur has been carried ventrally, this head will carry it dorsally as far as the horizontal plane.

A word with respect to the effect of tilting the pelvis dorsally. As has been explained elsewhere, when the thigh is horizontal, tilting the pelvis dorsally when the thigh is in the flexed position has the same effect on the action of the muscles as carrying the femur ventrally; therefore, when the frog is at rest in the sitting position, tilting the pelvis dorsally enables the dorsal head of the adductor magnus muscle, when it contracts, to help the ventral head to carry the thigh ventrally, — in other words, to raise the body from the ground at the same time that it is thrown forward by the extending thigh. When the thigh is extended, tilting the pelvis dorsally has the same effect on the action of the muscle as carrying the femur dorsally, and this head will carry the femur ventrally. Thus throughout the jumping movement this muscle would help not only to extend the thigh, but to propel the body upwards. If the femur is at 90° extension, tilting the pelvis dorsally acts in the same way as rotating the femur ventrally, and increases the power of the muscle to rotate

the femur dorsally. Thus, when the thigh is half extended, the muscle helps to keep the distal end of the femur dorsal to the proximal end of the crus, so that the extremity of the femur shall be properly supported, and at the same time it favors a backward rather than a lateral movement of the extending limb.

The *caput accessorium* arises from the slender tendon of the ventral head of the semitendinosus. It is composed of a few bundles of fibres lying median to the shaft of the femur between the two other heads of the adductor magnus, with which it is closely connected. It acts to extend the femur.

**Cruralis.**—The cruralis (*vastus internus* of Ecker), which forms the largest and strongest head of the triceps group, lies to the lateral and ventro-lateral sides of the femur, the ventral border of the muscle being covered by the adductor longus and sartorius muscles.

The cruralis arises by two heads. The outer or more lateral head arises by a very short tendon which is attached to the capsular ligament and partly to the cephalad border of the cartilage which helps to form the acetabulum. Some of the fibres of the inner head take origin from the capsular ligament by a short tendon, while the remainder spring from a Y-shaped ligament which arises from the capsule and is prolonged as a slender cord which runs along the median side of the muscle and is inserted into the common tendon of the triceps. The muscle runs parallel with the femur beneath the tensor fasciæ latæ, and is inserted into the common tendon of the triceps group.

The cruralis is a two-joint muscle which acts on the knee and the hip joints. It extends the os cruris on the femur, and this is perhaps its most important action. When the pelvis and the thigh are in the horizontal plane, and the leg in the resting semi-extended position, it flexes the femur until it forms an angle of about  $90^\circ$  with the long axis of the pelvis. If the crus be flexed on the femur and be kept from extending, the muscle will cause the femur to be completely flexed. This action is not, however, strong, because the origin of the muscle on the capsular ligament brings the strain close to the axis of rotation. On the other hand, the muscle tends to bind the head of the bone in the acetabulum from the cephalad side of the joint, just as the obturator internus and iliacus externus do from the caudad side. This action of the muscle is very important.

With the pelvis horizontal or tilted dorsally  $40^\circ$  and the thigh in the horizontal plane, or carried ventrally or dorsally out of the

horizontal plane, the action of the muscle is the same as above described.

**Gemellus.** — The gemellus lies somewhat dorsally and somewhat caudad to the hip joint. It is an extensor of the thigh in all positions of the limb, its power lessening rapidly as the thigh nears complete extension because of the lessening tension of the muscle. If the extensor action is prevented, the muscle will be seen to carry the thigh dorsally. In the sitting position the extensor action of the muscle is increased, at the expense of the power to carry dorsally. It tilts the pelvis ventrally if the femur is flexed, and tilts it dorsally if the femur is extended.

The *glutæus magnus* (vastus externus of Ecker) is a strong two-joint muscle lying on the dorsal and dorso-lateral surfaces of the thigh. It arises from the outer surface of the posterior process of the wing of the ilium, the point of origin lying cephalad, dorsal, and lateral to the acetabulum. The fibres take a course in general parallel to the femur, to be inserted into the common tendon of the triceps group. The muscle vigorously extends the os cruris on the femur. It acts strongly to carry the femur dorsally, in all positions of the femur and pelvis. Gad<sup>1</sup> says that the vastus externus flexes the already flexed thigh further, but extends it from a certain degree of extension. He goes on to say that he thinks that the adductors may have such a reversal of their action. "This lies in the fact that these muscles lie outside of the plane of rotation of the os femoris, and are so inserted above that the resultant of their pull comes to lie now on the flexor, now on the extensor side of the sagittally directed axis of rotation." We cannot altogether agree with this, as far as the *glutæus magnus* is concerned. When the pelvis and thigh are horizontal, the muscle causes the femur to be flexed in all positions of the leg. If the pelvis is tilted dorsally more than 20° and the thigh is in the horizontal plane, the point of origin of the muscle having been carried caudad with respect to the axis of rotation for flexion and extension of the hip joint, the flexor action of the muscle on the hip is changed to an extensor action, and it will extend the femur in all positions of the thigh, from complete flexion to complete extension. If the femur is fixed, the *glutæus magnus* will tilt the pelvis dorsally if the femur is extended, and carry the pelvis ventrally if the femur is flexed.

<sup>1</sup> GAD: Verhandlungen der physikalischen medicinischen Gesellschaft, in Würzburg, N. F., 1884, xviii.

In the sitting position, with the pelvis tilted dorsally and the thigh horizontal, the muscle acts not only as a powerful extensor of the knee, but as an extensor of the hip, and so aids in the leap. Aside from the extensor action on the knee, the most important action of the muscle is to carry the thigh dorsally. As the thigh extends and the trunk is raised from the ground, this muscle, by carrying the thigh dorsally, brings it more in line with the pelvis, and so favors the forward movement of the trunk. The carrying of the thigh dorsally also helps to raise the knee from the ground. As the femur comes into the plane of the pelvis, the muscle changes its action from being an extensor to being a flexor of the hip, and probably at the end of the leap helps to flex the thigh. Inasmuch as the muscle is a strong extensor of the knee, it is doubtful whether it can do more than help initiate the flexion movement.

**Gracilis major.** — This is a strong two-joint muscle, the proximal part of which lies on the median, and the distal part towards the medio-ventral side of the thigh. The ventral border of the muscle partly covers the adductor magnus, and its distal extremity is partly covered by the sartorius. Dorsally it is in contact with the semimembranosus. It arises caudad to the acetabulum from the most caudad part of the pelvic disk, in the region of the ischium, by a thin, ribbon-like tendon. It covers the ventral head of the semitendinosus, the tendon of which can be seen to emerge from beneath its dorsal border at the knee. The tendon of insertion of the gracilis major passes under the expansion of the sartorius tendon, over the ligamentum mediale of the knee joint, to be inserted on the ventral (median, Gaupp) surface of the median condyle of the os cruris. The muscle, as it descends the thigh, therefore crosses from the median to the medio-ventral side of the femur, and so acquires the power to carry the femur ventrally and rotate it ventrally. Two tendinous slips are given off from the main tendon, one of which passes across the median surface of the proximal end of the shaft of the os cruris, where it is inserted well towards the dorsal side, just distal to the point from which the lateral condyle springs; the other slip connects with the tendon of the semimembranosus (see Fig. 4).

The most important action of this muscle on the hip joint is to extend the femur on the pelvis; as has been said, it also has a slight action to carry it ventrally, and it rotates it ventrally. These effects are produced when both pelvis and femur are in the horizontal plane, and when either of them has been carried either dorsally or ventrally

out of this plane. In the sitting position the femur is rotated dorsally, which increases the power of the gracilis magnus to rotate it ventrally at the same time that it extends it. In this position it would also tend to tilt the pelvis dorsally, which would help to raise the trunk from the ground during the leap. When the pelvis is tilted dorsally, the origin of the muscle is carried more ventrally with respect to the head of the femur, which would increase the power of the muscle to carry the femur ventrally.

By its distal end the muscle either flexes or extends the os cruris on the femur, according to the position of the knee at the time that the muscle contracts. (*a*) If the degree of the extension of the knee is less than  $110^{\circ}$  when the muscle contracts, it will cause flexion, and this flexion movement, if continued, will result in an over-flexion. (*b*) If the degree of extension of the knee at the time that the muscle contracts is between  $120^{\circ}$  and  $140^{\circ}$ , the muscle causes extension. (*c*) If the degree of extension is more than  $140^{\circ}$ , the muscle will cause flexion. This latter effect is, however, only produced when the muscle is put under strong tension. There is a dead point between the positions *a* and *b*, and another between *b* and *c*. The dead point between *a* and *b* is about at the position the knee takes when in the resting semi-extended position, *i. e.*, extended  $110^{\circ}$ – $120^{\circ}$ .

When the action of the muscle is being tested by electrical excitation, a marked ventral rotation of the thigh occurs; and if this be prevented by holding the femur with forceps, it will be found that as the crus is flexed it is also rotated ventrally and carried dorsally, the rotation increasing as the flexion progresses; and in extreme over-flexion the crus may form an angle of  $45^{\circ}$  with the femur on its lateral side. The carrying dorsally begins to show itself when the crus is flexed about  $110^{\circ}$ . This action of the crus is partly due to the muscle being inserted on the ventral side of the median condyle, but more to the character of the joint surfaces and ligaments (see p. 27). These movements produced by the gracilis major resemble those caused by the semimembranosus; but its extension action on the knee is less, and its rotation effect on the knee more, than that of the semimembranosus. The flexion action is seen regardless of the extension at the hip, except in so far as flexion of the knee is interfered with by the muscles on the lateral surface of the thigh, which are put under tension by the extension of the hip and flexion of the knee. If the extension of the hip is not more than  $150^{\circ}$ , the flexion of the knee caused by the contraction of the gracilis magnus may be sufficient to carry the crus

past the femur into the position of extreme overflexion. This muscle, by extending the thigh and overflexing the lower leg, will bring the foot into the position which will enable it upon the contraction of the gastrocnemius to wipe a piece of paper off the back. Of course these two muscles would not be the only ones engaged in such a movement.

For the same reason that complete flexion of the crus cannot take place when the thigh is extended more than  $150^{\circ}$ , over-flexion of the crus prevents complete extension of the femur. During life, contraction of the muscles on the lateral side of the thigh, by shortening them, would still further limit the extension of the thigh with respect to the pelvis when the knee was in over-flexion, and would likewise limit the flexion of the crus on the thigh when it was extended.

The power of the gracilis magnus to flex the knee is important, because it opposes the extension of the knee at the beginning of the leap and consequently prevents the lower leg and foot from being driven out laterally with respect to the trunk during the early part of the stroke. The gracilis magnus is an extensor of the hip as well as a flexor of the knee. Moreover, it has greater leverage upon the hip than the knee, and helps to extend the hip at the expense of its flexor action on the knee. As the hip becomes more and more extended, the tendon action of the two-joint muscles which flex the thigh comes into play, and this fact, together with the direct extensor action of these muscles on the knee, overcomes the flexor effect of the gracilis magnus muscle on the knee. When the knee has been extended  $110^{\circ}$ – $120^{\circ}$ , the dead point between the sliding and rotation surfaces of the knee is passed, and the action of the gracilis magnus is suddenly changed from flexion to extension, and it assists in thrusting the leg backwards.

**Gracilis minor.**—The gracilis minor lies on the median surface of the thigh. It forms a thin narrow ribbon, and lies directly beneath the skin. The fascia covering the cephalad two thirds of the muscle is connected with the skin at frequent intervals by connective tissue bridges.

There is a very thin sheet of fibrous tissue attached in the median line to the cartilagio-marginalis, which covers the border of the pelvic disk. This delicate sheet of tissue extends ventrally as far as the origin of the rectus abdominis and dorsally to the insertion of the more caudad fibres of the sphincter ani muscle. The gracilis minor arises with its fellow of the opposite side from the middle of this sheet of tissue. The muscle is inserted at the knee to the tendon of the gracilis major.

During contraction, the muscle, by virtue of the connective tissue bridges which bind the cephalad two thirds to the skin, causes the skin to be drawn into minute folds. These are different from the large folds seen when the thigh is made to extend after the connections between the skin and the muscle have been severed. The contraction of the muscle puts the skin on the median side of the thigh under tension and holds it close to the thigh. The muscle tends to cause the same movements of the thigh and lower leg as the gracilis major, but is very much weaker.

**Iliacus externus.**— This muscle arises from the lateral surface of the wing of the ilium, and its fibres pass caudad and somewhat ventrally to be inserted on a little projection (trochanter) near the median and distal border of the dorsal flattened surface of the head of the femur, close to where the head joins the shaft. The tendon winds over the joint, and slides over the capsule freely as the bone is flexed and extended.

If the pelvis and femur are horizontal, the muscle can flex the femur to  $30^\circ$  extension, can rotate the flexed femur ventrally  $30^\circ$ , and if the flexed femur has been carried ventrally, can bring it back to the horizontal plane. The power to carry dorsally is increased, and the power to flex and rotate is lessened, as the leg is extended. If the pelvis is tilted dorsally, the power of the muscle to carry the femur dorsally is increased, but its power to flex and to rotate is lessened. When the thigh is fixed and flexed, the flexor action of the muscle would turn the body of the frog to the corresponding side, and the two muscles acting together would tilt the pelvis ventrally. When the thigh is extended, the muscle would tend to tilt the pelvis dorsally.

Although in certain positions the muscle is a strong flexor, the most important function of the muscle is to rotate the femur ventrally, the muscle being the antagonist of the obturator internus. The power of the iliacus to produce ventral rotation is greatest when the pelvis and the femur are in the usual sitting position, *i. e.*, when the pelvis is tilted dorsally  $30^\circ$ – $40^\circ$ , and the femur is horizontal, flexed to  $30^\circ$  extension, and rotated dorsally  $60^\circ$ – $70^\circ$ . In this position the iliacus, because of the dorsal rotation of the femur and the dorsal tilting of the pelvis, is under considerable tension, and its power correspondingly increased. This fact indicates that it is made use of when the leg is extended during the leap.

The muscle helps to keep the head of the femur in the acetabulum. The tendons of the iliacus externus and of the caudad and ventral

fibres of the obturator internus wind round the femur in opposite directions, that of the iliacus coming from cephalad and above, and that of the obturator from caudad and below the joint. The iliacus opposes ventral and caudad displacement, the obturator dorsal and caudad displacement, and both of the muscles act together, especially in the squatting position, to bind the head of the bone in the joint, and prevent it from being dislocated by the leap.

If the flexed femur be rotated dorsally, as in the sitting position, the tendon of the iliacus is wound round the head of the bone close to the shaft and prevents extension of the thigh in the horizontal plane unless ventral rotation occurs (Hering). The thigh can be extended if it be carried ventrally, however, for then the tendon, by sliding over the flattened surface of the head of the bone, acquires a new position with respect to it, the strain being more in the direction of the long axis of the shaft. The femur could be extended, therefore, if the surface on which the foot rests when the frog is squatting is removed, or if the body is raised away from the support. In jumping the latter occurs, the body being raised from the ground at the same time that it is thrown forward by the extension of the femurs.

If the femur be extended in the horizontal plane by any means, the head of the bone moving beneath the tendon brings a strain on the iliacus externus which causes ventral rotation, even though the muscle itself does not contract. Any muscle, therefore, which extends the femur in the horizontal plane, when the femur is flexed and rotated dorsally, acts through the iliacus externus to cause ventral rotation. The amount of rotation produced in this way is only enough to bring the femur into the position which it holds in the resting extended position. This passive action of the iliacus is important, because in swimming the femur must be rotated ventrally as it is thrust away from the trunk, so that the force exerted by the extending muscles may drive the foot backwards, instead of downwards.

It is because of the considerable amount of ventral rotation produced in the hip joint by this muscle, together with the fact that the lower leg can be overflexed at the knee, that the frog is able to brush his foot across his back.

**Iliacus internus (Ilio-psoas).** — This muscle arises dorsal and cephalad to the head of the femur, from the rim and cavity of the pelvis and from the cephalad part of the capsule of the hip, towards the dorsal side. The origin from the median surface of the root of the wing of the ilium within the cavity of the pelvis considerably increases the

length of the fibres. The muscle runs across the dorsal part of the capsule of the hip joint, then along the dorsal surface of the shaft of the femur, and is inserted on the dorso-median and median surface.

This is a very strong one-joint muscle, which acts as flexor of the hip in all positions of the thigh. Gad<sup>1</sup> writes: "To bring the extended leg to the position ready for the spring, the frog needs, at least with respect to the movements of the hip and knee joints, to innervate only one muscle, namely, the ilio-psoas." "This strong one-joint muscle is by the frog the flexor of the thigh  $\chi\alpha\tau'$   $\acute{\epsilon}\xi\omicron\chi\eta\nu$ . There can be no doubt of its flexor action by any position of the thigh."

The effect of the iliacus internus to flex the knee joint is produced indirectly, through the passive tendon action of the semitendinosus. The flexion of the knee ordinarily is quite complete, but overflexion is not produced in the case of the dead frog. The flexion of the knee causes a partial flexion of the ankle to about 90° extension, through the tendon action of the tibialis anticus longus.

When pelvis and femur are horizontal, if the femur has been extended, the muscle tends to produce flexion of the femur in the horizontal plane. If the femur has been partially flexed and carried dorsally, this muscle will carry it ventrally as far as the horizontal plane, and if the femur has been carried ventrally, the muscle will carry it dorsally as far as the horizontal plane. The power of the muscle to bring the limb into the horizontal plane is best seen when the flexor action is prevented by a smooth instrument placed cephalad to the shaft of the femur.

If the pelvis has been tilted dorsally, the origin of the muscle is carried dorsally, and if the femur is in the horizontal plane, the muscle flexes the femur and carries it dorsally. The power of the muscle to carry the femur dorsally when the pelvis has been tilted dorsally is important, as it helps to raise the knee off of the ground when the leg is being drawn into the sitting position.

When the femur is extended, the muscle acts to tilt the pelvis dorsally, but when the femur is flexed, it tilts the pelvis ventrally.

Since the insertion of the muscle extends round on to the median surface of the shaft of the femur, it gives to the muscle the power to rotate the femur ventrally, which, when the knee is partly flexed, tends to raise the ankle and foot from the ground. As flexion progresses, however, from 50° extension on to complete flexion, the capsule of the

<sup>1</sup> GAD: Verhandlungen der physikalischen medicinischen Gesellschaft in Würzburg, N. F., 1884, xviii, p. 44.

joint would inhibit ventral rotation more and more, and if the pelvis had been carried dorsally would help the obturator internus to produce the dorsally rotated position of the thigh belonging to the sitting position. In harmony with this is the fact that as the pelvis has been tilted dorsally the origin of the iliacus internus is carried dorsally, and the power of the muscle to rotate the femur ventrally is lessened. If the femur has been rotated dorsally, however, the muscle can rotate it ventrally.

Although the iliacus internus acts directly to rotate the thigh ventrally, if the pelvis has been tilted dorsally, the iliacus, like any flexing muscle, may make use of the tendon action of the obturator internus and cause dorsal rotation of the thigh. It of course could not do this in case its power to flex were not greater than its power to rotate ventrally, and the power of the obturator to rotate dorsally were not greater than the power of the iliacus to rotate ventrally. Naturally the obturator internus would have to be under tension, in order that this effect should be produced.

As the thigh is flexed, the iliacus externus and iliacus internus would, because of the nearing of their points of attachment, have less and less flexion action. On the other hand, as the thigh is rotated dorsally by the obturator internus, those muscles which rotate it ventrally are put under greater tension, and so acquire a condition favorable to their action, when the thigh has to be rotated ventrally during the extension of the hip by the leap. Undoubtedly the contraction power of a muscle is greatest when it is under tension and has its greatest length, and is least when its tension is least and it is shortest, as is the case when it has completed a movement which it is its function to produce. Thus the dorsal rotators of the femur have the least power when dorsal rotation is complete, while the ventral rotators are at this time in the most favorable position for action. Since the dorsal rotation of the femur which takes place at the close of the flexion of the thigh, when the pelvis has been tilted dorsally, would restore the tension of those flexors which are also ventral rotators, one would be inclined to think that at the moment of the leap they would by their flexor action interfere with the leap in case they contracted. It is probable, however, that the extensors of the thigh are sufficiently powerful to overcome the flexion action of these muscles, and to cause the whole force of their contraction to be directed to rotating the thigh ventrally. Indeed, we have here probably another example of tendon action. The extensors of the thigh, by the act of

extension which they produce, increase the tension of the contracting ventral rotators and thus help in producing the important movement of ventral rotation.

**Ilio-femoralis.** — This muscle carries dorsally and rotates ventrally in all positions of the thigh. These are the only effects when the limb is extended  $80^{\circ}$ ; if the extension is less than  $80^{\circ}$ , the muscle will flex, and if more than  $80^{\circ}$  will extend, as well as carry dorsally and rotate ventrally. Carrying the femur dorsally decreases, and carrying ventrally increases, the tension on the muscle, with corresponding effects on the strength of its action. If the pelvis be tilted dorsally  $40^{\circ}$ , and the femur be horizontal, the muscle extends, carries dorsally, and rotates ventrally in all positions of the bone. The pelvis is tilted ventrally by this muscle if the femur is flexed, and is tilted dorsally if the femur is extended at the time contraction occurs.

**Ilio-fibularis.** — The ilio-fibularis is a slender two-joint muscle which lies in the middle of the dorsal surface of the thigh, between the glutæus magnus and the semimembranosus. It arises as a tendon just caudad to the point of origin of the glutæus magnus, from the root of the wing of the ilium just below the processus superior. Thus the point of origin is dorsal and slightly cephalad and median to the acetabulum, and to the axis of rotation for flexion and extension in the head of the femur. The method of insertion at the knee brings the point of attachment dorsal and median to the distal end of the femur. When the femur is extended, the muscle lies dorsal and median to the shaft; when the femur is flexed, the muscle lies diagonally across the shaft, because in the process of flexion the tendon of origin is carried across the head of the femur from the dorsal, median, and caudad side to the dorsal, lateral, and cephalad side. This shifting of the tendon of origin from one side to the other of the head of the femur of course markedly alters the function of the muscle.

When the pelvis, femur, and os cruris are suspended horizontally, the muscle is found to flex the femur on the pelvis when the hip is extended  $90^{\circ}$  or less, but if it is extended  $100^{\circ}$  or more, the muscle extends the femur. If the pelvis be tilted dorsally, the femur being horizontal, the point of origin of the muscle is carried a little more caudad, *i. e.*, towards the extensor side of the hip joint, and the extending power of the muscle is increased at the expense of the flexing power, and flexion is rarely seen, even when the hip is completely flexed.

The ilio-fibularis can carry the femur dorsally, and so raise the knee from the ground, this action being most marked when the knee is extended more than  $90^\circ$ , and especially when the femur has been carried ventrally out of the horizontal plane. Moreover, the muscle can rotate the femur ventrally, this effect being greatest when the femur has been rotated dorsally, as in the sitting position. The power to carry dorsally and to rotate ventrally is most evident when flexion of the knee has been prevented by a pin thrust through the tendons on the extensor side of the knee, and when the muscle is excited electrically. The power of the muscle to carry dorsally shows best when the flexion and extension movements are prevented.

In the following description lateral condyle and lateral ligament are spoken of, although these in the frog lie dorsally to the knee joint. The ilio-fibularis is inserted at the knee by a tendon which divides into two branches which span the dorsal (lateral, Gaupp) side of the knee joint and lie superficially to the lateral ligament, acting as a second lateral ligament to bind the femur to the os cruris. The distal branch has a broad insertion on the dorsal side of the proximal end of the shaft of the os cruris, just distal to the lateral condyle, and the proximal branch, which forms a broad, delicate ribbon, passes round the dorsal surface of the lateral condyle of the femur and is inserted well towards the lateral surface of the condyle. The distal branch of the tendon flexes the os cruris on the femur. At the same time it has a distinct tendency to carry the crus ventrally and rotate it dorsally, this tendency being the greater the more the crus is carried dorsally and rotated ventrally by overflexion. In these respects it is therefore the antagonist of the semimembranosus, gracilis major, and semitendinosus. Since the crus is carried dorsally in the process of overflexion, this muscle would be of service in carrying the crus ventrally into the plane of the femur during the extension of the hind limb. The proximal branch of the tendon, through its attachment to the dorsal surface of the lateral condyle of the femur, enables the muscle to carry the femur dorsally.

Gad states that the resting length of the muscle is so small that, if the hip is flexed, the knee is passively flexed. This latter statement is questionable, for the origin of the muscle lies dorsally to the hip joint, and the tendon slides over the dorsal side of the joint when the hip is flexed, instead of being wound round the joint, as is the case with some of the muscles of the median side of the femur.

**Obturator externus.**—The origin of this muscle is a continuation

of that of the pectineus on to the ischium, *i. e.*, lies ventrally and slightly caudad with respect to the hip joint. The thigh is carried ventrally by this muscle in all the positions of the limb, the effect being the more marked the more dorsal the thigh at the time. If the thigh and pelvis are horizontal, and, the supporting thread being held, the thigh is prevented from moving ventrally, the muscle acts as an extensor when the thigh is extended more than  $70^{\circ}$ , and acts as a flexor when the thigh is flexed less than  $70^{\circ}$  extension. When the thigh is carried dorsally or ventrally, like effects are observed, except that when it is carried ventrally the action is less marked.

If the pelvis be tilted dorsally to the sitting position, the origin of the muscle is carried somewhat cephalad with respect to the head of the femur. If now the muscle be excited, the thigh is carried ventrally; or if this is prevented, it is flexed if extended and extended if flexed, the dead point being at about  $110^{\circ}$  extension.

If the femur is fixed, the muscle tilts the pelvis dorsally if the femur is flexed, and ventrally if it is extended.

**Obturator internus.**—This muscle surrounds two thirds of the acetabulum, from the cephalad and ventral to the dorsal side. The fibres take origin from the lateral surface of the pelvic disk, just inside the border, all the way from the anterior to the posterior spine. They all act on a tendon which is inserted in a slight groove which runs transversely across the dorsal flattened surface of the head of the femur, just proximally to the point of attachment of the tendon of the iliacus externus. The tendon is attached, not, as Gaupp says, to the capsule, but to the head of the bone. It soon penetrates the synovial membrane and the capsule, which surrounds it for a short distance as a sleeve, and spreads out upon the capsule over the head of the bone.

The tendon consists of a short and relatively broad ribbon, which is prolonged ventrally as a strip of fibrous tissue, which runs half-way round the joint on the caudal and ventral sides, in the form of a thin collar. The cephalad, ventral, and caudad fibres of the muscle run obliquely to join this collar, forming a whirl about the joint. If the fibres be cut away from their origin on the disk of the pelvis and the muscle be raised up, it will be seen that the fibrous collar on which the fibres are inserted lies in a groove on the cartilago remanens. The groove is formed by the ventral border of the acetabulum, where it projects laterally from the disk of the pelvis. As the ventral fibres of the muscle contract, the fibrous collar draws over the

smooth cartilage forming the groove as round a pulley. The dorsal and some of the caudal muscle fibres take a more direct course, and, converging from their point of origin, are inserted on the short broad head of the tendon. This latter group of fibres differs both in its anatomical relations and its physiological action, so greatly from the former group as almost to deserve another name. We shall refer to the two groups as the ventral and dorsal fibres.

The tendon glides over the capsule and the head of the femur as it is flexed and extended. Its position on the head of the bone is secured in part by loose bands of fibrous tissue which, though connecting it with the capsule, permit it to shift its position upon it within certain limits. It is also held in place by the fibres of the muscle itself, especially the dorsal and caudal fibres. Indeed the chief function of these fibres seems to be to act as guy ropes to prevent the tendon from sliding forwards over the head of the bone when the ventral fibres contract, and so enable the ventral and cephalad portions of the muscle to do its important work of rotating the thigh dorsally.

If the femur is horizontal and is extended, the tendons of the obturator internus and iliacus externus are seen to be attached to the median side of the dorsal flattened surface of the head of the bone, the obturator being inserted in a slight groove which lies median and somewhat cephalad to the little trochanter which forms the point of insertion of the iliacus externus.

As the extended bone is flexed to  $90^\circ$  extension, one sees the points of insertion of these muscles describe an arc about the centre of rotation for flexion and they come to lie caudad to this centre. As the bone is flexed to complete flexion, the points of insertion come to lie laterally from the centre of the head of the bone, and the groove where the obturator is inserted is seen to be caudad and median to the trochanter, instead of cephalad and median as when the femur is extended.

This change of position of the point of insertion of the obturator with respect to the axis of rotation for flexion and extension necessarily alters the action of the muscle. Moreover, if the femur remains in the horizontal plane, and the pelvis be rotated about its transverse axis, *i. e.*, be tilted ventrally or dorsally, the tension and the direction of pull of both the ventral and dorsal fibres of the muscle is changed with respect to the femur, and the action of the muscle is altered.

*The muscle helps to bind the bone in the socket* (see iliacus externus).—This action is very important, because the acetabulum is very shallow, taking in, indeed, less than a third of the head of the bone, and the capsule is so loose that, aside from securing the airtight closure of the joint, it does little to prevent the head of the bone from leaving the socket, except when it has been put under tension by marked ventral or dorsal rotation.

In the flexed position of the femur the cephalad and ventral fibres of the obturator internus by contracting draw the fibrous collar to which they are inserted firmly against the head of the bone, bind it in the socket, and oppose backward and dorsal displacement. The dorsal and caudad fibres not only assist by preventing the fibrous collar from sliding forwards over the head of the bone, but act directly to prevent ventral and forward displacement. This action of the muscle is most marked in the sitting position, since tilting of the pelvis dorsally puts the fibres under tension.

*Rotation effects.* — To study the action of the obturator internus, make a pelvis-femur preparation, with the os cruris cut off just below the knee, and all the other muscles divided or removed. Fix the pelvis in the horizontal position and suspend the femur so that it can move freely in the horizontal plane. When the preparation is thus placed, it will be found that the femur can be flexed and extended without any opposition being encountered, and with but very little rotation occurring. If, however, the pelvis is tilted dorsally  $40^{\circ}$  and the femur is passively flexed, a dorsal rotation of the bone is observed, this beginning when the flexion process has carried the femur beyond  $90^{\circ}$ , and increasing as complete flexion is approached. Tilting the pelvis dorsally puts the ventral fibres of the obturator internus on the stretch, and flexion of the joint increases the tension on the muscle. Any muscle or force, therefore, which will flex the femur horizontally when the pelvis is in the sitting position will tend to act through the ventral fibres of the obturator internus to rotate the femur dorsally, that is, help to produce the rotation which is normally present when the frog is in the ordinary squatting position.

The amount to which the ventral fibres of the obturator internus can rotate the femur by contracting differs in different positions of the hip joint. It is greatest when the femur is flexed and the pelvis is tilted dorsally, *i. e.*, in the squatting position, where the dorsal rotation may be  $70^{\circ}$ , as much as the ligamentum ventrale will permit. If the pelvis be brought into the horizontal plane, the dorsal

rotation can only be  $50^{\circ}$ . If the femur be extended, the amount of dorsal rotation possible lessens, until by complete extension it is almost absent.

The importance of the dorsal rotation produced by the ventral fibres of the obturator internus during the latter part of the flexion of the femur, when the thigh is being given the squatting position, is very great. It is through the rotating action of this muscle that the distal end of the femur comes to be supported on the central end of the os cruris instead of lying beside it. This muscle must act, moreover, during the early part of the extension of the thigh by the leap in order that the muscles extending the thigh may cause the trunk to be raised up and thrown forward, instead of driving the leg out sidewise.

The dorsal fibres may rotate ventrally when the femur is flexed, and dorsally when the femur is extended. The ventral rotation of the flexed femur is best seen when the femur is prevented from being carried dorsally. These fibres are weak, and neither of these rotation effects is strong.

*Flexor and extensor action.* — These effects can be ascertained best by electrical excitation of different parts of the muscle. If the pelvis and femur are horizontal, the muscle is found to extend the femur if it be flexed and to flex it if it be extended. The dead point is at from  $110^{\circ}$  to  $120^{\circ}$ . Both the ventral and the dorsal fibres act thus, and the reversal of action is due to the change in the position of the point of insertion of the muscle on the head of the bone with respect to the axis, about which flexion and extension occur. It is doubtful whether the muscle acts either as flexor or as extensor strongly enough to be of much service.

*Carrying the femur dorsally.* — The dorsal fibres of the obturator may produce this effect, and act the more, the more the femur has been carried ventrally. In the flexed position, as the bone is rotated dorsally, the part of the tendon connected with the dorsal fibres draws more and more across the central portion of the head of the femur, which is now directed backward and medially, and helps to carry the distal end of the bone dorsally.

*Pectineus.* — The muscle arising from the pelvic disk, ventrally and somewhat cephalad to the hip joint, runs obliquely across the ventral surface of the femur, and is inserted in a long line on the crista femoris almost as far as the middle of the bone.

The chief action of the muscle is to carry the thigh ventrally; in

addition to this it is a flexor. The power to carry ventrally is greatest when the thigh has been carried dorsally and extended, since carrying the thigh ventrally and flexing it relaxes the muscle. The flexor action comes more and more into prominence as the extended leg is carried ventrally.

If the pelvis is carried dorsally, the line of origin of the muscle comes to lie more cephalad to the head of the femur, and in the sitting position the flexor action of the muscle is increased at the expense of the power to carry ventrally.

**Pyramidalis.** — This feeble muscle acts to carry the thigh dorsally and to depress the urostyle. It has in addition to this a slight extensor action.

**Quadratus femoris.** — This weak muscle lies caudad to the hip joint, and acts as an extensor of the thigh in all of its different positions.

**Sartorius.** — The sartorius is a long-fibred, ribbon-like muscle which lies along the middle of the ventral surface of the thigh. It arises by a thin, broad tendon from the ilium, on the forward part of the ventral border of the pelvic symphysis, and terminates on the ventral side of the knee in a narrow, thin tendon, which spreads out to form a triangular expansion. The more proximal part of the expanded portion passes beneath the median border of the tendon of the triceps, with which it is connected by bands of fibrous tissue, and is inserted on the ventral surface of the median condyle of the crus, well forwards and towards the lateral side, close to the insertion of the gracilis major, the tendon of which is covered by the tendon of the sartorius (see Fig. 2, Plate I). The more distal part of the tendon of the sartorius is inserted on the middle and proximal part of the ventral surface of the expansion of the tendon of the semitendinosus. This method of insertion enables the muscle to make use of the peculiar tendon of the semitendinosus, and should give it a similar action to flex the extended crus, and, as it flexes, to carry it dorsally and rotate it ventrally.

In studying the action of this slender muscle by electrical excitation, the lower leg was cut off at about its upper third, so as to get rid of most of the weight of the leg and foot. The effect of the spread of current was avoided, in some cases by freeing the muscle from its neighbors and placing a strip of rubber membrane between the sartorius and the adjacent muscles. In other cases, all the other thigh muscles were cut away, except that the one-joint muscles of the hip and the central end of the cruralis where it is attached to the capsule were left, so that the direction of the strain of the contract-

ing sartorius should not be altered. Of course, care was taken to watch for spread of current. The following effects were obtained also by electrical excitation of the nerve :

*Effect on the hip.* — The muscle can flex the femur on the pelvis in all positions of the femur. If the muscle be cut across the middle when it is at rest and the hip and knee are extended, the cut ends slightly overlap, and when the hip and knee are flexed, the cut ends overlap markedly. Were it not that the fibres of the muscle are very long, and consequently capable of great shortening, the muscle would have little or no power as flexion of the hip and knee neared completion. As the flexion of the hip takes place, the thigh is at the same time carried ventrally. This latter action of the muscle is the stronger, the more the thigh is flexed and the more it has been carried dorsally. When the thigh has been carried dorsally, the power of the muscle to carry it ventrally is greater than its power to flex the thigh on the pelvis. Tilting the pelvis has no appreciable effect on the character of the movements produced.

*The effect on the knee joint.* — In the extended position of the knee the strain from the contracting sartorius is transmitted through the tendinous expansion of the semitendinosus tendon to the crus. The line of attachment of the semitendinosus tendon on the crus extends from distal and median, proximally and laterally, diagonally across the proximal part of the ventral surface of the crus, and when the muscle is excited it is seen to act like the semitendinosus, to flex the knee, and to carry the flexing crus dorsally, at the same time that it rotates it ventrally. The more proximal part of the triangular expansion of the sartorius tendon being inserted towards the lateral border of the ventral surface of the median condyle of the crus gives to the muscle a marked rotation effect when the knee is over-flexed.

**Semimembranosus.** — This is a strong two-joint muscle on the dorso-median side of the thigh. The dorsal border covers the proximal part of the ilio-fibularis, which in the distal part of the thigh shows itself between this muscle and the dorsal head of the triceps, the glutæus magnus. The median border is in contact with the gracilis major, and partly covered by the gracilis minor. The semimembranosus arises from a broad insertion from the lateral surface of the pelvic disk, chiefly from the ischium, and dorsal and slightly median to the acetabulum. The muscle lies more median than dorsal to the shaft of the femur, and is inserted towards the median side of the knee on the proximal end of the os cruris and the ligaments within the joint (see Plate II, Figs. 1, 2).

This muscle extends the thigh on the pelvis, when the pelvis and thigh are in the horizontal plane, or when either of these has been carried either dorsally or ventrally out of this plane. It also tends to carry the thigh dorsally in all positions of the thigh, this action showing itself most markedly when the thigh is prevented from extending and when it has been carried ventrally.

The action on the pelvis is not quite so simple. As has been explained elsewhere (p. 17), when the thigh is extended  $90^\circ$ , carrying the thigh dorsally has the same effect at the hip joint as rotating the pelvis about its long axis: when the thigh is flexed, carrying the thigh dorsally has the same effect as tilting the pelvis ventrally; and when the thigh is extended, carrying the thigh dorsally has the same effect as carrying the pelvis dorsally. Since this muscle always carries the thigh dorsally when it is free to move, one might expect that when the thigh was fixed and the pelvis was free, this muscle would produce the movements of the pelvis just described. Experiment shows that in all positions of the thigh the muscle tends to extend the pelvis on the thigh. It also tends to rotate the pelvis about its long axis, and, in most positions of the thigh, to tilt the pelvis dorsally; but if the thigh be flexed beyond  $50^\circ$  extension, the muscle will tilt the pelvis ventrally.

In the sitting position, that is, with the thighs in the horizontal plane and extended to  $40^\circ$ , and the pelvis tilted dorsally  $40^\circ$ , the muscle acts strongly to extend the thigh, to carry the thigh dorsally, and to tilt the pelvis ventrally. In the early part of the leap it would propel the body forwards, would raise the knee off of the ground, and would tend to tilt the pelvis ventrally, opposing those muscles which act to tilt it dorsally. By carrying the pelvis ventrally in the early part of the leap, it would help to keep the weight of the trunk well forward and oppose the action of the powerful gastrocnemius muscle, which by vigorously extending the ankle joint tends to throw the frog over on his back. When the thigh was extended more than  $50^\circ$ , and especially in the latter part of the leap, the muscle while still acting to extend the thigh, and to raise the knee from the ground by carrying the thigh dorsally, would act to carry the pelvis dorsally and thus give the body a position favorable to landing on the hind feet.

In addition to the above effects the muscle tends to rotate the thigh ventrally, since it winds round the femur somewhat in passing from the point of origin dorsal to the head of the bone, to its point of

insertion on the median condyle of the os cruris. This rotation effect is most when the femur is rotated dorsally in the sitting position.

The action of this muscle on the knee is a very remarkable one, and requires a brief statement concerning the relation of its tendon to the joint. The tendon of insertion divides into two short branches, these forming a Y, the arms of which pass to either side of the median condyle of the os cruris. One of the arms of the Y runs in the dorsal (lateral, Gaupp) direction, and is inserted beneath the tendinous arch forming the origin of the plantaris longus, in the most proximal part of the fossa intercondylia and on the ligaments within the joint, while the other arm runs in the ventral direction and is inserted on the most proximal part and to the ventral (median, Gaupp) side of the median condyle of the os cruris. These two branches of the tendon form an arch round the median condyle, which plays between them as it rolls on the distal end of the femur during the early part of the act of flexion. Still another branch which is given off dorsally (laterally) from the tendon is closely connected with the internal ligaments of the joint and passes to the most proximal and dorsal (lateral) side of the lateral condyle. When the crus is completely extended, the dorsal branch, especially of the Y tendon, which is attached to the os cruris in the most proximal part of the fossa intercondylia, slides the proximal end of the os cruris on the distal end of the femur and flexes the completely extended crus a short distance to  $150^{\circ}$  extension. If the crus is passively flexed beyond this amount, the rotation surfaces of the two condyles come to bear on the end of the femur, and the points of attachment of the tendon of the muscle are wound under them and carried into the joint. The tendon now draws over the distal end of the femur as round a pulley, and when the muscle contracts it causes the condyles of the os cruris to roll on the head of the femur and extends the os cruris as far as  $140^{\circ}$  extension. This happens if the thigh holds a position between  $100^{\circ}$  and  $140^{\circ}$  extension. If the os cruris be still further passively flexed to less than  $100^{\circ}$  extension, the flattened median surfaces, instead of the proximal rounded rotation surfaces of the condyles of the os cruris, come in contact with the head of the femur, which now lies in the intercondyloid fossa, and the muscle causes the end of the os cruris to slide on the femur in such a way as to flex the knee, rotate the os cruris ventrally, and carry it dorsally with respect to the femur. These results are largely due to the shape of the joint surfaces and the action of the lateral

ligaments of the knee, for, if either the dorsal or the ventral branch of the Y be cut, the other branch acting alone can produce all of these movements, — flexion, when the extension present is  $150^{\circ}$  or more, extension when the extension present is between  $100^{\circ}$  and  $140^{\circ}$ , and flexion, rotating ventrally and carrying dorsally, from  $90^{\circ}$  extension to complete over-flexion. These figures differ slightly by different preparations.

In the early part of the leap the semimembranosus acts to extend the hip, but opposes the extension of the knee. This is of use, because, if the knee were extended before the hip, the leg would be driven out sidewise instead of backwards. This opposition is overcome when the thigh is partly extended by the powerful extensors on the lateral side of the knee; and when the knee has been extended beyond about  $100^{\circ}$ , a dead point is passed, and the flexor action of the semimembranosus is suddenly changed to an extensor action. Up to this point the flattened median surfaces of the fossa intercondylea of the os cruris were sliding on the median surface of the head of the femur, but now the proximal rotation surfaces of the condyles suddenly come in contact with and roll on the distal end of the femur. In passing the dead point the contracting semimembranosus is put under elastic tension, and when its flexor action is changed to an extensor action, it behaves like a spring that has been stretched and is suddenly released and gives a sudden impulse to the extending leg. Towards the close of the leap, when extension has passed  $150^{\circ}$ , the flexor action of the muscle again shows itself and helps to check the extension movement, lessen the strain on the knee joint, and favor the beginning of the recovery of the leg to the flexed position needed for the next leap.

Gad<sup>1</sup> says that the semimembranosus and rectus internus (the gracilis major) cause, like the two-joint semitendinosus and ilio-fibularis (biceps), besides extension of the hip, flexion of the knee. He goes on to say that they do not have to be contracted to cause the latter movement, for their natural length even in the resting position is so small that by the dead animal the flexion of the hip produced by the ilio-psoas (iliacus internus) without anything further is associated with flexion of the knee. This effect belongs in the case of dead muscles only to the semitendinosus, and this can passively flex the knee by being wound up round the hip when that joint is flexed. If the pelvis of a frog be held with the cephalad end

<sup>1</sup> GAD: *Loc. cit.*, p. 172.

downwards, the thigh is flexed by its own weight, and the knee is seen to be flexed at the same time, provided the foot has been cut off and the muscles of the lower leg have been removed, to get rid of the stretching caused by their weight. If now the tendon of the semitendinosus be cut, the knee immediately straightens to the position of almost complete extension.

**Semitendinosus.**—This is a two-joint muscle which lies beneath the gracilis major on the median side of the thigh. It arises from the pelvis by two heads, one of which lies to the dorsal and the other to the ventral side of the head of the femur. The two bellies of the muscle come together distally, and are inserted by a single tendon on the median surface of the proximal end of the os cruris.

Both of the heads of the muscle act to extend the femur on the pelvis in all positions of the bone. The dorsal head, at the same time that it extends, carries the femur dorsally, and the ventral head, at the same time that it extends, carries the femur ventrally. When the two heads act together, simple extension is seen. The power of the two heads to carry the femur out of the horizontal plane is best seen when extension is prevented.

Both heads of the muscle act through the common tendon to flex the os cruris on the femur. The method of attachment of the tendon on the os cruris is so remarkable, and reveals the function of the muscle so clearly, that it deserves a special description (see Plate I and Plate II, Fig. 2). To appreciate the adaptation of the method of insertion to the work which the muscle has to do, one must recall that the flexion movement of the knee of the frog is not limited to  $180^\circ$  or less, as in the case of most joints, but that, as the flexing os cruris approaches the femur, it is carried dorsally and rotated ventrally, so as to be able to pass by the femur, and that flexion may be carried on into an over-flexion of  $40^\circ$ – $50^\circ$ .

As the crus passes from extreme extension to extreme over-flexion, it is first simply flexed, and then, as it approaches the thigh, is flexed, carried dorsally and rotated ventrally. The attachment of the semitendinosus is such as to enable it to act in harmony with the joint surfaces of the knee to produce all of these movements of flexion of the crus, of carrying it dorsally and rotating it ventrally, and to produce each of these effects most strongly at the time that it is most needed.

The slender tendon of this muscle divides near its termination, one branch being attached close to the proximal end, and well towards the lateral border of the ventral side of the os cruris, and the other

further down the bone and towards the median border of the ventral surface. Between these two branches there is a membranous expansion, which is attached along the line connecting the points of insertion, and inside of this there is a second membrane, the two membranes forming a pocket. The line of insertion runs, therefore, from before backwards diagonally across the ventral side of the crus, and from the lateral towards the median border. The relation of the triangular expansion of the semitendinosus tendon to the bone is such that when the knee is flexed the more distal fibres are slack, and when the knee is extended the more proximal fibres are slack. As the knee is extended, the tension is transferred from the proximal and lateral to the distal and median fibres, and as it is flexed, from the distal and median to the proximal and lateral fibres. By this arrangement the main tendon is kept always close to the joint. By extended leg the conditions are favorable to simple flexion, and, the power being applied to the os cruris at a considerable distance from the joint, the flexor action is a strong one. As the leg flexes, the power of the muscle to rotate the crus ventrally and carry it dorsally increases more and more at the same time that the flexor action lessens.

The method of action of the semitendinosus is of advantage during extension as well as flexion, and although the muscle is to be classed chiefly as a flexor of the crus, it probably contracts vigorously when the crus is being extended in the leap; the advantage of this has been explained in part in the description of the action of the semi-membranosus. When the leg is flexed in the sitting position, the muscle acts strongly to carry the crus dorsally with respect to the femur and to produce a ventral rotation at the knee, but has only a feeble flexor action; thus it can contract vigorously during the leap and help to hold the lower leg away from the thigh as it slides past it. As the extensor action approaches completion, however, the flexor action of the muscle becomes greater and greater, and it tends to lessen the strain which would be brought on the knee joint at the end of the leap, and aid in the rapid recovery of the limb to the flexed position required for the next stroke.

As Gad observed, the resting length of this two-joint muscle is so short that the knee is passively flexed by it when the hip is flexed. The flexion is not complete, however, or rather the muscle does not cause over-flexion unless in tonus. This action of the muscle can be readily seen by holding a pelvis limb preparation with the pelvis pointing downward. The thigh is then flexed by its own weight. If the foot

and muscles of the lower leg are present, their weight is sufficient to stretch the dead muscle and prevent its tendon action from showing itself; but if the foot and muscles of the lower limb are cut away, the os cruris is seen to flex to nearly complete flexion. That this effect is produced by the semitendinosus and not by the other two-joint muscles which may flex the knee, is proved by cutting the tendon of the semitendinosus, when the os cruris immediately extends.

**Tensor fasciæ latæ.** — The tensor fasciæ latæ, the median and weakest head of the triceps group, lies on the lateral side of the thigh. It arises from the ventral border of the wing of the ilium, a little cephalad to the belly of the iliacus externus. With pelvis and thigh horizontal, the fibres take a slightly ventral course, covering the dorsal border of the cruralis, and are inserted into the common fascia of the triceps. When the limb is in complete extension, the muscle is quite tense, but in complete flexion the fibres are very lax, and could exert but little traction power.

With the pelvis and thigh in the horizontal plane, and the leg in the resting, semi-extended position, the muscle causes the femur to be flexed and the os cruris to be extended until the limb forms an angle of about  $90^\circ$  with the long axis of the pelvis. If the crus be flexed on the femur and be kept from extending, the muscle will cause the femur to be completely flexed. If the limb be carried ventrally and the femur be prevented from flexing, the muscle will carry the thigh dorsally above the horizontal plane. Tilting the pelvis dorsally  $40^\circ$  increases the power of the muscle to carry the limb dorsally, but does not prevent the flexor action. In the sitting position the muscle acts the same as the cruralis, only is much weaker. This muscle tends to carry the pelvis ventrally, and opposes the action of the chief extensors of the hip, which tend to carry the pelvis dorsally.

**Triceps.** — The triceps consists of three muscles, glutæus magnus, cruralis, and tensor fasciæ latæ, which cover the lateral and a part of the dorsal and ventral aspects of the thigh. They all arise from the pelvis, cross over the hip and knee joints, and are inserted by a common tendon on the os cruris. The action of each of the three parts of the muscle has been described separately.

SUMMARY OF ACTION OF THE MUSCLES OF THE THIGH ON THE FEMUR, PELVIS,  
AND OS CRURIS.

**Adductor longus.**

Flexes femur and to a less extent carries ventrally (feeble).

**Adductor magnus.**

*Ventral head.*

Effect on femur.

Pelvis and femur horizontal.

Flexes femur if it is extended less than  $45^\circ$ .

Extends femur if it is extended more than  $45^\circ$ .

Carries femur ventrally (strong).

Pelvis tilted dorsally, and femur horizontal.

Flexes femur if it is extended less than  $90^\circ$ .

Extends femur if it is extended more than  $90^\circ$ .

Carries femur ventrally (stronger).

Effect on pelvis (strong).

Tilts pelvis dorsally if femur is flexed.

Tilts pelvis ventrally if femur is extended.

*Dorsal head.*

Effect on femur.

Pelvis and femur horizontal.

Extends femur (strong).

Rotates femur dorsally (strong).

Effect to carry femur dorsally or ventrally slight.

Femur flexed.

If it is already dorsal, carries it dorsally.

If it is already ventral, carries it ventrally.

Femur extended.

If already dorsal, carries it ventrally.

If already ventral, carries it dorsally.

Pelvis tilted dorsally and femur horizontal.

Extends femur.

Rotates femur dorsally (strong).

Carries femur ventrally.

**Cruralis.**

Effect on hip.

Flexes femur (not strong).

Binds head of femur in acetabulum from cephalad side.

Effect on knee.

Extends os cruris on femur in all positions of the limb.

**Gemellus.**

Effect on femur.

Extends femur; most when pelvis tilted dorsally.

Carries femur dorsally if it has been carried ventrally.

Effect on pelvis.

Tilts pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

**Glutæus magnus.**

Effect on femur.

Pelvis and femur horizontal.

Flexes femur in all degrees of extension.

Carries femur dorsally (strong).

Pelvis tilted dorsally and femur horizontal.

Flexes femur if pelvis tilted dorsally less than  $20^{\circ}$ .

Extends femur if pelvis tilted dorsally more than  $20^{\circ}$ .

Carries femur dorsally.

Effect on pelvis (strong).

Tilts pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

Effect on knee.

Extends os cruris on femur in all positions of the limb.

**Gracilis major.**

Effect on femur.

Extends femur on pelvis (strong).

Carries femur ventrally; most when pelvis tilted dorsally.

Rotates femur ventrally; most when femur rotated dorsally.

Effect on pelvis.

Tilts pelvis dorsally when already dorsal.

Effect on knee.

Flexes os cruris into over-flexion when extended less than  $110^{\circ}$ .

Extends os cruris when extension between  $120^{\circ}$  and  $140^{\circ}$ .

Flexes os cruris if extension more than  $140^{\circ}$ .

Rotates os cruris ventrally in over-flexion.

Carries os cruris dorsally in over-flexion.

**Gracilis minor.**

Puts the skin under tension.

**Iliacus externus.**

Binds head of femur in acetabulum from dorsal and caudad sides.

Effect on femur.

Flexes femur (strong); less when pelvis tilted dorsally.

Carries femur dorsally; most when femur ventral and extended.

Rotates femur ventrally (strong); most when pelvis is tilted dorsally and the femur flexed.

**Iliacus externus** (*continued*).

Effect on pelvis.

Tilts the pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

**Iliacus internus.**

Effect on femur.

Pelvis and femur horizontal.

Flexes femur, strong.

Carries femur ventrally if it is dorsal.

Carries femur dorsally if it is ventral.

Rotates femur ventrally.

Pelvis tilted dorsally and femur horizontal.

Flexes femur (strong).

Carries femur dorsally.

Rotates femur ventrally (but effect not strong).

Effect on pelvis.

Tilts pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

**Ilio-femoralis.**

Effect on femur.

Pelvis and femur horizontal.

Flexes femur if it is extended less than  $80^{\circ}$ .

Extends femur if it is extended more than  $80^{\circ}$ .

Carries femur dorsally.

Rotates femur ventrally.

Pelvis tilted dorsally and femur horizontal.

Extends femur.

Carries femur dorsally.

Rotates femur ventrally.

Effect on pelvis.

Tilts pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

**Ilio-fibularis.**

Effect on femur.

Pelvis and femur horizontal.

Flexes femur if extended less than  $90^{\circ}$ .

Extends femur if extended more than  $90^{\circ}$ .

Carries femur dorsally ; most if extended more than  $90^{\circ}$ .

Rotates femur ventrally.

Pelvis tilted dorsally and femur horizontal.

Extends femur.

Carries femur dorsally.

Rotates femur ventrally.

**Ilio-fibularis** (*continued*).

Effect on pelvis.

Tilts pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

Effect on knee.

Flexes os cruris of femur.

Carries os cruris ventrally when flexed.

Rotates os cruris dorsally when flexed.

**Obturator externus.**

Effect on femur.

Pelvis and femur horizontal.

Flexes femur when it is extended less than  $70^{\circ}$ .Extends femur when it is extended more than  $70^{\circ}$ .

Carries femur ventrally.

Pelvis tilted dorsally and femur horizontal.

Action much the same, except the dead point between flexion and extension is at  $110^{\circ}$ .

Effect on pelvis.

Tilts pelvis dorsally if femur flexed (strong).

Tilts pelvis ventrally if femur extended (strong).

**Obturator internus.***Ventral head.*

Binds head of femur in acetabulum, from ventral and caudad sides.

Effect on femur.

Pelvis and femur horizontal.

Flexes femur if extended more than  $110^{\circ}$ .Extends femur if extended less than  $110^{\circ}$ .

Carries femur dorsally, most if it has been carried ventrally.

Rotates femur dorsally (weak).

Pelvis tilted dorsally, and femur horizontally.

Flexes and extends about the same.

Rotates femur dorsally when extension  $90^{\circ}$  or less; the chief action, and the stronger the greater the flexion.

Effect on pelvis.

Tilts pelvis dorsally if femur flexed.

Tilts pelvis ventrally if femur extended.

*Dorsal head.*

Effect on femur.

Flexes femur if extended more than  $110^{\circ}$ .Extends femur if extended less than  $110^{\circ}$ .

Carries femur dorsally.

Rotates femur ventrally if it is flexed.

Rotates femur dorsally if it is extended.

**Pectineus.**

Effect on femur.

Flexes femur ; most when femur carried ventrally ; action increased when pelvis tilted dorsally.

Carries femur ventrally ; most when femur extended and carried dorsally ; action less when pelvis is tilted dorsally.

Effect on pelvis.

Tilts pelvis dorsally if femur flexed.

Tilts pelvis ventrally if femur extended.

**Pyriformis.**

Effect on femur.

Extends femur (feeble).

Carries femur dorsally.

Effect on urostyle.

Depresses.

**Quadratus femoris.**

Extends femur.

**Sartorius.**

Effect on femur.

Flexes femur on pelvis in all positions.

Carries femur ventrally ; most when flexed and carried dorsally.

Effect on pelvis.

Tilts pelvis dorsally if femur flexed.

Tilts pelvis ventrally if femur extended.

Effect on knee.

Like semitendinosus, flexes the os cruris, carries it dorsally and rotates it ventrally in over-flexion.

**Semimembranosus.**

Effect on femur.

Extends femur.

Carries femur dorsally.

Rotates femur ventrally when rotated dorsally.

Effect on pelvis.

Tilts pelvis dorsally if extension more than  $50^{\circ}$ .

Tilts pelvis ventrally if extension less than  $50^{\circ}$ .

Effect on knee.

Flexes os cruris into over-flexion when extension less than  $100^{\circ}$ .

Extends os cruris when extension between  $100^{\circ}$  and  $140^{\circ}$ .

Flexes os cruris when extension more than  $150^{\circ}$ .

Carries os cruris dorsally in over-flexion.

Rotates os cruris ventrally in over-flexion.

**Semitendinosus.**

Effect on femur.

Extends femur.

Carries femur dorsally by dorsal head.

Carries femur ventrally by ventral head.

Effect on pelvis.

Ventral head.

Tilts pelvis dorsally if femur flexed.

Tilts pelvis ventrally if femur extended.

Dorsal head.

Tilts pelvis ventrally if femur flexed.

Tilts pelvis dorsally if femur extended.

Effect on knee.

Flexes the os cruris in all positions (strong).

Carries the os cruris dorsally in over-flexion.

Rotates the os cruris ventrally in over-flexion.

**Tensor fasciae latae.**

Effect on femur.

Pelvis and femur horizontal.

Flexes femur.

Carries femur dorsally.

Pelvis tilted dorsally and femur horizontal.

Flexes femur.

Carries femur dorsally more strongly.

Effect on pelvis.

Tilts pelvis ventrally if femur flexed.

Tilts femur dorsally if femur extended.

Effect on knee.

Extends os cruris on femur in all positions of limb.

# FURTHER OBSERVATIONS ON THE RESUSCITATION OF THE RESPIRATORY NERVOUS MECHANISM.

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## I. THE AUTOMATISM OF THE RESPIRATORY CENTRE.

IN a recent number of this Journal we<sup>1</sup> published experiments on the resuscitation of certain of the bulbar centres, including observations on the automatism of the respiratory centre. We endeavored to isolate the centre from all afferent impulses by subjecting the brain, including the bulb and cervical spinal cord, to varying periods of anæmia. After restoration of the circulation a stage can be found during which the respiratory centre is discharging itself rhythmically, while as yet excitation of such afferent nerves as may be presumed to affect it most easily is ineffective. Although at this stage there is every reason to believe, both on general grounds and on certain special evidence already dealt with, that the connections of the bulbar centre with the upper parts of the brain have not yet become capable of conduction, it seemed worth while to supplement the experiments referred to by another series in which, in addition to eliminating totally, as was believed, both upper and lower paths by anæmia, the anatomical elimination of the most important of them was accomplished by the knife. In the previous paper the general result of these observations is mentioned, but only in the briefest way.

The experiments were performed on eleven cats and two rabbits. In all the animals (anæsthetized by ether) the upper paths were severed by dividing the brain stem above the level of the respiratory centre by means of a thin probe passed through a trephine hole. The level of the section varied in different experiments from about the middle of the fourth ventricle to the upper border of the pons. Where shock is avoided by making the section sufficiently late in the

<sup>1</sup> STEWART and PIKE: This journal, 1907, xix, p. 328.

occlusion period, or sufficiently early in the period of resuscitation, we find, without exception, that the behavior of the respiratory nervous mechanism is the same as that previously described when anæmia alone is relied on to temporarily isolate the centre from afferent impulses. For instance, if the brain stem be divided at a stage in the occlusion when, after the first cessation of respiration, the secondary gasps have returned and have been going on for some time, while stimulation of the vagus or brachial plexus has ceased to influence them, the gasps go on unaltered either in rhythm or in depth. The same is true if the section be made in the resuscitation period, soon after the return of respiration, when the afferent nerves tested are still ineffective. Section of both vagi, in addition to section of upper paths, is equally without influence on the respiratory movements at this time. We have already shown that elimination of the lower part of the spinal cord by ligation of the thoracic aorta does not essentially affect the return of respiration in resuscitation. In two experiments the cord was also divided with the knife in the lower cervical region.

It is completely in agreement with our view (that at the moment when respiration returns in resuscitation the respiratory centre is still isolated from afferent impulses, coming either from above or from below) that the initial rate of the respiratory movements in resuscitation is the same as after actual section of the upper paths and the vagi without anæmia. This initial rate is apparently dependent upon a property of the respiratory centre so fundamental that it is established as soon as the automatic respiratory discharge emerges in resuscitation, independently of the length or completeness of the previous occlusion. The character of the respiration reminds one strongly of that seen after double vagotomy, the inspiration being prolonged, but not exhibiting the exceedingly long spasmodic contractions of the diaphragm described by Marckwald. There is considerable disagreement among investigators as to the effect upon the respiratory movements of division of the higher paths without section of the vagi. Nikolaides<sup>1</sup> gives the literature. As a rule, anatomical section of the upper paths, when the respiratory centre has not been isolated by anæmia, causes a diminution in the respiratory rate, which is further diminished by subsequent section of both vagi, usually to between 4 and 5 a minute. Occasionally the upper paths do not seem to be exerting any influence on the respiratory movements, and then

<sup>1</sup> NIKOLAIDES: *Archiv für Physiologie*, 1905, p. 466.

section of the vagi alone brings down the rate at once to the minimum. Under experimental conditions it may in rare cases be observed that division of the upper paths, *plus* one vagus, slows the respiration to the minimum rate, section of the second vagus producing no effect on it.

It may be noted that all the characteristic reflex respiratory effects of stimulation of the vagus or brachial nerves are obtained through the bulbar centre after anatomical separation of the higher paths. The following extracts from the protocols of Experiments 1 and 13 illustrate some of these points :

*Experiment 1.* — Adult male cat. Ether. Tracheotomy. Central ends of right vagus and left brachial prepared for stimulation.

2.38 P. M. Stimulated vagus. Complete inhibition of respiration in expiration. Stimulated brachial; respiration much quickened.

2.39. Occluded head arteries. Trephined skull immediately.

2.45.30. Divided brain just below the tentorium. The cat is gasping spontaneously. Pupils well dilated.

2.48. No gasps for some time. None later so long as occlusion lasted.

2.50. Heart beat strong; 188 in a minute.

2.51.30. Stimulated brachial. No respiration.

2.52. Released head arteries.

2.53. Cut left vagus. Nose and tongue pink. Corneal tension increased. Pupils somewhat contracted.

2.57. Stimulated brachial. No respiratory effect.

3.01. First gasp. Stimulated brachial. No effect on respiration, but movements of hind legs occur.

3.03. Gasps  $5\frac{1}{2}$  a minute.

3.04. Stimulated brachial. Distinct acceleration of respiration.

3.05. Stimulated vagus. No noticeable effect. Respiration, 7 in a minute.

3.10. Stopped artificial respiration.

3.11. Stimulated brachial. Marked effect on respiration, especially increase in depth.

3.12. Stimulated vagus. Inhibition of the deeper gasps, while the shallower intermediate gasps go on, or are even increased in frequency.

3.13.30. Occluded head arteries again.

3.14.30. Left pupil dilating rapidly.

3.15. Gasps going on regularly; deep, 12 a minute.

3.20. Last gasp.

3.25. Released arteries.

3.39. Stimulated brachial. No respiratory effect. No respiratory movements have returned, although occasional movements of the lower abdominal wall occur, associated with hind-limb movements. These abdominal movements can also be elicited by striking the hind legs.

3.53. Pupils more slit-like. No corneal reflex.

4.05. Pulling on the tracheal cannula causes strong expiratory movements of the abdominal and intercostal muscles. Stimulation of the brachial causes contraction of the abdominal muscles.

4.08. Stimulated vagus. No effect. The reflexes from the upper part of the spinal cord are now good, but no respiratory movements have yet returned. Sucking air from the trachea does not give the expiratory phenomenon, which can be got every time the trachea is pulled out by means of the cannula. When traction on the trachea is continuous, the contraction of the abdominal and intercostal muscles is prolonged, but eventually relaxes.

4.20. No change. Asphyxia produced at this time causes general spasms, but no respiratory movements.

*Autopsy.*— Section complete, 8 mm. above the calamus scriptorius on the dorsal surface, but slanting further forward on the ventral surface, to pass through the pons just below the trigeminal roots.

*Experiment 13.*— Cat. Ether. Tracheotomy. Prepared and ligated (for stimulation) central ends of right vagus, and left brachial nerves.

3.27 P. M. Stimulated vagus. Complete inhibition of respiration in expiration. Stimulated brachial; great acceleration of respiration.

3.30. Occluded head arteries. Respiration and corneal reflex disappear promptly.

3.32.20. Pupils at maximal dilation. A gasp.

3.33. Gasps.

3.36.30. Divided brain posterior to tentorium. No respiratory movements for some time before this, and none after so long as occlusion lasted.

3.37.30. Struggles of hind end of animal.

3.40. Released head arteries.

3.44. First gasp; good jaw movements also. Gasps continue. Division of the left vagus has no effect.

3.45.30. Stimulated vagus. No effect on gasps, which continue regularly.

3.46. Stimulated brachial. Effect doubtful.

3.48. Gasps are at rate of 1 in thirteen seconds.

3.48.30. Stimulated vagus. Gasps are 1 in thirteen seconds, exactly the same as before stimulation.

3.50. Stimulated brachial. Gasps 1 in seven seconds.

3.51. Respirations are 1 in eight and one-half seconds.

3.52. Stimulated vagus. Gasps are 1 in sixteen seconds during stimulation.

3.53. Respirations 1 in ten seconds.

3.54. Stimulated vagus. Respiration distinctly inhibited.

3.55. Respirations 1 in nine seconds.

3.58 30. Stopped artificial respiration.

4.00. Respirations, 11 to the minute. Good regular gasps, including jaw movements, although these are somewhat feebler than before.

4.02. Stimulated vagus. Respiration 1 in twenty-eight seconds.

4.03. Stimulated brachial. Distinct inhibition in expiration.

4.05 All respirations include movements of the jaws. Respirations are slow, with prolonged inspiration, like those seen after double vagotomy. Spontaneous respiration was well established, and no doubt would have continued for a considerable time.

4.12. Divided spinal cord. Clamped aorta below diaphragm. Right pupil well contracted; left, wide.

4.21. Twitching of whiskers.

4.23. Stimulated vagus. Contraction of diaphragm occurs each time the vagus is stimulated; also, opening the mouth, protrusion of tongue, and contraction of oesophagus as in swallowing.

4.25. Twitching of right axilla. Asphyxia does not produce respiratory movements, but when artificial respiration is started again, the diaphragm contracts independently of the artificial respiration (*i. e.* with a different rhythm), as shown by feeling the diaphragm with the finger.

4.35. Left pupil still wide, right very narrow. Right fore limb gives reflex contraction on striking it.

4.55. Stopped artificial respiration. Movements of diaphragm begin after a time and continue regularly. Fair respiration, rate 3 in twenty seconds, apparently unaffected by stimulation of vagus or brachial. A little earlier, stimulation of central end of vagus caused contraction of diaphragm, while stimulation of brachial did not. Verified several times. Contraction of diaphragm caused by vagus stimulation was not tonic, but a single twitch. Not due to escape of current, since it could not be obtained later on.

5.25. Spontaneous respirations still occur at intervals, involving diaphragm, thorax, and jaw. Stimulation of the vagus now causes contraction of diaphragm, accompanied by jaw and throat movements. Animal has lived thirty minutes without artificial respiration. Weak respiratory movements of diaphragm and jaw alternate with strong, one strong respiration and one weak occurring in fifteen seconds. Sometimes there are several weak respirations to each strong one, but the rate of the strong movements is practically constant, about 4 in the minute.

5.31. Condition much the same. Stimulation of the central end of the vagus still causes respiratory movements; spontaneous movements also occur. Stopped experiment.

*Autopsy.*—Section of spinal cord was at level of the seventh cervical pair of nerves. Complete. Upper section was through pons above roots of trigeminus. Section was complete except for a thin layer on the dorsal surface.

The effects of stimulation of a nerve like the brachial on the respiratory muscles through the reflex centres of the cord are to be sharply discriminated from the effect produced on the bulbar respiratory centre. We have seen abundant instances of reflex excitation of the intercostal muscles and the muscles of the abdominal wall through the cord at a time when there was no evidence whatever that the bulbar respiratory centre had resumed its functions. For example, stimulation of the central end of the brachial very frequently causes a strong and long-continued contraction of these muscles when no spontaneous respirations have appeared, and even in cases where respiration never returns. The diaphragm does not participate in these movements, and their character, although undoubtedly they may be capable of altering the intrathoracic pressure, and therefore of aiding pulmonary ventilation, is totally different from that of the true respiratory movements. One characteristic is their tendency in the earlier stages of resuscitation to involve only the lower abdominal muscles and the hind legs, movements of which are also easily elicited at this time by stimulation of the sciatic. It is possible that the strong and long-continued expiration which we have frequently observed, for example, on making steady traction on the tracheal cannula is a reflex effect of this nature. It can be obtained under conditions which preclude the activity of the bulbar respiratory centre.

As a rare phenomenon, an apparently reflex contraction of the diaphragm may be caused by stimulation of the central end of the vagus at a time when no spontaneous respirations are occurring. The contraction consists of a single twitch at the beginning of stimulation involving both halves of the muscle, and is not due to escape of current on to the phrenic (see Experiment 13). Here respiration had returned after section through the pons and occlusion.

## II. THE MECHANISM OF SPINAL SHOCK.

We have said that shock must be avoided in making the upper section. We can entirely confirm the statement of Asher and Lüscher,<sup>1</sup>

<sup>1</sup> ASHER and LÜSCHER: *Zeitschrift für Biologie*, 1899, xxxviii, p. 499.

who used Kronecker's method of paraffin emboli, that elimination by anæmia of the brain and cervical cord does not cause shock in the portion of the cord whose circulation is still maintained, and can add that complete section of the encephalon at the level mentioned, after a certain duration of the anæmia, is also innocuous in this regard. The same is true at an early period in resuscitation. Where the section is made so early in occlusion as to be accompanied by visible signs of stimulation it produces shock. The experiments already quoted illustrate the avoidance of shock by dividing the brain stem six and one-half minutes after the beginning of occlusion. In Experiment 4 the section was made four minutes after the beginning of occlusion; in Experiment 3, one and one-half minutes from beginning of occlusion, while Experiments 5 and 6 show the result of a division at the very beginning of occlusion, and Experiment 10 the effect of section before occlusion. The condensed protocols of these experiments follow:

*Experiment 3.* — Adult cat. Ether. Tracheotomy. Prepared central ends of right vagus and left brachial nerves.

3.25 P. M. Stimulated vagus. Respiration inhibited in expiration.

3.30.30. Occluded head arteries. Respiration stopped in about forty-five seconds.

3.32. Pupils well dilated. Some secondary gasps. Divided brain.

3.41.30. Released arteries. Hind end very active. No respiration ever returned, either spontaneously or in response to stimulation of nerves.

4.10. Produced asphyxia. No contraction of upper limbs or diaphragm even in two minutes. Pupils remain narrowly contracted, but, on resuming artificial respiration, they dilate partially and then soon become slit-like again.

Experiment terminated.

*Autopsy.* — Section was complete; passed through the pons above trigeminus roots, and dorsally through posterior part of the posterior corpora quadrigemina.

*Experiment 4.* — Adult cat. Ether. Tracheotomy. Prepared for stimulation central ends of right vagus and left brachial. Divided both vagi.

10.38 A. M. Stimulated brachial. Quickening of respiration. Stimulated vagus. Quickening of respiration, but gasps are shallower.

10.41. Occluded head arteries.

10.45. Divided brain.

10.46. Spontaneous respiration going on, not spasmodic. Pupils maximal. No corneal reflex. Intra-ocular tension low.

10.49. Last gasp.

10.51. Released the head arteries.

11.24. First gasp.

11.30. Deep gasps going on. Stimulated brachial. Strong tonic contraction of the abdominal muscles follows. Respirations 4 in fifty-five seconds without stimulation, and exactly the same during stimulation.

11.32. Stimulated vagus. There is exactly the same rate of respiration, 4 in fifty-five seconds, as before stimulation. Strong tonic contraction of abdominal muscles.

11.39. Now got complete inhibition of respiration. After stopping stimulation, respiration returned, but was shallower than before.

11.40.30. Spontaneous respiration ceased.

No true respiratory movements occurred after this, either spontaneously or in response to stimulation of nerves.

11.55. Produced asphyxia. No true respiratory movements. Spasms of hind end.

*Autopsy.* — Section complete. Situated just above tentorium. Passed above the upper edge of the pons, nearly 5 mm. above the trigeminus roots.

*Experiment 5.* — Cat. Ether. Tracheotomy. Prepared central ends of right vagus and left brachial nerves.

2.43 P. M.. Stimulated vagus. Complete inhibition of respiration in expiration. Stimulated brachial; great increase in respiration.

2.45:30. Occluded head arteries. Divided brain and spinal cord immediately, the bone having been removed before occlusion.

2.53. Released arteries.

3.27. Stimulated vagus. Good retraction of nictitating membrane, and bulging of eye, but no dilation of pupil unless stimulation is very strong, when a slight dilation occurs, increased somewhat by increasing the strength of stimulation. Confirmed. No respiratory effect. No respiration has returned as yet.

Slight reflex movements of hind limbs on striking them. Also, on striking hind leg, get fairly good contractions of abdominal muscles on the same side, extending to the median line but not crossing. The reflex contraction of the hind limbs crosses slightly.

3.51. Clamped abdominal aorta below the diaphragm. The head arteries are now much better filled. Intra-ocular pressure increases almost at once. The nictitating membrane, which had covered the eyes, retreated, and the eyes, which had been strongly rotated inward and downward, returned to their normal position.

4.12. Stimulated vagus; slight dilation of pupil; no respiratory effect. No respiratory movements ever returned. Stopped experiment.

*Autopsy.* — The lesion of the cord involved the dorsal half, but only a little of the ventral half, at level of the sixth cervical pair. Section through the brain was complete, through pons just above trigeminus roots.

*Experiment 6.* — Cat. Ether. Tracheotomy. Central ends of right vagus and left brachial prepared for stimulation.

10.45 A. M. Stimulated brachial. Great acceleration of respiration. Stimulated vagus; stronger expiration.

10.47. Occluded head arteries. Divided brain immediately, the skull having been trephined before occlusion.

10.53. Released arteries.

10.54. Divided left vagus.

12.12. Stimulated right vagus. No effect except on eye. Stimulated brachial. Great contractions of lower end of animal; no true respiratory effect.

12.45. Reflex movements of right fore limb are got on pulling or striking it, or pinching toes. Strong spasms of almost the whole animal occur from time to time, with opisthotonus, the head being strongly drawn back. Respiration did not return. Experiment discontinued. Asphyxia caused a strong general spasm.

*Autopsy.* — Section complete, through posterior part of anterior corpora quadrigemina.

Apart from the problem with which they were more particularly connected, these observations have a certain interest in relation to the mechanism of spinal shock. Sherrington,<sup>1</sup> in discussing the causation of spinal shock, sums up as follows: "I think, therefore, that spinal shock is neither due to irritation by trauma nor in the main a phenomenon of inhibition. The rupture of certain aborally conducting paths appears to induce it. Which these paths exactly are is matter for research." It is difficult to reconcile our results with this conclusion. For undoubtedly these hypothetical aboral paths are physiologically ruptured by cerebral anæmia of a certain duration, and they are anatomically ruptured when at this stage of anæmia the bulb or pons is divided, yet shock is not produced in the part of the spinal cord below the anæmic region. If, however, an earlier stage in the anæmia be chosen for the anatomical lesion, or if this be produced prior to occlusion of the head vessels, the symptoms of bulbar and spinal shock promptly appear. If we admit the validity of Sherrington's argument, that the negative result of a second spinal transection, made after the shock produced by the first has passed off, is inconsistent with the idea that the stimulation caused by the trauma is the effective factor, then we would seem shut up to the conclusion

<sup>1</sup> SHERRINGTON: The integrative action of the nervous system, New York, 1906, p. 246.

that it is neither the rupture of conducting paths by itself nor the traumatic stimulation by itself, but the combination of the two, which determines the production of shock.

So far as the respiratory centre is concerned, we have observed that section of the upper paths with the knife, when their conductivity and excitability were still intact or but slightly depressed by a very short total occlusion or by a longer imperfect occlusion, acted prejudicially on the power of resistance of the centre to a continuance of the anæmia for a period which under normal circumstances would have left it capable of prompt and perfect resuscitation. An example is seen in Experiment 10:

*Experiment 10.* — Cat. Ether. Tracheotomy.

3.29 P. M. Respiration 9 in fifteen seconds.

3.30. Divided brain. The respiration goes on for a little, somewhat slower, then stops. Started artificial respiration. Some spontaneous respiration soon came back.

3.40. Fair spontaneous respiration.

4.00. Respiration 7 in sixty seconds, deep and prolonged inspiration, just like the respiration following double vagotomy.

4.17. Respiration 2 in sixteen and one-half seconds. Prepared central ends of right vagus and left brachial for stimulation.

4.18. Stimulated vagus. Strong inhibition of respiration in expiration. Stimulated brachial. Strong inhibition in expiration with shallow gasps toward end of stimulation. Two swallowing movements occur.

4.21. Occluded head arteries.

4.25. Released head arteries.

4.36. First respiration occurs (including movement of diaphragm). Only one gasp seen. Tongue is protruded occasionally.

4.46. Produced asphyxia. No respiratory movements.

4.54. Reflex can be got in shoulder by striking fore limb of same side. No respiration has returned.

4.58. Stimulation (pinching) of right ear causes strong scratch movements of right hind limb. No movements of fore limb occur on pinching ear, although good contractions of the fore limb can be obtained on striking it.

5.08. Stimulated vagus. No effect except on eye. The pupil becomes maximal, with bulging of the eye and retraction of the nictitating membrane.

5.09. Stimulated brachial. Strong contractions of hind end of animal, including abdominal muscles.

5.10. Cut left vagus (in case absence of respiratory movements should be due to apnoea). No respiratory movements occur. Reflex contrac-

tions of abdomen and thorax can be got from any part of the abdomen or thorax on striking that part.

5.55. Stopped artificial respiration. Struggles occurred, but no true respiration. Discontinued experiment.

*Autopsy.* — Section was between the anterior and posterior corpora quadrigemina on the right side, but spared the greater part of the left posterior corpus quadrigeminum, slanting backwards from right to left. Ventrally, the section passed through the pons above the level of the trigeminus roots. The connection of the left corpus quadrigeminum with the bulb was not completely destroyed.

In our experience, section of the cord as low as the upper and mid-dorsal region at a time in the resuscitation when the bulbar centres are just beginning to function is always fatal, the bulbar discharge ceasing in a very short time. It is not, perhaps, surprising that this should be the case where the blood pressure is not maintained after section by ligation of the thoracic or abdominal aorta. But in one experiment (13) section of the cord at the seventh cervical segment, although accompanied by occlusion of the aorta just below the diaphragm, was followed by immediate cessation of the respiratory movements, which had been thoroughly re-established after section of the pons and occlusion. It was not the cutting off of (hypothetical) impulses from below, normally indispensable to the activity of the respiratory centre, which was responsible for the stoppage of respiration, since, more than forty minutes later, regular diaphragmatic respiration was established upon stopping the artificial respiration. It would seem that, under the conditions of the experiment, the still more or less crippled respiratory centre succumbed temporarily to shock, spreading in this instance up the cord from the lesion. It is generally stated that spinal shock involves only the nervous apparatus peripheral to the lesion. But it may very well be that even under normal circumstances the influence which we call shock is propagated in both directions, although less readily centripetally. Normal, undeteriorated centres may not show any obvious ill effect of the relatively weak centripetal shock influence, while centres previously crippled by anæmia may do so; just as the normal spinal cord is rendered more excitable by strychnia, whereas a portion of the cord recovering from anæmia is, at a certain stage in its resuscitation, rather depressed than excited.<sup>1</sup>

<sup>1</sup> STEWART, GUTHRIE, BURNS, and PIKE: *Journal of experimental medicine*, 1906, viii, p. 309.

In Experiment 10, although respiration did not return, good reflexes could be elicited from the previously anæmic cervical cord. The conduction of impulses by this part of the cord and by the bulb was early established (twelve minutes after release). Stimulation of the left brachial at this time, although it caused no movements of the opposite fore limb, caused strong contractions of the hind limb and movements of the tongue. Thirty-three minutes after release, pinching the right ear was followed by strong scratch movements of the right, and feeble movements of the left hind limb. The right fore limb did not contract at all, although it gave good movements on being struck. Asher and Arnold<sup>1</sup> observed that, at a certain stage in total anæmia of the spinal cord below the upper thoracic region, excitation of the central end of the sciatic caused movements of the fore limbs without exciting intervening spinal segments. In this case the impulses were possibly carried up the long afferent paths in the posterior column without passing over a synapse in the anæmic area. In our observations the impulses must have passed over at least one synapse in the previously anæmic region before reaching the long proprio-spinal fibres in the lateral column described by Sherrington<sup>2</sup> as the path through which the scratch reflex is liberated in the spinal dog when the skin of the shoulder is stimulated, if indeed this is the path concerned under the conditions of our experiments.

In Experiment 9, where the brain was divided in a cat, above the tentorium, without occlusion, and respiration stopped permanently after a few breaths, tickling either ear (thirty-five minutes after section of the brain) caused strong scratch movements of the corresponding hind limb, not involving the contralateral foot. No movements of the fore limbs were caused during the passage of these impulses down the cervical cord, whose reflex functions were indeed still almost submerged by the shock, as only feeble movements of the fore limb could be elicited by stimulating it mechanically.

We<sup>3</sup> have previously described the severe tonic convulsions which appear in the period of recovery from cerebral anæmia. It is of interest to note in this connection that similar spasms, involving the muscles of the trunk limbs, neck, and jaws, may occur in the period of recovery from cerebral anæmia combined with transection of the brain stem above the medulla oblongata. The same hyper-

<sup>1</sup> ASHER and ARNOLD: *Zeitschrift für Biologie*, 1900, xl, pp. 271-287.

<sup>2</sup> SHERRINGTON: *Loc. cit.*, p. 52.

<sup>3</sup> STEWART and PIKE: *Loc. cit.*, *Journal of experimental medicine*, p. 307.

excitable condition of the spinal reflex mechanisms, simulating that seen in strychnine poisoning, previously described, is met with also in animals whose brain has been divided. Whatever share the higher parts of the brain may take in maintaining the hyperexcitability of the lower parts of the central nervous system at a particular stage in the resuscitation, they are not indispensable for the condition in animals subjected to anæmia combined with section of the higher paths, although these centres may add to the effects produced by the lower centres in animals subjected to anæmia but whose brain has not been divided.

An observation (in Experiment 5) on the effect of stimulation of the vago-sympathetic on the pupil during resuscitation, after an occlusion of the head arteries and division of the brain stem and cervical spinal cord, is worthy of mention. While retraction of the nictitating membrane and protrusion of the eyeball were readily obtained, and to the maximum degree, no dilation whatever of the pupil was caused unless the current was very strong, when it dilated slightly. The dilatation was increased somewhat when the stimulation was strengthened still more, but nothing like maximal dilatation could be obtained with any strength. This is an instance in which the statement of Mulert,<sup>1</sup> that the "all or nothing" law holds approximately for the dilator pupillæ, could not be verified. It is possible that the pupillo-dilator fibres somewhere on their course had suffered relatively more from the anæmia than the other groups.

<sup>1</sup> MULERT: *Archiv für die gesammte Physiologie*, 1894, lv, p. 550.

## THE ACUTE EFFECTS OF GASTRIC AND PERITONEAL CAUTERIZATION AND IRRITATION ON THE BLOOD PRESSURE AND RESPIRATION.

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IN the course of a series of experiments on the treatment of carbolic acid poisoning, it was observed that the administration of concentrated phenol by a stomach tube, to dogs, produces a very prompt, considerable, and lasting fall of blood pressure. It became necessary for us to decide whether this fall is caused by a direct action of the phenol, following its absorption; or whether it is a condition of reflex shock, resulting from the gastric irritation. As one means to this end, we experimented with other corrosives and irritants, and found, to our surprise, that the acute effects of gastric irritation are absolutely negative, at least with anæsthetized dogs.

We employed violent corrosives, and strong and mild irritants, all in large doses, administered either by the stomach tube, or painted on the inner and outer surface of the exposed stomach, and on the denuded submucosa, and on the parietal peritoneum. We also perforated the stomach with actual cautery. A number of these measures were applied successively to the same animal. When given by the stomach tube, the simple irritants were made to precede the corrosives; with the local applications, a fresh surface was taken for each application.

The experiments were made on dogs, anæsthetized with morphin and ether and having the carotid connected with a mercury manometer. The anæsthesia was made light in the case of the milder irritants, such as mustard; otherwise it was sufficiently deep to exclude all expressions of pain, although the lid reflex was often preserved. The publication of the protocols and tracings seems superfluous, since they are so largely negative.

The results of the experiments are classified and summarized in Table I.

TABLE I.

CLASSIFICATION OF EXPERIMENTS AND SUMMARIZED RESULTS.

**I. Administration by stomach tube.**

Substance administered.	No. of tracing.	Effect on blood pressure.	On respiration.	Remarks.
<b>Water at about 20° C. :</b>				
Stomach filled	49	No effect.	....	There is a gradual rise of pressure throughout the procedure, but we are inclined to attribute this to the withdrawal of the ether.
“ evacuated				
“ filled				
“ evacuated				
500 c.c. in 3 min.	53	No effect.	No effect.	
250 c.c. evacuated				
500 c.c. at 23° C. in 5 min.	56	“ “	“ “	The blood pressure rises slowly by 10 mm., but this is probably due to lessening of ether.
250 c.c. evacuated				
<b>Hot water :</b>				
75 c.c. at 70° C.	51	Slight rise.	....	
250 c.c. at 65° C. in 1½ min.	57	No effect.	No effect at first, later decrease in excursions.	
235 c.c. evacuated				
200 c.c. at 60° C. in 2 min.	57	“ “	Decrease of excursions.	
Evacuated				
<b>Cold water :</b>				
300 c.c. at 10° C. in 3 min.	57	No effect.	No effect.	
Evacuated				
<b>NaOH :</b>				
10 per cent, 12 c.c. }	32	Insignificant fall with recovery.	....	Vagi divided.
40 “ “ 23 “ }				
40 “ “ 23 “ }	32	No effect.	....	
40 “ “ 10 “ }	49	Slight vagus stimulation and slight irregularity of blood pressure.		
<b>H<sub>2</sub>SO<sub>4</sub> :</b>				
25 per cent, 12 c.c. concentrated, 12 c.c. }	46	No effect.	....	
“ “ 8 “ }				
“ “ 8 “ }	51	Slight fall (15 mm.).	Excursions slightly increased.	
<b>HCl :</b>				
Concentrated, 12 c.c. }	46	No effect.		
“ “ 10 “ }				
“ “ 25 “ }	50	“ “		
<b>Phenol :</b>				
95 per cent, 12 c.c. }	47	No effect.	....	Vagi divided.
95 “ “ 12 “ }				

TABLE I (continued).

Substance administered.	No. of tracing.	Effect on blood pressure.	On respiration.	Remarks.
Phenol (continued):				
95 per cent 12 c.c. }	48	No effect.		
95 " " 12 " }				
95 " " 10 " }	50	" "	....	Vagi divided.
95 " " 10 " }				
Formaldehyde:				
13 per cent, 15 c.c.	49	No effect?	....	There is a rise due to withdrawal of ether.
40 " " 5 "	49	Slight irregularity.		Vagi divided.
40 " " 10 "		Vagus stimulation?		
40 " " 10 "	50	No effect.	....	
40 " " 10 "	51	" "	No effect.	
Mustard spirits:				
6 c.c.	47	No effect.		
9 c.c.	49	Slight irregularity?		
6 c.c.	51	No effect.	No effect.	

## 2. Local applications.

### (a) *Tip of tongue.*

HCl: Concentrated, drop 49 No effect.

### (b) *Gastric mucosa.*

Red-hot iron	54 } 58 }	No effect.	No effect.	Applied twice, once to perforation.
NaOH, 40 per cent	58	" "	" "	Local change manifest.
H <sub>2</sub> SO <sub>4</sub> , concentrated	54 } 58 }	" "	" "	Immediate corrosion.
HNO <sub>3</sub> " "	54	" "	" "	Turns yellow at once.
Phenol, 95 per cent	54 } 55 } 58 }	" "	" "	Blanches instantly.
Formaldehyde, 40 per cent	53 } 58 }	" "	" "	
Mustard spirits	53 } 58 }	" "	" "	
Peppermint spirits.	58	" "	" "	
Alcohol, 95 per cent } Acetic acid, 5 per cent }	58	" "	" "	

### (c) *Gastric submucosa.*

Melted sealing-wax	52	No effect.		
Red-hot iron	54	" "	No effect.	
Formaldehyde, 40 per cent	53	Slight rise.	Stimulation.	
" 40 " "	53	No effect.	No effect.	
Mustard spirits	54	Very slight fall.	Slight decrease.	
H <sub>2</sub> SO <sub>4</sub> , Conc.	54	No effect.	No effect.	

TABLE I (continued).

Substance administered.	No. of tracing.	Effect on blood pressure.	On respiration.	Remarks.
<i>(d) Gastric serosa.</i>				
Red-hot iron, twice	54	No effect.	No effect.	Local change manifest.
NaOH, 40 per cent	58			
" 10 " "	54			
H <sub>2</sub> SO <sub>4</sub> , concentrated	51	Slight rise? probably no effect.		
" "	54	No effect.	No effect.	Strong contraction of muscle.
" "	58			
HNO <sub>3</sub> "	54	" "	" "	Turns yellow.
Formaldehyde, 40 per cent	51	Probably no effect.	...	The blood pressure rises, but this is doubtless asphyxial.
" 40 " "	53	Slight rise.	Stimulation.	
" 40 " "	54	No effect.	No effect.	
" 40 " "	58			
Mustard spirits	53	Very slight rise.	Very slight increase.	
" " "	58	No effect.	No effect.	
Peppermint spirits	} 58	"	"	"
Alcohol, 95 per cent				
Acetic acid, 5 per cent				
<i>(e) Parietal peritoneum.</i>				
Red-hot iron	54	No effect.	No effect.	
NaOH, 40 per cent	58			
" 10 " "	55			
H <sub>2</sub> SO <sub>4</sub> , concentrated	54	Slight mo-	Momentary arrest.	
" "	54	mentary fall.		
" "	58	No effect.	No effect.	
HNO <sub>3</sub> "	55			
" "	55			
Phenol, 95 per cent	55			
Formaldehyde, 40 per cent	54	No effect.	No effect.	
Mustard spirits	54			
" "	58			
Acetic acid, 5 per cent	58			

Arranging the experiments into three groups, namely:

- GROUP I. *Corrosives.* — NaOH, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, HCl, Phenol, Actual Caутery.
- GROUP II. *Strong Irritants.* — Formalin, Spir. Mustard, Hot Water.
- GROUP III. *Mild Irritants.* — Alcohol, Spir. Peppermint, 5 per cent Acetic Acid, Cold Water, Simple Distention and Evacuation.

We obtain the following totals:

Groups.	Blood pressure.				Respiration.			
	No of exp's.	No effect.	Slight fall.	Slight rise.	No. of exp's.	No effect.	Slight decrease.	Slight increase.
Corrosives . .	45	41	4	0	28	26	1	1
Strong irritants .	26	21	1	5	19	13	3	3
Mild irritants .	12	12	0	0	10	10	0	0
Total .	83	74	5	5	57	49	4	4

It will be seen that in about nine tenths of the experiments no effect whatsoever was obtainable, either on the blood pressure or on respiration. In the remaining tenth the effect was insignificantly small, and as often in the direction of an increase as of a decrease. It may be interesting to remark, incidently, that very considerable distention of the stomach with water, or sudden evacuation of the fluid, is also without effect.

In the above summaries each application is considered as a separate experiment, together with the very short after-period elapsing before the application of another irritant. This after-period was generally less than five minutes; — a very short time, it is true, but ample for any ordinary reflex effects, such as might affect the general blood pressure or the respiration, or produce shock. In fact, however, a number of these experiments were made on each animal. Even should we grant the possibility of a delayed reflex action, this would therefore also be indicated on the curves; for the experiments were continued for half an hour to an hour, and during this entire period there was no sudden change. Furthermore, the successive application of the irritants to the same animals would be an ideal method of emphasizing any cumulative action which might be assumed. A brief enumeration of the procedures on each animal, together with the initial and final blood pressure, is of interest in this connection, and will also serve to emphasize the absolutely reckless severity (as we should have supposed *a priori*) of irritation to which the stomach may be subjected.

IRRITANTS APPLIED TO EACH ANIMAL.

- Dog No. 12.** *Procedures:* By stomach tube: 12 c.c. of 10 per cent NaOH; 46 c.c. of 40 per cent NaOH; Vagotomy. Blood pressure at the beginning, 132 mm.; at end, 80 mm. Duration of experiment, one hour.
- Dog No. 18.** *Procedures:* By stomach tube: 12 c.c. of 25 per cent H<sub>2</sub>SO<sub>4</sub>; 12 c.c. of conc. H<sub>2</sub>SO<sub>4</sub>; 6 c.c. of Spts. Mustard; 12 c.c. of conc. HCl; 12 c.c. of conc. Phenol; Vagotomy; 12 c.c. of conc. Phenol; Distention of stomach; 24 c.c. of conc. Phenol. Blood pressure at beginning, 115 mm.; at end, 75 mm. Duration of experiment, one hour.
- Dog No. 19.** *Procedures:* By stomach tube: 9 c.c. of Sp. Mustard; 5 c.c. of Formalin; 10 c.c. of 40 per cent NaOH. Gastric lavage; 5 c.c. of Formalin; 35 c.c. of conc. HCl; Vagotomy; 10 c.c. of Formalin; 20 c.c. of Phenol. Blood pressure at beginning, 150 mm.; at end, 140 mm. Duration of experiment, thirty-five minutes.
- Dog No. 20.** *Procedures:* By stomach tube: 6 c.c. of Sp. Mustard; 10 c.c. of Formalin; 8 c.c. of conc. H<sub>2</sub>SO<sub>4</sub>; Hot water (70° C.). Abdomen opened; Formalin, H<sub>2</sub>SO<sub>4</sub>, and melted sealing-wax on outer coat of stomach. Stomach cut open, mucosa partly removed; melted sealing-wax on submucosa. Blood pressure at beginning, 105 mm.; at end, 77 mm. Duration of experiment, twenty-six minutes.
- Dog No. 21.** *Procedures:* Abdomen and stomach cut open. Local applications to gastric mucosa, submucosa, and serosa and to parietal peritoneum, of Formalin; Sp. Mustard; Red-hot iron to perforation; conc. H<sub>2</sub>SO<sub>4</sub>; Formalin; 10 per cent NaOH; conc. Phenol; conc. HNO<sub>3</sub>. Blood pressure at beginning, 132 mm.; at end, 78 mm. Duration of experiment, one hour.
- Dog No. 22.** *Procedures:* Distention of stomach with hot (65° C.) and cold (10° C.) water. Abdomen and stomach cut open. Local application to gastric mucosa and serosa and parietal peritoneum of 95 per cent Alcohol; Sp. Peppermint; Sp. Mustard; 5 per cent Acetic Acid; Formalin; conc. H<sub>2</sub>SO<sub>4</sub>; 40 per cent NaOH; conc. Phenol; Actual cautery. Blood pressure at beginning, 90 mm.; at end, 45 mm. Duration of experiment, one hour. Sciatic stimulation at end causes rise of pressure and increase of respiration.

Dog No. 19 is especially interesting. It would be difficult to imagine a more formidable array of corrosives than those which were poured into the stomach of this animal during the course of half an hour; and notwithstanding, the experiment was concluded with a blood pressure of 140 mm., only 10 mm. lower than the initial pressure. The other five animals showed a very gradual fall, the final pressure

ranging from 45 to 80 mm., with an average of 71 mm.; representing a fall of 28 to 54 mm., with an average of 33 mm. This, however, is still far removed from a condition of shock; indeed, in the animal with the lowest blood pressure (dog No. 22) the vaso-motor and respiratory centres responded to sciatic stimulation in a perfectly normal manner, at the conclusion of the experiment. In view of the behavior of dog No. 19, the gradual fall of pressure is, in our opinion, fairly attributable to the exposure, the operation of opening the abdomen, and especially to the duration and the generally increasing depth of the anæsthesia, and not to the irritants. In brief, therefore, it would seem that the most violent corrosion and irritation of the stomach or parietal peritoneum, in the anæsthetized dog, produces no acute reflex effects. It is, of course, very probable that the later effects would be more serious. This is a portion of the subject which our experiments do not touch. It is self-evident that the measures to which we subjected these animals would inevitably have led eventually to intense and probably fatal inflammatory reactions.

Experiments now under way tend to show that this insensibility to strong corrosives is not shared by the mucous membrane of the mouth and by the larynx and trachea. These seem to respond by much more violent reflexes to all kinds of irritation. We mention this fact at the present time, so that our negative results from irritants administered by the stomach may not be misconstrued as applying to irritants taken by mouth.

#### CONCLUSIONS.

Corrosion or violent or mild irritation of the gastric mucosa, submucosa, or serosa, or of the parietal peritoneum, has generally no acute reflex effect upon the blood pressure or respiration in anæsthetized dogs. In the few cases in which a response was obtained this was slight, and about as often in the direction of an increase as of a decrease.

A succession of violent irritant measures applied to these structures is also without definite effect on circulation and respiration, the observation extending over an hour.

## CHEMICAL STUDIES ON GROWTH.—I. THE INVERTING ENZYMES OF THE ALIMENTARY TRACT, ESPECIALLY IN THE EMBRYO.

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IN the ordinary study of nutrition the phenomena of growth receive only incidental consideration, since developmental changes are not usually conspicuous features in the activities investigated. From a chemical standpoint growth is distinguished by a preponderance of synthetic, or anabolic, reactions leading to the replacement of disintegrated parts or the accumulation of new tissue constituents. There are periods in the life of every organism which are especially characterized by processes of growth. Any comprehensive appreciation of nutrition in its broadest sense, therefore, calls for adequate recognition of the nature of the metabolism which distinguishes them.

A knowledge of the chemico-physiological peculiarities of growing tissues and organisms finds direct application to the practical problems of nutrition. What are the materials at the expense of which growing structures build? What is the equipment of a developing organism for the special metabolism which it experiences? What chemical and physiological adaptations contribute to a relative preponderance of constructive activities? To what extent does growth have unusual biochemical facilities at its disposal? Questions like these remain for the most part unanswered in the familiar literature on nutrition. They demand special researches which appear timely at present, in view of the profound interest awakened among physiologists in the study of metabolism. It is this which has encouraged the present series of experiments, the expenses of which have been met largely by a grant from the Carnegie Institution of Washington. The data, most of which have been obtained through a study of

embryonic material, will be presented for publication as contributions to the solution of specific problems.<sup>1</sup>

It is well established that the familiar disaccharides, maltose, sucrose and lactose, can be inverted to six-carbon sugars before or during the act of absorption from the alimentary tract. It is, further, understood that the hydrolysis is accomplished by enzymes which act specifically upon the individual disaccharides. Whether such agencies — maltase, sucrase, lactase — are always present in the intestinal secretions as well as in the secretory cells, and act within the lumen of the digestive tube upon the sugars; or whether the hydrolytic reactions occur mostly within the cells of the lining mucosa, is by no means so clearly demonstrated. The fact that some of the disaccharides referred to are rarely, if ever, introduced into the intestinal tract of certain species, gives a new interest to the significance of the occurrence of the enzymes which invert them, *i. e.*, to the question as to whether specific enzymes are developed only in response to the individual requirement. The problems regarding the adaptation of secretory glands are likewise here suggested. We shall see that the specific inverting enzymes furnished in the same species may vary at different periods in its life history, while the distribution also varies in the different groups of animals. It is too early, however, to offer an adequate explanation, not to say a teleological theory, for these facts.

The sucrose-inverting properties of the intestinal mucosa were the first of these enzyme activities to be pointed out, in 1871. We shall refer briefly to some of the literature indicating the distribution of the individual enzymes. The bibliography in the appendix includes many of the more significant contributions.

**Maltase.** — The most universal of the sugar-inverting enzymes, maltase, was first recognized in animals in the mucosa of the small intestine of the pig by Brown and Heron. Bourquelot soon thereafter clearly distinguished between maltase and sucrase, and showed that the activity of intestinal extracts is lost by filtration through porous clay. Since then maltase has been shown to have a very wide

<sup>1</sup> Brief notes regarding some of the studies have already been published. *Cf.* MENDEL: "The alimentary enzymes of the embryo," *This journal*, 1906, xv, p. xiii; "Chemical studies on growth," *British medical journal*, 1906, p. 1787 (December); "Embryo-chemical studies — the purine metabolism of the embryo," *This journal*, 1907, xix, p. xvii; *Journal of biological chemistry*, 1907, iii, p. xxxiv.

distribution in many tissues and secretions of animals as well as their blood serum. In extracts of the stomach alone was it missed by Fischer and Niebel. Maltase has been found in the intestinal juice of man (by Tubby and Manning, and others) and animals (by Röhmann, Mendel, and others); although, according to Röhmann and Nagano, the mucosa extracts are far more active than the secretion itself. Bierry has reported the occurrence of maltase in the embryonic intestines of sheep and cattle.

**Sucrase.**—It is interesting to note that Paschutin, the earliest investigator of the sucrose-inverting power of the intestinal mucosa, reported distinct differences in different species of animals. Whereas extracts prepared from the dog, pig, rabbit, and rat were active, those from the calf and sheep were not. Precisely similar observations regarding the latter animals were made much later by Fischer and Niebel.<sup>1</sup> The occurrence of sucrase as described above has been verified by the work of Brown and Heron, Bourquelot, Grünert, Röhmann, Mendel, and others, and extended to include the intestine of birds, the horse, both young and old (Fischer and Niebel), and the newly born infant (Miura). It is present in the intestinal secretion itself in the dog (Röhmann, Bastianelli, Mendel). In the intestinal extracts of the embryos of cattle and sheep sucrase has been missed by Bierry, and its occurrence regarded as doubtful by Krüger. It is not ordinarily present in the blood and tissues as are maltase and amylase.

**Lactase.**—With reference to the distribution of lactase the statements are less concordant; and about this enzyme much of the recent controversial literature regarding adaptation in glands has turned. Observations of Röhmann and Lappe indicated its absence in the mucosa of the small intestine of the cow, although it is present in that of the calf; and in the case of the dog they likewise found the enzyme present in greater abundance in *young* animals. Similar distinctions between young and old individuals of the same species have been claimed for the rabbit by Orbán and Weinland, for the guinea pig and cat by Plimmer. In birds and amphibia lactase has been uniformly missed. A survey of the numerous data now available in the literature which is compiled in the appendix indicates a preponderance of lactase in younger animals wherever comparative investigations are at hand. In the succus entericus itself lactase has

<sup>1</sup> Positive results with the succus entericus of sheep have been reported by PREGL. The experiments were conducted without adequate antiseptic precautions.

always been missed (Pregl, Mendel, Röhmann and Nagano, Frouin and Porcher). Its presence in the embryos of various species, as well as in the fæces of sucklings, has been reported.

Plimmer, who has published a careful study of the distribution of lactase since most of our experiments were completed, reaches these conclusions: "Neither frog nor fowl have lactase in their intestine, and we may conclude that animals lower than mammals do not possess this ferment." "Of the mammals the carnivora and omnivora have lactase present during the whole of their lives, but the herbivora only when they are young, with the exception of the rabbit." With reference to this animal, divergent views have been expressed. In the case of the adult pig, also, Plimmer's positive results differ from those reported by Portier. As an illustration of the influence of age, Plimmer found that lactase appears in the intestine of the rat between two days and twelve hours before birth. In the guinea pig it is lost already five weeks after birth. Bierry and Salazar found lactase in the fœtus of the cow at the fourth month; in that of the sheep at the end of the second month. It is interesting to note that Frouin and Thomas<sup>1</sup> have found the intestinal contents of the fœtus capable of splitting various glucosides. With regard to artificial adaptation by feeding lactose or milk to animals Plimmer concludes, contrary to Bainbridge and Weinland: "Neither the pancreas nor the intestine of animals can be made to adapt themselves to any particular diet."<sup>2</sup> Lactase is not present in the tissues in general, according to Portier.

Attention has been directed primarily to the occurrence of these enzymes in the embryonic intestine. The cells lining this portion of the alimentary tract are among the earliest to develop into a characteristic and distinct membrane with many of the structural features of the tissue subsequently formed. In the earliest stages they exist as a single layer of simple columnar epithelial cells lining the dorsal wall of the yolk sac, later becoming differentiated into the specialized varieties of cells characteristic of the future alimentary canal.

**Methods of investigation.**—Before describing the technic of our experiments certain factors contributing to the discrepancies between

<sup>1</sup> FROUIN and THOMAS: *Comptes rendus de la société de biologie*, 1907, lxii, p. 227.

<sup>2</sup> In the case of the salivary glands in relation to amylase, MENDEL and UNDERHILL have reached a similar conclusion. Cf. *Journal of biological chemistry*, 1907, iii, p. 135; also GARREY: *ibid.*, p. xl.

different investigators, or essential to any satisfactory procedure, may be pointed out. Some of these have been particularly emphasized by Plimmer (1907). Competent observers, for example, Röhmann and Nagano (1903), have maintained that the inverting enzymes may be unequally distributed in the different portions of the small intestine, not to mention the remainder of the alimentary tract. This suggests the desirability of examining the entire membrane, where possible. The succus entericus contains these enzymes in weak concentration, if at all. The epithelium of the intestine, however, is decidedly richer in active ferment. A reasonable time is required for any satisfactory extraction. The enzyme will not pass through a Berkfeldt filter; in view of the endocellular character of the inverting ferments it is advantageous to use strained extracts without attempting a perfect separation of the cellular débris. Obviously, with weak extracts a reasonable digestion period must be allowed in order to obtain convincing results.

To determine whether inversion has taken place, the osazone method cannot be depended upon where the degree of hydrolysis is slight.<sup>1</sup> We have employed this only as a confirmatory test. For lactose the changes in rotatory power in the solutions are not always satisfactory indications of digestion, owing to the small variations which even a considerable conversion of lactose ( $(\alpha)_D = +52.5^\circ$ ) to dextrose ( $(\alpha)_D = +52.7^\circ$ ) and galactose ( $(\alpha)_D = +80.3^\circ$ ) brings about. More striking in any case is the increased reducing power of the digested solutions, upon which we have especially depended. Like Plimmer, we prefer the use of the gravimetric Allihn method in place of volumetric copper processes. For filtering the cuprous precipitate Gooch crucibles are regularly employed in this laboratory, the copper being weighed as cupric oxide.

The tissues examined include the small intestine of the pig at embryonic, suckling, and adult ages; the unhatched chick and adult hen; and the newly born and suckling dog. The extracts were uniformly prepared by treating the finely comminuted tissue with about three volumes of 2 per cent sodium fluoride solution at room temperature during twenty-four hours. In the case of the embryo material the entire gut was used; in the adult tissues the mucosa was scraped off. The comparatively-long period of extraction was selected in order to make certain that the enzymes present would be removed. In the

<sup>1</sup> Cf. BIERRY: *Comptes rendus de la société de biologie*, 1905, lviii, p. 700; also PLIMMER: *Journal of physiology*, 1907, xxxv, p. 24.

earlier experiments the extracts were filtered through paper; later they were merely strained through cloth, following the procedure recommended in the most successful investigations on the inverting enzymes.

Most of the digestion trials, unless otherwise stated, were carried out as follows: 5 c.c. of the intestinal extracts were added to 100 c.c. of the sugar solutions, the latter of approximately 1 per cent strength and containing 2 per cent of sodium fluoride. Control experiments with boiled extract were simultaneously carried out in every case under precisely comparable conditions. The digestions were allowed to proceed at 38° C. during from forty-eight to seventy-two hours, a period which the experience of previous investigators has shown to be adequate to indicate digestive changes. The digesting mixtures were then heated to coagulate the proteins present, slightly concentrated on a water bath, filtered, the coagulum washed, and the filtrates made up to 100 c.c., *i. e.*, the original volume of the sugar solution used. The occurrence of inverting enzymes was determined by a comparison of the reducing power of the sugar solution before and at the end of the digestion. The losses incidental to the manipulation, retention of sugar in the coagulum, etc., can be estimated from the data obtained with the (boiled) control digestions.

The reducing power of the solutions was estimated in some early preliminary trials by Pavy's volumetric method.<sup>1</sup> Our conclusions are, however, based upon data obtained with the gravimetric Allihn method. The results are expressed in terms of cupric oxide obtained from an aliquot portion of the final solution. Typical protocols are summarized below.

**Intestinal mucosa of the adult pig.**—The extracts were prepared as already described. The letters (*a, b, c*, etc.) refer to extracts obtained from different animals (Table I).

In another series mucosa from an adult pig was extracted during *ten* days with 2 per cent sodium fluoride solution before the digestion trials were begun, in order to obtain any lactase present without fail.

<sup>1</sup> Sixty cubic centimetres of PAVY'S solution were employed in each estimation concordant duplicate titrations being made in every case. About 6 to 7 c.c. of the sugar solutions were necessary in the case of maltose and lactose. The variations in duplicate experiments were expected not to exceed 0.3 c.c. We do not regard the volumetric method as entirely satisfactory, and have always repeated the experiments, using the gravimetric Allihn process.

TABLE I.  
ADULT PIG.

Sugar used.	Source of the extract.	CuO from 25 c.c.		Results.
		Before digestion.	After digestion.	
Maltose	Pig <i>a</i>	gm. 0.1200	gm. 0.2160	} <i>Maltase</i> present in the intestine
	Pig <i>a</i> (boiled)	0.1200	0.1190	
Sucrose	Pig <i>a</i>	none	heavy reduction	} <i>Sucrase</i> present in the intestine
	Pig <i>b</i>	"	" "	
	Pig <i>c</i>	"	" "	
	Pig <i>a</i> (boiled)	"	no "	
	Pig <i>b</i> (boiled)	"	" "	
Lactose	Pig <i>c</i> (boiled)	"	" "	} <i>No lactase</i> found
	Pig <i>a</i>	0.3153	0.3049	
	Pig <i>a</i> (boiled)	0.3153	....	
	Pig <i>b</i>	0.3153	0.3144	
	Pig <i>c</i>	0.3153	0.3056	

In view of the positive results recorded by others for the existence of lactase in the gut of the adult pig, we have considered the possibility of a localization of the enzyme in some portion of the small intestine. To determine this we did not employ our usual method as described above, but substituted the procedure suggested by Plimmer. The entire length of this organ was divided into six parts, each of which was separately examined. The mucosa was scraped from six feet of gut from each section so as to furnish 75 gm. of

TABLE II.

Solution.	CuO from 25 c.c.	Solution required to reduce 60 c.c. Pavy's solution.
Original lactose solution . . .	gm. 0.406	c.c. 5.3
Digestion solution . . . . .	0.392	5.6
" " (boiled extract)	....	5.8

material. This was ground with clean sand and extracted twenty-four hours with 200 c.c. water and 2 c.c. toluene. After straining through cloth, boiled and unboiled portions (50 c.c.) of each extract were digested with 100 c.c. 4 per cent lactose solution, in the presence of toluene (2 c.c.). At the end of the experiment the solutions were made up to 200 c.c., and 20 c.c. of mercuric nitrate solution were added. After six to twelve hours' standing, 125 c.c. of clear filtrate were exactly neutralized with potassium hydroxide, the unboiled extract and its control experiment with boiled extract being kept at the same volume throughout. After filtration of the neutralized mixtures, 100 c.c. of the filtrate were treated with hydrogen sulphide gas. After removal of the mercuric sulphide, just sufficient copper sulphate solution was added to 75 c.c. of the solution to completely remove the excess of hydrogen sulphide. The volumes of the mixtures were then equalized with water and the reducing power determined by Allihn's gravimetric method in duplicate in aliquot portions (20 c.c.) of the final filtrates.<sup>1</sup> The experimental results are summarized in Table III.

TABLE III.  
LACTASE IN INTESTINE OF ADULT FIG.

Animal.	Portion of intestine used for extracts.	CuO obtained (averages).	Remarks.
Fig 2	Upper six feet, next to pylorus (boiled)	<sup>gm.</sup> 0.2828	Control expt.
	“ “ “ “	0.3804	Lactase present
	Second “ from “	0.5738	“ “
	Third “ “ “	0.3797	“ “
	Fourth “ “ “	0.3722	“ “
	Fifth “ “ “	0.3274	“ “
Sixth “ “ “	0.2928	Lactase?	

In view of these positive results, portions of small intestine just beyond the pylorus and just before the cœcum were used for comparison (see Table IV).

In these experiments the positive results were verified by the osazone method, phenyl-glucosazone crystals being obtained only in those

<sup>1</sup> Cf. PLIMMER: *Journal of physiology*, 1907, xxxv, p. 23.

cases where the increased reducing power also gave evidence of an inversion of the disaccharide.

TABLE IV.

Animal.	Portion of intestine used for extracts.	CuO obtained (averages).	Remarks.
Pig <i>x</i>	Upper part (boiled)	<sup>gm.</sup> 0.3157	Control expt.
	“ “	0.4050	Lactase present.
	Lower part (boiled)	0.3145	Control expt.
	“ “	0.3164	No lactase.
Pig <i>y</i>	Upper part (boiled)	0.3470	Control expt.
	“ “	0.4241	Lactase present.
	Lower part (boiled)	0.2763	Control expt.
	“ “	0.2806	Lactase ?

Inasmuch as lactase was regularly found missing in the lower portions of the small intestine, we are inclined to attribute the discrepancies between the results already reported by Portier, Weinland, and Plimmer regarding the presence of lactase in the adult pig's gut, to the use of different parts of the alimentary tube. We have no record regarding the exact location of the portions of intestine used in our earlier negative experiments. The subsequent experience makes it appear likely that the lower portions where lactase is usually missing were used in those cases. A similar explanation may apply to the experiments of the investigators quoted above.

**Intestinal mucosa of suckling pigs.** — An extract was prepared from the small intestine of a suckling pig seven weeks old. The analytical data are summarized in Table V, page 90, the analyses being made in duplicate. The presence of all three enzymes, maltase, sucrase, and lactase, was thus demonstrated in the young pig.

**Experiments with embryonic pigs.** — The experiments upon embryonic pigs were conducted with extracts from embryos of varying ages. In order to afford an approximate idea of the degree of development the specimens were measured in length of body, in which terms they are referred to in the protocols. The variations in size between the individuals of the same litter are not inconsiderable. We are indebted to our colleague, Professor Coe, for the following statement regarding the size of the pig embryo at various stages of development:

"We have not been able to find in the literature exact data concerning the relation between length of body and age of embryo in the pig, so that our estimate of the actual age of the embryos of any particular size has been influenced by the available data concerning the rate of development of the embryos of other mammals. Furthermore, it is well known that the size of the pig embryo at any given

TABLE V.  
SUCKLING PIG.

Sugar used.	Character of the extract.	CuO from 25 c.c.		Results.
		Before digestion.	After digestion	
Maltose	boiled	gm. 0.2678	0.4441	} <i>Maltase</i> found.
	unboiled	0.2678	0.2570	
Sucrose	boiled	no reduction	heavy reduction	} <i>Sucrase</i> found.
	unboiled	" "	no "	
Lactose	boiled	0.2692	0.3613	} <i>Lactase</i> found.
	unboiled	0.2692	0.2569	

age is subject to great variation, doubtless depending to some extent on the number of young in the litter and the size and general condition of the mother; and, moreover, the individual embryos of a single litter exhibit a remarkable variation in size. Within certain wide limits, however, it is possible to estimate the age of the embryo of any size from a measurement of its body length.

"The figures show the estimated age of the embryos used

Average length of body	Estimated age of embryo
mm.	days.
25	32
50	44
75	54
100	62
125	68
175	80
200	88
230	96
280	110
300	112

TABLE VI. (EMBRYO FIG.)

Sugar used.	Source of the extract.	Size of embryo.	CuO from 25 c.c. solution.		60 c.c. Pavy's solution require.		Results.	
			Before digestion.	After digestion.	Before digestion.	After digestion.		
Maltose	intestine	mm. 230	gm. 0.2429	gm. 0.2469	c.c. ..	c.c. ..	<i>Maltase?</i>	
	“ boiled	“	0.2429	0.2339	..	..		
	“	200	0.3215	0.3250	6.5	5.9	<i>Maltase?</i>	
	“ boiled	“	0.3215	0.3080	6.5	6.8		
	“	175	....	....	7.1	5.9	<i>Maltase</i>	
	“ boiled	“	....	....	7.1	7.1		
	“	120	0.3215	0.363	6.5	4.7	<i>Maltase</i>	
	“ boiled	“	0.3215	0.313	6.5	6.9		
	“	120	....	....	7.1	5.3	<i>Maltase</i>	
	“ boiled	“	....	....	7.1	7.2		
	“	120	0.2429	0.2704	..	..	<i>Maltase</i>	
	“ boiled	“	0.2429	0.2360	..	..		
	“	75	....	....	6.0	5.2	<i>Maltase</i>	
	“ boiled	“	....	....	6.0	6.0		
Maltose	“	50	0.3215	0.310	6.5	6.4	No maltase	
	“ boiled	“	....	....	6.5	6.5		
	liver	200	0.3215	0.331	6.5	6.1	No maltase?	
	“ boiled	“	0.3215	....	6.5	6.6		
	kidneys	120	0.3215	0.311	6.5	6.7	No maltase	
	“ boiled	“	....	....	6.5	6.9		
	Sucrose	intestine	280	none	none	..	..	No sucrase
		“ boiled	“	none	none	..	..	
“		175	none	none	..	..	No sucrase	
“ boiled		“	none	none	..	..		
“		120	none	none	..	..	No sucrase	
Lactose	“	“	none	none	..	..		
	intestine	230	0.2811	0.3674	..	..	<i>Lactase</i>	
	“ boiled	“	0.2811	0.2760	..	..		
	“	200	0.4060	0.4255	5.3	4.3	<i>Lactase</i>	
	“ boiled	“	0.4060	0.3950	5.3	5.4		
	“	175	....	....	5.4	3.6	<i>Lactase</i>	
	“ boiled	“	....	....	5.4	5.6		
	“	120	....	....	6.0	4.5	<i>Lactase</i>	
	“ boiled	“	....	....	6.0	6.1		
	“	120	0.2811	0.3621	..	..	<i>Lactase</i>	
	“ boiled	“	0.2811	0.2930	..	..		
	“	75	....	....	5.6	4.8	<i>Lactase</i>	
	“ boiled	“	....	....	5.6	5.4		
	“	50	0.4060	0.3990	5.3	5.4	No lactase	
“	“	0.4060	....	5.3	5.3			
liver	200	0.4060	0.4000	5.3	5.3	No lactase		
“	“	....	....	5.3	5.3			
kidneys	120	0.4060	0.3900	..	..	No lactase		

in the present experiments, the length of the body being the measurement from tip of snout to base of tail. The period of gestation is about one hundred and twelve days (sixteen weeks)."

Experiments were also made with extracts of tissues other than the intestine, in order to determine, to what extent, if at all, the specific inverting enzymes are early developed in various parts of the embryo. The protocols appear in Table VI, page 91.

The figures give evidence of the presence of lactase and maltase in the embryonic intestine of the pig at an early age, these enzymes being missed only in the smallest embryos (50 mm.) examined. The constant failure to find sucrase in the embryonic intestine is in striking contrast with the presence of the other inverting enzymes and with the conditions prevailing in the pig after birth. The negative results with embryo liver and kidney extracts suggest the specific localization of the inverting enzymes in the embryonic intestine.

**Intestinal mucosa of the suckling dog.** — In view of the differences just recorded in the occurrence of the inverting enzymes at various stages in the life history of the pig, we have made experiments with intestinal extracts from the puppy in order to complete the data already available in the literature. Quantitative variations in the content of lactase in young and old dogs have been noted by Röhmann and Lappe, and Orbán. We are unaware of comparative observations respecting the other inverting enzymes.

The entire small intestine was extracted during forty-eight hours. In each digestion trial 100 c.c. of the sugar solution + 5 c.c. extract were used. Duration of digestion = 3 days.

TABLE VII. (NEWLY BORN DOG.)

Source of extract.	Sugar used.	CuO from 25 c.c. solution.			Results.
		Before digestion.	After digestion.	In boiled control after digestion.	
2 puppies 6 hours after birth	Maltose	0.2431	0.3938	0.2428	<i>Maltase.</i>
	Sucrose	none	much	none	<i>Sucrase.</i>
	Lactose	0.3322	0.4360	0.3281	<i>Lactase.</i>
2 puppies 2 weeks old	Maltose	0.2428	0.3881	0.2460	<i>Maltase.</i>
	Sucrose	none	much	none	<i>Sucrase.</i>
	Lactose	0.3058	0.3936	0.3050	<i>Lactase.</i>

All three enzymes were found as in the suckling pig (Table V). Pautz and Vogel, Miura, and Weinland have described the occurrence of them in newly born infants also.

**Intestinal mucosa of the full-grown hen.** — The entire intestine, cleaned and comminuted immediately after death, was extracted thirty hours. In each trial 100 c.c. of the sugar solution + 10 c.c. extract were digested forty hours.

TABLE VIII.  
FULL GROWN HEN.

Sugar used.	CuO from 25 c.c. solution.			Results.
	Before digestion.	After digestion.	In boiled control after digestion.	
Maltose . .	gm. 0.1201	gm. 0.2093	gm. 0.1218	<i>Maltase.</i>
Sucrose . .	none	much	none	<i>Sucrase.</i>
Lactose . .	0.3153	0.3052	...	<i>No lactase.</i>

Corresponding with the observations of Weinland, Portier, and Plimmer on birds, no lactase was detected (Table VIII).

**Intestinal mucosa of the chick at birth.** — The entire intestine of nineteen chicks at birth was extracted in the usual way during seventy-two hours. In the digestion trials 100 c.c. of sugar solution + 5 c.c. extract were kept at 38° during forty-eight hours (Table IX).

TABLE IX.  
NEWLY BORN CHICK.

Sugar used.	CuO from 25 c.c. solution.			Results.
	Before digestion.	After digestion.	In boiled control after digestion.	
Sucrose . . . .	gm. none	gm. 0.1890	gm. none	<i>Sucrase.</i>
Lactose . . . .	0.2458	0.2422	0.2407	<i>No lactase.</i>

These results on the newly born chick, quite contrary to the findings in the embryonic pig and other newly born animals, give additional evidence of the biological individuality of this group of animals,

and warn against broad generalizations on the basis of observations made on single species. We are not aware that the presence of sucrase in embryo birds has previously been noted.

#### SUMMARY AND CONCLUSIONS.

The early appearance of inverting enzymes in the intestine of the embryo corresponds with the relatively early specialization and histological development of the portion of the alimentary tract here investigated. The alimentary proteolytic enzymes, like the special glands which elaborate them, come into evidence at a comparatively late period. Maltase is the most universally distributed of all inverting enzymes. In the embryo pig maltase and lactase are found in the intestine, while sucrase is missing. After birth all three enzymes are present. In the full-grown pig lactase is not regularly found in all portions of the small intestine. We attribute the differences reported by the various investigators (Plimmer, Weinland, Portier) to the probability of their having used different portions of the small intestine, since the experiments recorded by us show an unlike distribution of the enzyme in various regions of the intestine. In the newly born puppy all the enzymes are found.

In birds other conditions prevail. Lactase is not found at any period; sucrase, on the other hand, is uniformly present in the newly hatched chick and the adult hen. One might be inclined toward a teleological explanation for the absence of lactase from the intestine of non-mammalian animals, and similarly for the absence of sucrase from the embryos of the pig, sheep, and cattle. Such considerations apply with less force, however, to the subsequent formation of sucrase, or its embryonic occurrence in birds. For the present, the statistics of the occurrence of the alimentary inverting enzymes must await a more adequate interpretation with respect to their functional significance. At any rate, it is safe to conclude that the alimentary tract of the young mammal is, as a rule, even more adequately equipped to digest and utilize the sugar of the milk than are the adults of the same species.

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## CHEMICAL STUDIES ON GROWTH.—II. THE ENZYMES INVOLVED IN PURINE METABOLISM IN THE EMBRYO.<sup>1</sup>

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THE progress of physiological research has demonstrated that the metabolism of nucleoproteins is characterized by features which are specific and peculiar to these compounds, in distinction from the simple proteins. To this may be added the recognition that the chemical changes involved are facilitated by unique enzymes found widely distributed in both animal and plant tissues. The experimental studies up to the present time have been concerned almost entirely with the chemical transformations of the purine derivatives associated with the nucleoproteins.<sup>2</sup> Sufficient data have already been accumulated in such investigations to encourage the belief that diverse pathological conditions may be connected with disturbances in the metabolism of the purines and referable to alterations in the occurrence or activities of the enzymes just referred to. This view is fortified by the increasing importance which is being attributed to the *rôle* of enzymes in the nutritive exchanges of living organisms.

The purine metabolism of developing tissues has scarcely been investigated. It is generally conceded that a synthesis of purines is unusual, if it occurs at all, in adult mammals.<sup>3</sup> In growing animals and during embryonic development other conditions prevail.<sup>4</sup> The growth and metamorphosis of tissues during developmental periods

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> The recent extensive and almost complete *résumé* of the literature on this subject, by BLOCH: *Biochemisches Centralblatt*, 1906, v, pp. 521, 561, 817, 873, makes a detailed reference to individual papers unnecessary.

<sup>3</sup> The evidence on this point has been reviewed by MENDEL: *The Harvey lectures* 1905-6, p. 208; *Journal of the American Medical Association*, March 31, 1906.

<sup>4</sup> The experimental results of our study of purine synthesis in the embryo will be reported in a subsequent paper of this series.

are likely to be attended by an appropriate capacity to alter the available materials. The methods of study which have been applied to the adult consist in an investigation of the changes in the body as a whole, the end products of purine metabolism being considered; or in observations on the action of individual organs or tissue extracts upon nucleoproteins and purines. We have employed the latter method in the present research upon the equipment of the embryonic organism (of the pig) in respect to the purine-transforming enzymes.

Those transformations of the nucleoproteins in which the purines are involved are at present referable to several distinct types of enzymes which appear to be specific:

1. Nuclease — which liberates the purines from the nucleic acid molecule.
2. Deamidizing enzymes; adenase and guanase — which act upon amino-purines to liberate ammonia by hydrolysis, forming oxypurines (hypoxanthine and xanthine).
3. Xantho-oxidase — transforming these oxypurines to uric acid by oxidative change.
4. Uricolytic enzyme — effecting a destruction of uric acid, and forming allantoin as one of the probable intermediary products.

**Nuclease.** — Enzymes of this class unquestionably have a wide distribution. They have been described in animal and plant tissues.<sup>1</sup> Jones showed the close association of nucleases with nucleoproteins, and Sachs has given evidence that they are not identical with trypsin. In the blood nuclease appears to be missing. Numerous experiments upon autolysis have, as is well known, given evidence of the liberation of purines from their nucleic acid complexes. The specific character of the nucleases is, however, not proved thereby. With respect to the participation of nucleases in the possible degradative changes in embryonic tissues<sup>2</sup> little is known. Sachs has reported the presence of nuclease in the pancreas of newly born dogs.

<sup>1</sup> Cf. ARAKI: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 84; IWANOFF: *Ibid.*, 1903, xxxix, p. 31 (the term *nuclease* first used here); PLENGE: *Ibid.*, 1903, xxxix, p. 190; JONES: *Ibid.*, 1904, xli, p. 101, xlii, p. 35; NAKAYAMA: *Ibid.*, 1904, xli, p. 360; SCHITTENHELM: *Ibid.*, 1904, xlii, p. 257; SHIGA: *Ibid.*, 1904, xlii, p. 502 (yeast); SACHS: *Ibid.*, 1905, xlvi, p. 337 (nuclease and trypsin); ABDERHALDEN and SCHITTENHELM: *Ibid.*, 1906, xlvii, p. 452 (digestive juices); KIKKOJI: *Ibid.*, 1907, li, p. 201 (fungi); FOA: *Zentralblatt für Physiologie*, 1907, xxi, p. 24 (intestinal juice).

<sup>2</sup> The extent of these autolytic changes will be discussed in a subsequent paper.

**Adenase and guanase.**—The significance of these enzymes which transform adenine and guanine into hypoxanthine and xanthine, respectively, involves the question as to what purine groups occur preformed in the nucleic acid molecule. Although in the earlier studies the four familiar purines mentioned above were reported as decomposition (cleavage) products of nucleoprotein materials, the refinement of analytical methods has tended more and more to limit the yield to the two aminopurines, adenine and guanine. The very recent investigations of Jones and Austrian<sup>1</sup> give convincing evidence in this direction. They find that thymus nucleic acid fails to produce xanthine by the action of nuclease, and show that hydrolysis at high temperature (as conducted by most previous experimenters) gives results that are misleading.<sup>2</sup> The writer is quite in accord with the views of these authors; and the earlier experience of Levene<sup>3</sup> on the hydrolysis of fresh glands points in a similar direction. The appearance of hypoxanthine and xanthine under conditions favorable to enzyme activity is therefore referable to the aminopurine precursors. Such reactions have been abundantly demonstrated by the work of Schittenhelm,<sup>4</sup> Jones and Levene. Active enzyme preparations have already been obtained from extracts of various tissues. Upon one point the views are somewhat conflicting. Jones postulated the existence of distinct adenase and guanase enzymes because extracts of certain organs employed by him failed to convert guanine into xanthine, although adenine was readily transformed into hypoxanthine.<sup>5</sup> The contrary results of Schittenhelm<sup>6</sup> were shown by Jones<sup>7</sup> to be due to the differences in the animal species employed in their experiments. Thus, the spleen of the pig lacks guanase, which is abundant in the same organ of cattle. Without further review of the controversial discussion on this point<sup>8</sup> we may add that our own ex-

<sup>1</sup> JONES and AUSTRIAN: *Journal of biological chemistry*, 1907, iii, p. 1.

<sup>2</sup> The destruction of purines (with the probable formation of pyrimidines) under certain conditions of hydrolysis with strong acids has been shown quite lately by BURIAN: *Zeitschrift für physiologische Chemie*, 1907, li, p. 438.

<sup>3</sup> LEVENE: *This journal*, 1904-5, xii, p. 276.

<sup>4</sup> See their numerous papers in the *Zeitschrift für physiologische Chemie* since 1904.

<sup>5</sup> JONES and PARTRIDGE: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 343 (guanase); JONES and WINTERNITZ: *Ibid.*, 1905, xlv, p. 1 (adenase).

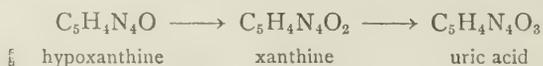
<sup>6</sup> SCHITTENHELM: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 152.

<sup>7</sup> JONES: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 84.

<sup>8</sup> Cf. JONES and AUSTRIAN: *Zeitschrift für physiologische Chemie*, xlvi, p. 110; SCHITTENHELM: *Ibid.*, 1906, xlviii, p. 571; SCHITTENHELM and SCHMID: *Ibid.*, 1906, l, p. 30.

periments corroborate the experience of Jones in showing the absence of guanase and the presence of adenase in the same species and organs. Both investigators have contributed valuable data to our knowledge of the distribution of the purine-transforming enzymes in various animals. The unlike equipment of different organisms in this respect is indicative of noteworthy variations in the purine metabolism of different species, and at once dispels the idea that these characteristic enzymatic properties are common to all active tissues.

**Xantho-oxidase.**—When glandular tissues are allowed to autolyze, or aminopurines are acted upon by tissue extracts or appropriate enzyme solutions in the absence of oxygen, the final purine products are oxypurines (hypoxanthine and xanthine). With an abundant supply of air, however, uric acid may be formed by some tissues. The facility with which this reaction can be accomplished depends in turn upon a specific enzyme to which Burian<sup>1</sup> has applied the name xantho-oxidase, xanthine presumably being the intermediary product which experiences oxidation, thus:



**Uricolytic enzyme.**—The destruction of uric acid by organ pulp and tissue extracts is likewise well established. Since uric acid is quite readily oxidized in pure solution in the presence of alkalis, some doubt has been expressed<sup>2</sup> as to how far, if at all, the destruction experimentally observed by previous investigators—notably Schittenhelm,<sup>3</sup> who used alkali solutions of uric acid in his experiments—is attributable to enzyme action rather than the destructive action of the alkali present in excess. We have given careful consideration to this criticism in relation to the present experimental work. In another place<sup>4</sup> one of us has already considered the action of alkalis upon

<sup>1</sup> BURIAN: *Zeitschrift für physiologische Chemie*, 1906, xliii, p. 497. This mode of formation of uric acid had, of course, long been recognized. Cf. SPITZER: *Archiv für die gesammte Physiologie*, 1899, lxxvi, p. 192; WIENER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1899, xlii, p. 373; SCHITTENHELM: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 251, etc.

<sup>2</sup> Cf. AUSTIN: *Journal of medical research*, 1906, xv, p. 309; 1907, xvi, p. 71.

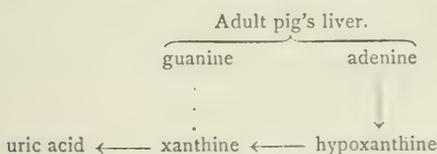
<sup>3</sup> SCHITTENHELM: *Zeitschrift für physiologische Chemie*, 1904, xliii, p. 239; 1905, xlv, p. 121 (term "uricolytic enzyme" introduced); p. 161 (enzyme isolated) Cf. also WIECHOWSKI and WIENER: *Beiträge zur chemischen Physiologie*, 1907, ix, p. 247.

<sup>4</sup> MITCHELL: *Journal of biological chemistry*, 1907, iii, p. 145.

uric acid, and found, in conformity with previous observations, that the compound can easily be destroyed in alkaline solution. It was shown, however, that this does not occur when protein is present, since alkali-protein is relatively inert. Furthermore, the quantities of alkali used to dissolve the uric acid in all our experiments quoted below was minimal in excess of that necessary to form soluble alkali urate. The evidence in favor of a specific uricolytic enzyme is strengthened by the fact which we have repeatedly verified, namely, that uric acid is not destroyed by extracts of certain embryonic organs, although comparable extracts from adult tissues readily destroy it. Finally, the destructive action is lost when the extracts are boiled,—an observation quite in accord with the behavior of enzymes. Levene and Beatty<sup>1</sup> have observed uricolysis by tissue extracts in acid solution.

EXPERIMENTAL PART.

Preliminary experiments were undertaken to ascertain whether any of the familiar enzyme transformations of the purines can be demonstrated in embryonic tissues. The livers of embryo pigs were employed for this purpose. In the adult liver of this species Jones and Austrian<sup>1</sup> noted adenase and xantho-oxidase, while guanase was missing. Their results may be indicated by the following graphic scheme:



in which the enzyme reactions observed are indicated by an arrow, those missing by the interrupted line. The purines present in fresh embryo livers and autolyzed embryo livers were at first isolated by the procedure suggested by Levene,<sup>3</sup> after hydrolysis of the materials with acid. This method is at best unsatisfactory and only approximate from a quantitative standpoint.

**Method.**<sup>4</sup>—The finely pulped livers were mixed with two or three parts of water, toluene was added in abundance, and the mixture

<sup>1</sup> LEVENE and BEATTY: Proceedings Society for Experimental Biology and Medicine, 1907, iv, p. 109.

<sup>2</sup> JONES and AUSTRIAN: Zeitschrift für physiologische Chemie, 1906, xlvi, p. 120.

<sup>3</sup> LEVENE: This journal, 1904-5, xii, p. 276.

<sup>4</sup> The experiments upon the fresh and autolyzed organs were carried out by Mr. C. S. LEAVENWORTH.

subjected to autolysis, with occasional shaking, in a closed vessel at 38°. After a period varying from three weeks to two months the mixtures, which showed decided change in the direction of solution, were treated with sulphuric acid to the extent of about 5 per cent of the entire solution. The mixture was heated until the solution failed to give a biuret reaction. This usually required about forty-two hours. After cooling the mixture was filtered, the residue was thoroughly washed with water, and the united solutions precipitated with Hopkins' mercuric sulphate solution. The mercury precipitate was decomposed in the manner followed by Levene.<sup>1</sup> The guanine was removed from the resulting solution of the purine bases by precipitation with ammonia. The guanine was converted into the hydrochloride, weighed and analyzed in this form. The remaining purine bases were separated by means of an ammoniacal solution of silver chloride, and the silver-purine precipitate was decomposed according to the well-known directions of Krüger and Salomon.<sup>2</sup> Hypoxanthine was identified as the nitrate, adenine as picrate (m.p. 276°-283° C.), and xanthine was searched for in the residues. Fresh tissues were decomposed directly with 5 per cent sulphuric acid and analyzed in precisely similar manner.

**Adult pig liver.**—For purposes of comparison estimations of the purines in 200 gm. (64 gm. of dry substance) of the liver of a full-grown pig were made in fresh tissue and after seventeen days' autolysis (Table I). These autolyses were subsequent to those reported below

TABLE I.

Found.	Fresh liver.	Autolyzed liver.
	gm.	gm.
Guanine hydrochloride	0.181 <sup>1</sup>	0.135
Adenine picrate	0.228 <sup>2</sup>	none
Hypoxanthine nitrate	0.058	0.046
	found	calculated
	<sup>1</sup> C <sub>5</sub> H <sub>5</sub> N <sub>5</sub> O · HCl · 2H <sub>2</sub> O : H <sub>2</sub> O = 15.9% ; 16.1%	
	after drying at 105° : N = 37.24 ; 37.3	
	<sup>2</sup> m. p. 281° C.	

<sup>1</sup> LEVENE: This journal, 1904-5, xii, p. 278.

<sup>2</sup> KRÜGER and SALOMON: Zeitschrift für physiologische Chemie, 1899, xxvi, p. 373.

on embryo livers, and the precipitation of the purines was made by the copper sulphate-sodium bisulphite method in this experiment instead of by the procedure just described.

Averages calculated for 100 gm. of dry tissue substance, in grams :

TABLE II.

Purine bases.	Before autolysis.	After autolysis.
Guanine . . . . .	0.191	0.142
Adenine . . . . .	0.125	none
Hypoxanthine . . . .	0.057	0.045

**Embryo pig liver.**—The livers were obtained from embryos of 50 mm., 75 mm., and 100 mm. in length. The variations in the size of pig embryos and their relation to age have been discussed in an earlier communication.<sup>1</sup> The average weight of the embryo pig liver at various ages is estimated from our data as follows :

TABLE III.

Age of embryo.	Length of embryo from crown of head to base of tail.	Weight of liver.	Water content of liver.
days	mm.	gm.	per cent
44	50	1.4	80
54	75	2.4	79
62	100	4.0	80
Adult pig	....	....	68

The isolated purines were identified by analysis in cases where the quantities obtained permitted. The analytical data are summarized in Table IV, page 104.

The figures presented plainly indicate the preponderance of guanine and adenine in the native nucleoproteins of the embryo liver. The small quantities of hypoxanthine isolated from the fresh livers may well be attributed to incipient enzymatic changes already begun, to purine material accumulated free in the liver tissue (as occurs in adult muscle), or to the action of the reagents in the method of decomposition employed.<sup>2</sup> The changes effected by autolysis are char-

<sup>1</sup> MENDEL and MITCHELL: This journal, 1907, xx, p. 90.

<sup>2</sup> Cf. the criticisms of JONES and AUSTRIAN: Journal of biological chemistry, 1907, iii, p. 1.



acteristic in each case, and correspond with the experience with adult pig's liver. The content of guanine is scarcely altered, especially when the unsatisfactory adaptation of the methods used for *quantitative* work is taken into account. Corresponding with this is the failure to find xanthine. We have united the residues obtained from 1127 livers (488 fresh and 639 after autolysis) in the treatment of the silver-purine precipitates by the Krüger-Salomon method, under the conditions in which xanthine is usually separated by this process, without obtaining any of this base whatever from the combined traces of insoluble material. Guanase is therefore undoubtedly missing in the liver of the pig embryo, — a finding which we have verified by other methods to be described later. This peculiarity of the tissues of the pig thus fully corroborates the results already described by Jones and Winternitz<sup>1</sup> for the adult pig liver, as does our observation on the occurrence of adenase. The existence of this enzyme is plainly indicated in the experiments recorded above, by the marked disappearance of adenine with simultaneous increase in the content of hypoxanthine. The divergent results obtained by Levene<sup>2</sup> are undoubtedly attributable to the use of the glands of some other species than the pig.

*The specific characteristics of the purine-transforming enzymes of the liver are thus already demonstrable in the embryonic organ. Nuclease and adenase are present in the liver at a very early stage in embryonic life.*

#### EXPERIMENTS WITH EXTRACTS OF EMBRYONIC TISSUES.

In extension of the observations already recorded we have searched for nuclease, adenase, guanase, xantho-oxidase, and uricolytic enzymes in some of the tissues of the embryo pig at various stages of development. The materials used were always quite fresh, never more than two or three hours elapsing until the experiments were begun. As a rule, the embryo livers were used in one set of experiments, while the remaining viscera, including lungs, heart, alimentary tract, spleen, pancreas, and kidneys, were used together in a comparable series. The material was always comminuted in a small hashing-machine, and treated with five times its weight of water, with an abundance of toluene. The extraction was continued with frequent stirring at room temperature for periods varying, in different experiments, from

<sup>1</sup> JONES and WINTERNITZ: *Zeitschrift für physiologische Chemie*, 1905, xliv, p. 8.

<sup>2</sup> LEVENE: *This journal*, 1904-5, xii, p. 295.

twenty-four to forty-eight hours. The mixture was allowed to settle, the supernatant fluid strained through cloth, and then filtered quite clear by suction through paper pulp. The digestions were conducted at about 38°, an excess of toluene always being present.

**Analytical methods.** — The purine compounds were separated by the copper sulphate-sodium bisulphite process of Krüger and Schmid.<sup>1</sup> Reprecipitation was usually adopted after decomposition of the purine precipitate with hydrogen sulphide. We have found the use of aluminium acetate to effect clear solutions in filtration very helpful.<sup>2</sup> The quantitative estimations of total purines were conducted according to the directions of Krüger and Schmid.<sup>3</sup> When purine compounds were precipitated by the silver method, the separation of the individual constituents followed the process of Krüger and Salomon.<sup>4</sup> Guanine was weighed as the hydrochloride or the free base; adenine as the picrate; hypoxanthine, xanthine, and uric acid were separated and identified in various ways indicated below:

**Nuclease.** —

*Experiment 1.* — The livers of 150 mm. pig embryos were extracted twenty-four hours at room temperature. The filtered extract was analyzed in 500 c.c. portions for free purine-base nitrogen (by double precipitation with the Krüger-Schmid reagents with subsequent Kjeldahl-N estimation), as follows:

(a) Fresh extract . . . . .	0.036 gm. purine N.
(b) Extract after three days' autolysis with air drawn through . . . . .	0.046 " " "

*Experiment 2.* — The same experiment was repeated with an extract of livers from 175 mm. embryos, using 500 c.c. portions.

(a) Fresh extract . . . . .	0.038 gm. purine N.
(b) Extract after three days' autolysis with air drawn through . . . . .	0.052 " " "

*Experiment 3.* — In this experiment an extract of *adult* pig's liver was used in 500 c.c. portions.

(a <sub>1</sub> ) Fresh extract . . . . .	0.026 gm. purine N.
(a <sub>2</sub> ) " " . . . . .	0.026 " " "
(b <sub>1</sub> ) Extract after three days' autolysis with air drawn through . . . . .	0.016 " " "
(b <sub>2</sub> ) " " " . . . . .	0.017 " " "

<sup>1</sup> Cf. HOPPE SEYLER-THIERFELDER: *Handbuch der chemischen Analyse*, p. 435.

<sup>2</sup> Cf. SCHITTENHELM: *Archiv für klinische Medizin*, lxxxi, p. 429, 1904.

<sup>3</sup> KRÜGER and SCHMID: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 1.

<sup>4</sup> KRÜGER and SALOMON: *Zeitschrift für physiologische Chemie*, 1898, xxvi, p. 350.

Experiments 1 and 2 show an appreciable increase in the free purines of the embryo liver extracts, and thus *indicate the presence of nuclease*. Despite the current of air steadily maintained through the solutions, there is no loss of free purines, — quite in contrast with what was observed with extracts of *adult* livers in Experiment 3. This speaks against the presence of uricolytic activity in the *embryo* extracts, — an inference which is justified by other experiments specially directed towards this question. Calculated on the basis of xanthine (36 per cent N), the quantity of free purines contained in 500 c.c. of the embryonic extracts was about 0.1 gm.

**Guanase.** — It will be recalled that the failure to find guanase in the liver of the pig was one of the observations which led Jones<sup>1</sup> and his co-workers to postulate the distinction between the two deamidizing enzymes, adenase and guanase. Schittenhelm<sup>2</sup> admits that the action of extracts of the liver of the adult pig upon guanine is feeble at most, although he is apparently unwilling to allow the specific distinction made by Jones. Our experience with adult livers of pigs corresponds with that of Jones.

*For example:* One kilo of the finely comminuted liver of an adult pig was mixed with 2.5 litres of water to which liberal amounts of chloroform and toluene were added, and allowed to autolyze during thirty days at room temperature. The mixture was then heated to boiling, treated with a little acetic acid to effect complete coagulation, and filtered. In the filtrate purine bodies were precipitated by copper sulphate and sodium bisulphite and liberated in the usual way with sodium sulphide. The material was then evaporated to dryness, and the residue was thoroughly extracted with 1 per cent ammonia. The portion thus remaining undissolved after further washing with dilute ammonia was taken up in sodium hydroxide, filtered, and treated with a slight excess of acetic acid. The precipitate thus formed weighed (after drying at 105° C.) 0.38 gm. It was shown to be guanine by the fact that by proper recrystallization 0.35 gm. of pure guanine hydrochloride was prepared from it. In the ammoniacal filtrate from the separation of guanine, examination for other purine bases disclosed *no adenine*, mere *traces of hypoxanthine*, and an amount of xanthine nitrate equivalent of 0.35 gm. of *xanthine*.

This result confirms the presence of adenase and oxidase in the liver of the pig, but indicates the absence of guanase. Identical results were obtained in the above-mentioned experiments by Jones and Winternitz.

<sup>1</sup> JONES and WINTERNITZ: Zeitschrift für physiologische Chemie, 1905, xlv, p. 8; JONES and AUSTRIAN: *Ibid.*, 1906, xlviii, p. 120.

<sup>2</sup> SCHITTENHELM: Zeitschrift für physiologische Chemie, 1905, xlv, p. 368.

The data already reported in the preliminary autolysis experiments pointed strongly to the absence of guanase in the liver of the embryo pig, since the guanine content of the autolyzing organs was unaltered after several weeks' digestion in the absence of air. In what follows it will be shown how extracts of embryonic organs act towards guanine added directly to them.

*Experiment 4. Extracts of embryo livers.* — Livers of 150 mm. and 175 mm. embryos were extracted at room temperature during thirty-six hours. Portions of 500 c.c. of filtered extract were allowed to digest (*a*) with and (*b*) without added guanine four days without air supply. The purines were then isolated by a modification of the Krüger-Salomon process. The copper-free filtrates were evaporated to dryness and the residues extracted twenty-four hours with 2 per cent ammonia. After repeated washing with ammonia the undissolved material was dissolved in sodium hydrate solution and guanine precipitated with acetic acid. It was converted into the beautifully crystalline hydrochloride and weighed as such.

(*a*) 500 c.c. extract + 0.19 gm. guanine hydrochloride<sup>1</sup> (converted into the free base by repeated evaporation with water and alcohol and then dissolved in a little dilute sodium hydrate solution).

(*b*) 500 c.c. extract alone.

Guanine was recovered:

(*a*) 0.18 gm. guanine hydrochloride.

(*b*) 0.04 " " "

The salt from (*a*) dried at 105° C. was analyzed:

N = 37.1 per cent; calculated 37.3 per cent.

Making allowance for the guanine present in the extract itself, at least 0.14 gm. of the hydrochloride was recovered. We attribute the discrepancy to the inevitable losses incidental to the analytical process used rather than to any enzyme activity. The filtrates from the guanine were examined by the silver precipitation method for other purines. No adenine picrate could be separated, but hypoxanthine was obtained as the nitrate. Adenase was therefore undoubtedly present.

*Experiment 5.* — Filtered liver extracts from embryos 120 mm. to 200 mm. in length were used. The digestions, without air supply, lasted one week. The guanine was weighed as the free base.

(*a*) 500 c.c. extract + 0.30 gm. guanine hydrochloride (representing 0.225 gm. guanine) dissolved in 15 c.c. 2 per cent sodium hydrate solution. Recovered: 0.26 gm. guanine.

<sup>1</sup> Prepared from an acid hydrolysis of pancreatic glands.

(b) 1000 c.c. extract alone. Recovered: 0.10 gm. guanine.

The guanine was converted into the hydrochloride, dried at 105°C. and analyzed:

(a) 37.1 % N; calculated: 37.3 % N.

(b) 37.5 % N.

Correcting for the guanine content of the extract  $\left(\frac{0.10}{2} = 0.05 \text{ gm.}\right)$

the quantity recovered was 0.21 gm., or 93 per cent.

*Experiment 6. Extracts of other viscera.*—The viscera (except the livers) of 150 mm. and 200 mm. embryos were extracted 36 hours. Filtered extracts were employed, without air supply, and digested during 6 days.

(a) 700 c.c. extract + 0.21 gm. guanine (dissolved in sodium hydrate solution).

(b) 700 c.c. extract alone.

The purines in *b* were negligible in amount. From *a* 0.198 gm. of xanthine was obtained. It was converted into the nitrate, then the free base, dried at 105°C. and analyzed: N = 36.7 %; calculated: N = 36.8 %.

The conversion to xanthine was therefore practically quantitative. *Guanase is present.*

*Experiment 7. Control experiment.*—To a boiled and strained extract of similar viscera 0.15 gm. of guanine (dissolved in sodium hydroxide solution) was added and a digestion of six days' duration carried out.

The purine bases were separated according to the method of Krüger and Schittenhelm,<sup>1</sup> which is more delicate for small quantities of xanthine. No xanthine was obtained. 0.136 gm. of guanine was recovered and converted into the characteristic hydrochloride.

*Experiment 8.*—The viscera of 120 mm. embryos were extracted forty-eight hours, and the extract digested five days with 0.23 gm. guanine hydrochloride. Analysis as in Experiment 7. Recovered:

0.04 gm. guanine hydrochloride.

0.157 " xanthine.

The xanthine was converted into the nitrate and then the free base.

N = 36.6 %; calculated: 36.8 %.

*Experiment 9.*—An extract of 230 mm. embryos was digested four days with 0.36 gm. guanine hydrochloride (= 0.27 gm. guanine). Recovered: 0.26 gm. xanthine.

*Under comparable conditions, therefore, the presence of guanase in embryonic tissues other than the liver has thus been demonstrated.*

<sup>1</sup> KRÜGER and SCHITTENHELM: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 153.

**Adenase.** — The adult liver, spleen, and pancreas<sup>1</sup> of the pig have been shown to contain an enzyme capable of converting adenine into hypoxanthine. Our preliminary autolysis experiments indicated the existence of adenase in the embryo liver also. We have made further studies regarding its distribution.

*Experiment 10. Extracts of embryo livers.* — An extract of the livers of 200 mm. embryos was allowed to undergo autolysis during five days. One litre of the filtered extract, equivalent to about 200 gm. tissue, yielded 0.075 gm. guanine.

*No adenine could be separated with picric acid.* Hypoxanthine was isolated as the silver nitrate salt, and converted into the characteristic nitrate. Recovered: 0.12 gm. Dried at 110° C. : N = 34.7 per cent; calculated: 35.2 per cent.

*Experiment 11.* — The preformed bases present in the fresh livers of 200–230 mm. embryos were separated after hydrolysis in a steam sterilizer during sixteen hours with 5 per cent sulphuric acid. 200 gm. of tissue, equivalent to the quantities used in some of the extraction experiments, were decomposed. The purine bases recovered were:

0.09 gm. guanine hydrochloride.

0.16 gm. adenine picrate, m. p. 280° C.

These data may be compared with some of those following.

*Experiment 12.* — Livers of 150–200 mm. embryos were extracted sixty hours. The filtered extracts were digested without air supply during five days.

(a) 1000 c.c. of the extract alone.

(b) 500 c.c. extract + 0.19 gm. adenine sulphate.<sup>2</sup>

The products isolated were:

	(a)	(b)
guanine	0.18 gm.	0.11 gm.
hypoxanthine nitrate	} 0.12 (N = 34.6%)	} 0.17 (N = 35.7%)
calculated		
$C_5H_4N_4O \cdot HNO_3$ N = 35.2%		

Making allowance for 0.06 gm.  $\left(\frac{0.12}{2}\right)$  hypoxanthine nitrate obtainable from 500 c.c. of extract alone, 0.11 gm. hypoxanthine nitrate was obtained from 0.19 gm. adenine sulphate added. The guanine remained practically unchanged, as has repeatedly been found in the preceding section.

*Experiment 13.* — Extracts from livers of embryos of 120–230 mm. length.

(a) 1000 c.c. of the extract alone.

(b) 500 c.c. extract + 0.10 gm. adenine<sup>3</sup> (dissolved in sodium hydrate solution).

<sup>1</sup> Cf. JONES and WINTERNITZ: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 1 (liver); SCHITTENHELM: *Ibid.*, 1905, xlvi, p. 354 (spleen); JONES and PARTRIDGE: *Ibid.*, 1904, xlii, p. 343 (pancreas).

<sup>2</sup> Prepared by Dr. Mitchell from thymus glands.

<sup>3</sup> Furnished by C. F. BOEHRINGER und SÖHNE, Mannheim.

Recovered :	(a)	(b)
Hypoxanthine nitrate	0.11 gm.	0.21 gm.
Nitrogen in $C_5H_4N_4O \cdot HNO_3 \cdot H_2O$ (calculated)	32.4%	32.1%
	(N = 32.3%)	

*Experiment 14.* — 500 c.c. of a filtered extract of livers of 150 mm. embryos were digested with 0.17 gm. adenine (dissolved in sodium hydrate solution).

Recovered : 0.25 gm. hypoxanthine nitrate.

*Experiment 15. Extracts of other viscera.* — From 500 c.c. of an extract of the viscera (excluding the liver) of 230 mm. embryos, digested five days at  $38^\circ$ , no adenine could be separated. 0.04 gm. hypoxanthine nitrate was isolated.

A similar experiment with 120 mm. embryos furnished 0.05 gm. hypoxanthine nitrate and no adenine.

*Adenase is therefore present in both the liver and other viscera of the embryo pig.*

**Oxidase.** — The uric-acid-forming xantho-oxidase has been demonstrated in the liver of the adult pig, but missed in the pancreas, spleen, and lung.<sup>1</sup> The experiments must, of course, be conducted in the presence of an abundant oxygen supply. In our experiments an air current was driven through the solutions during about ten hours of each day. Uric acid was separated by the method of Krüger and Schmid.<sup>2</sup>

*Experiment 17. Extracts of embryo livers.* — A filtered extract was prepared from the livers of 175 mm. and 230 mm. embryos. 800 c.c. were digested three days, a current of air being driven through the solution during thirty hours of that period. No uric acid or xanthine could be isolated. 0.07 gm. guanine and 0.096 gm. hypoxanthine nitrate were isolated. The latter was identified air-dry as  $C_5H_4N_4O \cdot HNO_3 \cdot H_2O$ . N = 32.1 per cent; calculated : 32.3 per cent.

*Experiment 18.* — Livers of 175–230 mm. embryos were extracted twenty-four hours. The filtered extract was then used for digestions, air being driven through during twenty hours of the two and one-half days' experiment.

(a) 1000 c.c. extract alone.

(b) 500 c.c. extract + 0.20 gm. adenine (dissolved in sodium hydrate solution).

No uric acid or xanthine was obtained from either solution.

(a) yielded a small quantity of guanine and 0.108 gm. hypoxanthine nitrate.

<sup>1</sup> Cf. JONES and AUSTRIAN : Zeitschrift für physiologische Chemie, 1906, xlviii, p. 120 (liver, pancreas); SCHITTENHELM : *Ibid.*, 1905, xlvi, p. 354 (lung, spleen).

<sup>2</sup> KRÜGER and SCHMID : Zeitschrift für physiologische Chemie, 1905, xlv, p. 1.

(b) yielded a small amount of guanine, 0.275 gm. adenine picrate (equivalent to 0.102 gm. adenine) and 0.208 gm. hypoxanthine nitrate. The failure to effect a complete change of the adenine to hypoxanthine was doubtless due to the brief period of digestion. Over 95 per cent of the adenine which disappeared was recovered as hypoxanthine.

*Experiment 19.* — A comparable extract (500 c.c.) from the livers of 150 mm. and 200 mm. embryos was digested with 0.17 gm. adenine (dissolved in sodium hydroxide solution) during five days, air being driven through nine hours each day. No adenine or uric acid were found at the end of the experiment. 87 per cent of the adenine added was recovered as hypoxanthine (0.299 gm. hypoxanthine nitrate, air dry, N = 32.0 per cent; calculated: 32.3 per cent).

*Experiment 20.* — 500 c.c. of a boiled and strained extract of the livers of 200 mm. embryos + 0.16 gm. adenine (dissolved in sodium hydrate solution) were digested five days, air being driven through during thirty hours of that time. A very small xanthine fraction was isolated, together with a trace of guanine and 0.35 gm. adenine picrate (m. p. 281° C.) representing 0.13 gm. adenine, *i. e.*, over 80 per cent of the added adenine could be isolated, despite the difficulties of exact quantitative recovery.

*Xantho-oxidase is therefore missing in the liver of the embryo pig during a considerable period of embryonic life.* Adenase is present and adenine can be converted almost quantitatively into hypoxanthine. *Uric acid is not formed, even in the presence of oxygen.* That the failure to detect uric acid is not due to simultaneous uricolytic action, by which any of the compound formed is at once destroyed, will be shown in experiments recorded below.

*Experiment 21. Extracts of other viscera.* — 1000 c.c. of a filtered extract from 230 mm. embryos + 0.38 gm. guanine hydrochloride (representing 0.285 gm. guanine) were digested four days, air being driven through during nine hours of each day. *No uric acid was found.* The xanthine fraction (weighing 0.278 gm.) yielded 0.256 gm. xanthine nitrate which was converted into the free base and analyzed. N = 36.5 per cent; calculated: 36.8 per cent.

*Experiment 22.* — 1000 c.c. of a filtered extract of viscera of 150–200 mm. embryos + 0.4 gm. guanine hydrochloride (equivalent to 0.30 gm. guanine) were digested (with air) during four days. Analysis yielded:

No uric acid.  
0.07 gm. guanine.  
0.22 gm. xanthine.

The latter was purified by conversion into nitrate, and then the free base, and analyzed:

N = 36.4 per cent; calculated: 36.8 per cent.

*Guanase is present in the extracts of the embryonic viscera other than the liver. Uric acid could not be detected.*

The changes in the equipment of enzymes incidental to growth are shown by comparison of the data furnished by a suckling pig about seven weeks old.

*Experiment 23. Extracts of the liver of the suckling pig.*—(a) 500 c.c. filtered extract + 0.24 gm. adenine sulphate. (b) 1000 c.c. extract alone. The digestion proceeded five days, air being driven through at intervals during forty hours.

Recovered.	(a)	(b)
Uric acid . . . . .	0.050 gm.	0.007 gm.
N in other purines . . . . .	0.037 gm.	0.024 gm.

The uric acid was crystalline and gave the murexide test. The formation of this compound and the *marked disappearance of the other purine bases* give evidence of the occurrence of xantho-oxidase in the liver in early adult life.

*Experiment 24. Extract of the liver of the adult pig.*—1000 c.c. of a filtered extract were digested with 0.25 gm. adenine (dissolved in sodium hydrate solution) with an air current during three days. There were recovered: No uric acid or adenine, a trace of xanthine, a small amount of guanine, and only 0.03 gm. hypoxanthine nitrate. The disappearance of the adenine and the failure to find a comparable quantity of hypoxanthine or xanthine indicate the presence of a xantho-oxidase. The absence of uric acid is attributable to the well-known uricolytic action of extracts of adult pig's liver. The experiment was carried out under conditions strictly comparable with those pertaining in the case of the embryo organs; the differences are thus emphasized by contrast.

**Uricolytic enzyme.**—This enzyme has been demonstrated in the liver of the adult pig by Schittenhelm<sup>1</sup> and Wiener,<sup>2</sup> and in the kidney by Pfeiffer.<sup>3</sup> *We have failed to detect its occurrence in extracts of embryonic tissues.* In searching for the enzyme tissue extracts have been digested with uric acid (dissolved in a minimal excess of sodium hydrate solution) at 38°, with a current of air driven through the mixtures, following the procedure adopted by Schittenhelm.<sup>4</sup> The

<sup>1</sup> SCHITTENHELM: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 354.

<sup>2</sup> WIENER: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xlii, p. 375.

<sup>3</sup> PFEIFFER: Beiträge zur chemischen Physiologie und Pathologie, 1906, vii, p. 463.

<sup>4</sup> SCHITTENHELM: Zeitschrift für physiologische Chemie, 1905, xlv, p. 121.

criticisms of this method, with the possibility of destruction of uric acid by the reagents employed, have already been considered on page 100.<sup>1</sup>

*Extracts of embryo livers.* — 500 c.c. of filtered extract + 0.15 gm. of uric acid were used in each case. The details are summarized below:

Experiment.	Size of embryos.	Uric acid recovered.		Remarks
		gm.	per cent.	
26	mm. 200	0.139	92	} Four days' digestion N = 33 %; calcu- lated: 33.3 %
27	175	0.137	91	
28	175	0.122	81	
29	175	0.127	85	

At later stages the liver contains a uricolytic enzyme.

*Experiment 30. Extracts of the liver of suckling and adult pigs.* — 500 c.c. filtered extract of the liver of *suckling pigs* about two months old + 0.17 gm. uric acid were dissolved and digested in the usual way during four days. 0.095 gm. uric acid = 55 per cent only was recovered.

*Experiment 31.* — 500 c.c. filtered extract of *adult liver* + 0.15 gm. uric acid were employed in a series of trials.

Conditions.	Uric acid recovered.	
	gm.	per cent.
(a) Digestion one day	0.048	32
(b) " three days	0.021	14
(c) " four "	none	..
(d) " " "	none	..
(e) Control, boiled extract, } digestion three days }	0.139	92

The control experiment (31e) here reported clearly indicates that the uricolysis observed in the other trials is not attributable to the reagents used. At precisely what age the uricolytic power is developed, *i. e.*, whether prior to birth or later, was not determined. The oldest embryos (200 mm. = 88 days) obtained by us showed entire absence of the enzyme at that age. It is of interest to note that

<sup>1</sup> Cf. also MITCHELL: *Journal of biological chemistry*, 1907, iii, p. 145.

Wiechowski<sup>1</sup> has lately identified allantoin among the products of uricolysis by tissues.

*Extracts of other embryonic viscera.*—The uricolytic enzyme could not be detected in the extracts of these viscera.

Experiment.	Size of the embryo. mm.	Uric acid	
		added. gm.	recovered. gm.
33	large	0.15	0.13
34	200	0.25	0.235 (N = 33.4%)

### RÉSUMÉ.

The nucleic acid of the liver of the embryo pig probably contains only two purine complexes: adenine and guanine.

The liver is capable of undergoing autolytic changes at an early age. Nucleases are present which liberate purine bases from the nucleic acid complexes.

The liver of the embryo pig contains adenase, even in its early stage of development, but no guanase. In this respect it shows the specific character of the liver of the adult animal.

An extract of embryo viscera, other than the liver, readily gives indication of the presence of guanase at an early age.

The unlike distribution of these two enzyme reactions under comparable conditions of experiment give further evidence in favor of the existence of two distinct and specific deamidizing enzymes.

It has not been possible to demonstrate the formation of uric acid from preformed or added purine bases (adenine or hypoxanthine) by extracts of embryonic tissues. The preliminary oxidative transformation of hypoxanthine to xanthine is likewise doubtful. Xanthoxidase is not present in the embryo visceral organs of the pig; it is found, however, in the livers of the full-grown and suckling animals of the same species.<sup>2</sup> The latter readily form uric acid from purines under suitable conditions.

The uricolytic enzyme has not been found in extracts of embryo pig tissues under conditions in which it is readily identified in the adult organs. The enzyme appears either shortly before or after birth. These observations speak in favor of the *specific* uricolytic

<sup>1</sup> WIECHOWSKI: Beiträge zur chemischen Physiologie, 1907, ix, p. 295.

<sup>2</sup> Comparable data obtained from a study of the embryo were reported by JONES and AUSTRIAN at a meeting of the American Society of Biological Chemists in Washington, May, 1907; Journal of biological chemistry, 1907, iii, p. 227.

power of tissue extracts, and indicate that the destruction of uric acid in such solutions is not solely due to the alkaline reaction, etc., of the digesting medium.

The tardy appearance of the oxidative and katabolic enzymes concerned in the transformation of the purines is suggestive as a characteristic of growing, synthetic organisms.

## CHEMICAL STUDIES ON GROWTH.—III. THE OCCURRENCE OF GLYCOGEN IN THE EMBRYO PIG.<sup>1</sup>

BY LAFAYETTE B. MENDEL AND CHARLES S. LEAVENWORTH.

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IN summarizing the results of his earliest classic investigations upon the occurrence of glycogen in embryonic tissues, Claude Bernard wrote, in 1859: "Il est permis de penser que chez le foetus cette matière glycogène a un rôle important à remplir dans le développement organique."<sup>2</sup> Since then a peculiar importance has been assigned by many writers to the existence of glycogen in growing organs and tissues. This view has been supported by the apparent richness of these parts in the distinctive storage carbohydrate. Indeed, it has been assumed quite generally that the embryonic structures have a proportionately larger supply of glycogen than is the case in the fully grown organism. The presence of glycogen in rapidly growing neoplasms, especially such as partake of an embryonic character, lends additional support to the suggestion that this carbohydrate plays a characteristic part in the phenomena of growth. In his "Specielle Physiologie des Embryo," Preyer expressed this belief, in 1885, in these words: "Vielmehr ist es wahrscheinlich, dass alles junge Protoplasma Glykogen bildet und dass Leukocyten es dahin bringen, wo nicht schon die noch nicht differenzirten embryonalen Zellen es erzeugt haben. . . . Jedenfalls gehört diese stickstofffreie Verbindung zu denen, welche im Fötus selbst entweder ihrer ganzen Menge nach oder zum grossen Theil entstehen und vergehen. Das Vogelei enthält

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> BERNARD, CLAUDE: *Journal de la physiologie de l'homme et des animaux*, 1859, ii, p. 336. Many years later BERNARD wrote: "From all these facts and all these examples, especially from those which relate to the embryo, we should conclude that the amylaceous material, in animals as well as in vegetables, is indispensable to the histological synthesis, and that its presence in certain tissues is related to the evolution of the cellular elements which compose them." (*Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux*, 1879, ii, p. 80).

kein Glykogen, der ganz junge Embryo gibt aber bereits die charakteristische Jod-Reaktion" (p. 272). In 1898 Schäfer wrote in his *Text Book of Physiology*: "In the embryo glycogen is much more widely distributed and occurs in much larger proportion than after birth, especially in the developing muscles. At this time the liver may contain very little" (i, p. 918).

So long as the purely biochemical relationships of glycogen were not clearly appreciated, it was inevitable that its exact chemical function in the embryonic cells and tissues should be obscure. An extreme view deprived glycogen of any histogenetic significance whatever; it was looked upon as "a by-product in the splitting of complex albumens to build up the tissues," — a product of disintegrative metabolism. In distinct contrast with such an opinion, glycogen has been classed as an "anaplastic" rather than "kataplastic" substance, stored like fats and used functionally in the growth of the organism.<sup>1</sup> One may think of the substance in this connection either as a stored material, furnishing energy as it is required, or as a compound, entering into the protoplasm in some more intimate way as an integral part of the differentiating bioplasm. Creighton<sup>2</sup> has ventured the following interpretation: "Briefly expressed, the formative property of glycogen is analogous to or parallel with that of hæmoglobin. . . . I have been led to the conclusion that glycogen plays the part of a carrier to the tissues, that it contributes somewhat to the building up without losing its own molecular identity, that it is present at the formation of tissues, and employed therein without becoming part of them, and that it acts thus, in some cases as the precursor or deputy of hæmoglobin, and until such time as the vascularity of the part is sufficiently advanced; in other cases as the substitute of hæmoglobin from first to last, — in those tissues which are built up in whole or in part without direct access of blood. . . . The placental and amniotic glycogen I believe to have a different meaning from that of the embryo itself, to mean, in fact, the throwing down of a formative medium no longer needed."

The older literature on glycogen has lately been reviewed with considerable care.<sup>3</sup> A few careful microscopic observations have

<sup>1</sup> PREYER: *Specielle Physiologie des Embryo*, 1885, p. 272.

<sup>2</sup> CREIGHTON: *Microscopic researches on the formative property of glycogen*, 1896, i, pp. 8-9.

<sup>3</sup> Cf. CREMER: *Ergebnisse der Physiologie*, 1902, i, 1, p. 803; PFLÜGER: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 1; RICHTER'S *Dictionnaire de*

more lately been made on pig embryos of 15 and 50 mm. by Gierke.<sup>1</sup> They confirm the finding that not all embryonic cells contain glycogen. The main store of embryonic glycogen is noted microscopically to be in the muscle and cartilage. The liver is devoid of glycogen, as is the nervous system. Lubarsch<sup>2</sup> has also arrived at similar conclusions. He points out especially the fact that the organ particularly prominent in relation to glycogen in extra-uterine life, namely, the liver, is glycogen-free in embryo pigs at an age when many other tissues show the carbohydrate in abundance. A review of the literature on the glycogen content of embryonic organs leads Lubarsch to summarize in part:

1. The glycogen content varies with the age and species of the embryo.
2. Most epidermal epithelia, striated muscles and cartilages uniformly contain glycogen.
3. Glycogen is uniformly absent in the blood, spleen, connective tissues, bones, and nervous substance in all embryonic stages.

Gierke and Lubarsch agree in emphasizing the fact that it is not always the most rapidly developing cells which tend to be richest in glycogen.

Statements like those just quoted are based in large part upon microscopic observations made on tissues stained by the iodine method. The micro-chemical test for glycogen is, however, one which demands most careful application in order to give a conclusive answer regarding the exact distribution and identification of glycogen; and its limitations are even more obvious when the quantitative aspects of the subject are under consideration.<sup>3</sup> A corroborative study of the distribution of the carbohydrate by adequate quantitative methods seems almost imperative before any far-reaching conclusions can be reached. Some of the earlier investigators, notably Claude Bernard, actually isolated glycogen from the tissues. The inadequacy of the earlier extraction methods has repeatedly been pointed out by Pflüger,<sup>4</sup> who has found that small quantities may easily be overlooked unless the tissues are completely dissolved in a hot solution of potas-

physiologie: article *Glycogène*; also GIERKE: ZIEGLER'S Beiträge zur pathologischen Anatomie, 1905, xxxvii, p. 502 (literature on microchemical studies on glycogen).

<sup>1</sup> GIERKE: *Loc. cit.*, p. 512.

<sup>2</sup> LUBARSCH: Archiv für pathologische Anatomie, 1906, clxxxiii, p. 192.

<sup>3</sup> Cf. also the criticism of ADAMOFF: Zeitschrift für Biologie, 1905, xlvi, p. 283.

<sup>4</sup> PFLÜGER: Archiv für die gesammte Physiologie, 1904, cii, p. 305.

sium hydrate, and glycogen precipitated by means of alcohol according to his method.

Under Leon Asher's guidance, Adamoff<sup>1</sup> has investigated the quantitative occurrence of glycogen in embryonic life, using the Pflüger method. The food factor in connection with the nutritive condition of the mother, — a feature which has repeatedly been discussed in relation to mammalian embryos — was eliminated in one series of experiments by using chicks, which develop independent of a variable food supply. Newly born rabbits were also analyzed, as well as the livers of human fœtuses prematurely delivered. It was found that chicks which have just left the shell contain, at most, insignificant traces of glycogen. After the fourth day, when they have utilized the ingested egg residues and are fed, the content of glycogen increases. Newly born rabbits contain about 0.4 per cent of glycogen; compared with well-nourished, full-grown dogs, this quantity is rather small. The glycogen in the human liver at a late fœtal period does not exceed that of an adult unfed animal in quantity. From these facts the conclusion is reached that abundance of glycogen is not a characteristic feature of embryonic organs. Energy of growth and content of glycogen bear no direct relation to each other.

The paucity of glycogen in certain specific embryonic tissues was pointed out by Claude Bernard.<sup>2</sup> He failed to find it in the liver in early embryonic life, and notes (p. 335) that this exception deserves special mention, because of the peculiar rôle which the organ plays in glycogen metabolism in adult life. Not until later in the development of the embryo, toward the middle of intra-uterine life, did Bernard find evidence of a glycogenic function in the liver. Paschutin<sup>3</sup> is reported to have missed glycogen in the liver of the embryo calf before the period when a length of 400 mm. is reached. In contrast with the small quantities of glycogen found in the liver of the newly born animal by previous investigators, Demant<sup>4</sup> reported as much as 11 per cent in the livers of newly born dogs. He stated that the quantity speedily diminishes immediately after birth. The analyses were made by Brücke's method.

<sup>1</sup> ADAMOFF: *Zeitschrift für Biologie*, 1905, xlvi, p. 281.

<sup>2</sup> BERNARD, CLAUDE: *Journal de la physiologie*, 1859, ii, p. 326.

<sup>3</sup> PASCHUTIN, quoted by DEMANT: *Zeitschrift für physiologische Chemie*, 1887, xi, p. 142.

<sup>4</sup> DEMANT: *Loc. cit.* The conflicting data of others are there reviewed.

Pflüger<sup>1</sup> has reinvestigated the occurrence of glycogen in the embryo liver by means of his improved method. He reports the finding of glycogen in all the embryo livers examined. These were obtained from the cow, sheep, and pig, the smallest embryo (calf) measuring 130 mm. The proof was in some cases qualitative only, owing to the mere traces of glycogen present. Pflüger's view is best summarized in his own words: "Die wichtigste Thatsachenreihe besteht darin, dass die embryonale Leber ganz ausserordentlich grosse Schwankungen im Glykogengehalt darbietet, so dass bald reichliche Mengen, bald nur Spuren gefunden werden, während die Muskeln stets beträchtliche Vorräthe an Glykogen darbieten. Es ist genau dasselbe Verhältniss, wie ich es bei dem erwachsenen Pferde gefunden habe, und hier liegt die Ursache unzweifelhaft in dem Ernährungszustande des Thieres. Die Leber ist eine grosse Vorrathskammer, welche bei guter Nahrungszufuhr Massen von Glykogen aufspeichert, welche zur Zeit des Mangels mit grösster Freigebigkeit an die verschiedenen Organe des Körpers abgegeben werden. Es ist etwas ganz Gewöhnliches, dass die Muskeln der geschlachteten Pferde 2% Glykogen, die Leber aber nur einige Zehntel Procent enthält. Es liegt deshalb nahe, anzunehmen, dass es sich beim Embryo genau ebenso verhält, weil auch seine Leber die uneigennützig Vorrathskammer zur Ernährung des übrigen Körpers darstellt."<sup>2</sup> One must not draw the conclusion that in the earlier embryonic period the liver has a behavior different from that of later life, after birth. The low content of glycogen in the foetal liver is ascribed by Pflüger to the deficient feeding of the mother animal; and he suggests that the animals killed for sale which furnish the embryos for analytical study often fail to receive food before being slaughtered.

Since our own experiments were completed<sup>3</sup> Lochhead and Cramer<sup>4</sup> have made a report on the glycogen metabolism of the foetus. They completed quantitative estimations of the carbohydrate in the placenta, foetal liver, and remainder of the foetal body in an age series of pregnant rabbits from the eighteenth day to the end of gestation. "The results show that at the earlier dates the maternal placenta possesses

<sup>1</sup> PFLÜGER: *Archiv für die gesammte Physiologie*, 1903, xcv, p. 19; 1904, cii, p. 305.

<sup>2</sup> *Ibid.*, 1904, cii, p. 307.

<sup>3</sup> Some of the results were briefly presented to the section in physiology, British Medical Association, at Toronto, 1906. Cf. MENDEL: *British medical journal*, 1906, p. 1787 (December).

<sup>4</sup> LOCHHEAD and CRAMER: *Journal of physiology*, 1907, xxxv, p. xi.

a considerable store of glycogen, its percentage amount being quite comparable with that of a normal adult liver. It remains constant until the twenty-fourth day, and then a distinct and progressive decrease occurs each day till the end of gestation. . . . The results present a striking relation to those obtained from the analyses of foetal livers. In the latter, though definite amounts of glycogen are found at dates when histological examination proves negative, the percentage is very low up to the twenty-fifth day, when for the first time it rises above that of the rest of the foetal bodies. This, therefore, represents the date at which the liver assumes its adult glycogenic function. It will be noted that the time coincides accurately with the beginning of the decrease in the placental glycogen. After this date the percentage of liver glycogen rapidly increases, but even at birth it falls short of the average found in the liver of an adult rabbit.

“Neither the placental store of glycogen nor that of the foetal liver is affected by feeding the animals on a diet rich in carbohydrates.

“There is a marked correspondence between the foetal weight and the percentage amount of foetal glycogen, suggesting that a close relation exists between the growth of the foetus and the glycogen metabolism.”

#### EXPERIMENTAL PART.

We have made a large number of analyses in order to determine the distribution of glycogen in the embryo pig, especially at the earlier ages. The method followed was that described by Pflüger<sup>1</sup> for embryonic tissue. We have found the long heating with alkali, recommended by him, necessary to avoid the difficulties presented by this kind of tissue. The precautions more recently suggested<sup>2</sup> were also carefully observed. The measurements of the embryos given below were taken from the crown of the head to the root of the tail. Embryos larger than 230 mm. are rarely available here. The material was brought directly from the slaughter-house and examined at once. (See Table I, page 123.)

**Glycogen in the entire embryo.**—Schöndorff<sup>3</sup> found variations of 0.7–3.7 per cent in the glycogen content of adult dogs. Several factors may contribute to the comparatively low figures reported

<sup>1</sup> PFLÜGER: *Archiv für die gesammte physiologie*, 1904, cii, pp. 307–308.

<sup>2</sup> *Ibid.*, 1906, cxi, p. 307.

<sup>3</sup> SCHÖNDORFF: *Archiv für die gesammte Physiologie*, 1903, xcix, p. 191.

here for the embryo pig, foremost among which is the higher water content of the embryonic tissues. There is a progressive increase in the glycogen, but the quantities in any event give no occasion to assume a special richness in this carbohydrate.

TABLE I.

Size.	Estimated age.	Number of embryos used.	Total weight.	Glycogen content.	
				Total tissue.	Per embryo.
mm.	days.		gm.	per cent.	gm.
50	44	12	104	0.25	0.022
75	54	4	115	0.37	0.107
100	62	2	97	0.33	0.163
137	71	1	120	0.50	0.601
150	74	1	136	0.56	0.771
187	84	1	267	0.57	1.521
212	90	1	484	0.69	3.339

**Glycogen in the embryo liver.**— In the livers of embryos ranging in size from 85 to 230 mm. we have uniformly failed to detect glycogen, *i. e.*, to obtain any reduction of alkaline copper solutions with the hydrolysis products obtained by Pflüger's method, using about 100 gm. of liver in each trial. No color test was applied; nor have we attempted any microchemical examination. It is noteworthy that Pflüger<sup>1</sup> obtained so little glycogen from 62 gm. of liver from six pig embryos of an age of two months that the iodine test alone was positive. Our failure to demonstrate glycogen in any measurable amount in the embryo liver can scarcely be due to any loss occasioned by long heating with alkali; for in control trials, carried out with embryo muscle heated during long and shorter periods, no loss was observed by prolonged heating when undue concentration of the alkali was carefully avoided.

**Glycogen in embryo nervous tissues.**— Claude Bernard failed to obtain glycogen from the nervous or osseous tissues of the embryo, and it is not ordinarily included among the constituents of adult nervous tissues. Contrary to the opinion of Gierke and of Lubarsch,

<sup>1</sup> PFLÜGER: Archiv für die gesammte Physiologie, 1903, xcv, p. 20.

Gage<sup>1</sup> has recently expressed the conclusion that at some period in the growth of nervous tissue glycogen plays as important a part as in the other tissues of the body. "A series of pig embryos from 7 to 70 mm. were sectioned, and plentiful glycogen was found in the cells of the dorsal ganglia in embryos from 7 to 20 mm. In the 20 mm. embryo glycogen was also present in the developing nerve trunks, and in older embryos it nearly or quite disappeared from the ganglion cells, but became exceedingly abundant in the nerve trunks."

Our observations were made upon the brain substance of embryos 85 to 230 mm. in length. The material from the different sizes was examined in lots of about 100 gm. each, with *uniformly negative results*.

**Glycogen in the embryo muscle and skeletal structure.** — The heads were removed and the bodies completely eviscerated. The brain and liver — both deficient in glycogen — were thereby eliminated. Corresponding with this fact the percentage of glycogen in the bulk of the residual tissue structures appears somewhat larger than that of the *entire* body reported above.

TABLE II.

Size.	Number of embryos used.	Weight of tissues used.	Glycogen found.
mm.		gm.	per cent.
62	12	95	0.46
75	7	115	0.48
75	8	102	0.47
100	4	115	0.46
125	2	92	0.73
150	1	87	0.33
150	1	70	0.44
175	1	132	0.42
190	1	197	0.58
215	1	297	1.11
215	1	297	1.10

<sup>1</sup> GAGE: Science, 1906, xxiv, September 7.

These data indicate a fairly uniform content of glycogen in the embryonic muscle mass at definite ages when the inevitable variations in such material are borne in mind. That it belongs primarily to the non-osseous structures is shown in the experiments which follow.

**Glycogen in embryo skeletal structures.** — Creighton<sup>1</sup> has enunciated the principle that "those cartilages which are destined to continue as such through life, including the septum varium, the ensiform, etc., are, generally speaking, without glycogen in the fœtus. . . . On the other hand, the embryonic cartilages in which one finds uniformly a large amount of glycogen are those destined to an early transformation or absorption. . . . In the cartilages of the long bones, vertebræ, etc., glycogen first appears exactly in the spots that afterwards become 'centres of ossification.'"

We have isolated the skeletal structures in small embryos as carefully as the texture of the growing osseous tissues will allow, and analyzed them. The skull was not included.

TABLE III.

Size.	Number of embryos used.	Weight of material used.	Glycogen.
mm. 100	6	gm. 58	per cent. none
125	5	110	0.10
150	3	98	0.54
175	2	104	0.40
212	1	65	0.40

**Conclusions.** — The preceding experiments cannot be interpreted as giving evidence that a large glycogen content is a characteristic of embryonic structures or developing tissues; for the tissues, examined by adequate methods, show no unusual richness in this carbohydrate. The distribution is not markedly different from what pertains in the adult animal, except that the liver does not assume its glycogen-storing function early, at least in the pig. This conclusion seems as reasonable, in the absence of direct contradictory evidence, as to attribute the uniformly noted poverty in hepatic glycogen entirely to the defi-

<sup>1</sup> CREIGHTON: Microscopic researches on the formative property of glycogen, 1896, i, pp. 80, 81.

cient nutrition of the maternal animal. The experience of Lochhead and Cramer also favors this view. The metabolism of glycogen in the embryo is doubtless comparable with its rôle in the nutrition of the adult; and it seems unnecessary to postulate any special "formative" property to account for its presence. Glycogen may thus be regarded simply as a store of nutrient energy rather than as a peculiar mark of histogenesis.

# THE INFLUENCE OF ELECTROLYTES AND OF CERTAIN OTHER CONDITIONS ON THE OSMOTIC PRESSURE OF COLLOIDAL SOLUTIONS.

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## I. INTRODUCTORY AND CRITICAL.

IT is somewhat remarkable that so much disagreement has existed and still appears to exist in reference to the osmotic properties of colloidal solutions. Graham<sup>1</sup> in 1861 made the explicit assertion, "colloid bodies in general are highly osmotic." The fact is indeed easily observed that colloidal solutions placed in a dialyser absorb water often rapidly and in considerable quantity, — an observation implying the existence of osmotic pressure, and the one on which Graham's statement was based. It is true that in his time no definite theory or even conception of an osmotic pressure existed, and that the phenomenon of endosmose was destined to remain only imperfectly intelligible until many years later, when the work of Pfeffer and van t' Hoff laid the foundation for an exact theory of solution. Yet the early recognized fact that colloids have a "high endosmotic equivalent" in reality implies the existence of a certain osmotic pressure. It is actually easier to demonstrate such pressure in colloidal than in crystalloidal solutions, on account of the ease with which membranes impermeable to colloids may be prepared. Why, then, are there still to be found investigators who deny that substances in true colloidal solution exert osmotic pressure?

The chief reason for this state of uncertainty has been the difficulty of freeing colloids from adhering crystalloid substances. The purest preparations of proteids always contain a certain proportion of inorganic ash; and colloids, in general, absorb and retain with great tenacity foreign substances present in their solutions. The observed pressures or depressions of freezing-point are usually very

<sup>1</sup> GRAHAM: *Philosophical transactions*, 1861, cli, p. 183.

low and most readily accounted for as due, not to the colloid, but to the associated impurities. In a recent investigation, conducted with great care, Reid<sup>1</sup> found that proteids purified by repeated crystallization, re-solution, and re-crystallization yielded in many cases solutions having no appreciable osmotic pressure. The natural inference—if these results are accepted as correct—is that the pressures observed in determinations conducted without such precautions are due, not to the colloid, but to the impurities present in solution with it; and such, in fact, has been Reid's inference from his experiments.

The observed experimental results may, however, be attributed to other causes; such treatment, as Moore and Roaf<sup>2</sup> have urged in criticism of Reid's conclusions, certainly alters the state of aggregation of the proteid, and conceivably in such a manner as to give solutions of too low pressure for determination by mercury manometers. The experiments of Moore and Roaf, and my own which follow, show clearly that the osmotic pressure of a colloid changes with its state of aggregation; and I shall give below measurements in which a great reduction of the normal pressure is produced by addition of small quantities of salt to a proteid solution. This reduction of pressure can, of course, be carried much further without producing precipitation; and a state of aggregation gained in this way tends to be retained, by a kind of hysteresis, after the removal of the conditions that produced it; so that a more or less permanent condition of reduced osmotic pressure, due to treatment with concentrated salt solutions, is certainly a possibility.

The other chief series of facts frequently adduced as showing that colloids have either a negligibly small osmotic pressure or none at all, consist in demonstrations that the freezing and boiling points of pure solutions, as determined with the Beckmann apparatus, are practically identical with those of the pure solvent; this, however, as both Starling<sup>3</sup> and Moore and Roaf<sup>4</sup> have pointed out, merely indicates that the osmotic pressure cannot exceed a certain upper limit; the method is, in fact, incapable of demonstrating with any certainty pressures of less than the very considerable value of 40 to 50 mm. Hg. Hence such determinations merely show that the osmotic

<sup>1</sup> REID: *Journal of physiology*, 1904, xxxi, p. 438.

<sup>2</sup> MOORE and ROAF: *Biochemical journal*, 1906, ii, p. 34.

<sup>3</sup> STARLING: *Journal of physiology*, 1899, xxiv, p. 316.

<sup>4</sup> MOORE and ROAF: *Loc. cit.*

pressure of such solutions is small, and have no bearing in the question of its existence or non-existence.

Moore and Roaf have urged another objection to the frequently employed method of reasoning which ascribes directly observed osmotic pressures to the salts or other crystalloids associated with the colloid. They point out — what ought to have been self-evident — that such substances cannot possibly produce permanent pressures in osmometers having parchment paper or gelatine membranes, since all crystalloids readily traverse such membranes. This simple reasoning seems perfectly conclusive; substances giving permanent osmotic pressures with such membranes must in fact be in colloidal solution; it is at least impossible that they should be the simple crystalloidal substances to which the pressures have usually been ascribed.

What part, then, if any, do the crystalloid substances present in colloidal solutions play in the production of the observed osmotic pressures? Moore and Roaf propound the following hypothesis at some length: that the associated crystalloid substances, while not directly responsible for the pressure, are nevertheless necessary to its production; in brief, that in some unexplained manner they *confer* on the colloid the power of exerting osmotic pressure, which in their absence would be altogether lacking. It is difficult to see the justification of any such view; the fact that the presence of certain "crystalloid" substances (the term uniformly employed by Moore and Roaf) has its influence on the osmotic pressure of the solution by no means implies that such substances *condition* the existence of the pressure, and that with their complete removal the osmotic properties of the solution would vanish. Prolonged dialysis of albumin solutions certainly does not produce such a proportionate change in osmotic pressure as the acceptance of such a view would lead us to expect, although by far the greater portion of the crystalloids may be thus removed. The view seems to imply a belief in the existence of a fundamental difference between the colloidal and the crystalloidal states of matter, the latter alone being capable of independently maintaining a state of solution and of exerting osmotic pressure. But it will, I think, be generally agreed that the progress of research affords less and less reason for drawing the sharp distinction between colloids and crystalloids that it was once the custom to observe — possibly in pursuance of the example and precept of Graham himself, with his comparison of the two states to two different worlds of

material, differing as do organized and unorganized beings.<sup>1</sup> There are at least a few colloidal solutions that can be prepared free from admixture with crystalloids, namely, the metal hydrosols, prepared by fine subdivision of the pure metal in pure water. The hydrosols of platinum and gold are, in fact, notoriously the more stable the greater the freedom from electrolytes,<sup>2</sup> and if such solutions are stable, they certainly exert osmotic pressure. It seems at times to be forgotten that the very conception of solution implies the existence of such pressure, since the primary criterion of this condition is a persistent and self-conserving homogeneity of composition. This signifies that inequalities of concentration, however occasioned, are equalized by diffusion; hence, if a partition permeable to solvent but not to solute is interposed between two unequally concentrated portions of a solution, the diffusing particles of solute necessarily exert pressure on this partition. To imply otherwise would be to deny that the property of diffusion (and hence of the dependent homogeneity of composition) is essential to a solution. In view of such considerations, the inference seems clearly unavoidable that since colloids are capable of independent solution, they must be capable also of independently exerting osmotic pressure; and we may conclude that though the presence of electrolytes may alter the pressure, they are not necessary to its production.

The readiness to attribute the state of solution of colloids to the action of electrolytes seems to have had its origin in the conception, due largely to the work of Hardy,<sup>3</sup> that the stability of such solutions depends on the charge carried by the colloid particles. The function of the electrolyte is to provide the particles with the necessary charge. Ions precipitate colloids by neutralizing the charge; the increased surface tension of the particles due to removal of the potential difference between particle and medium is, according to Bredig,<sup>4</sup> the direct cause of the separation of the colloid from solution. On the whole, subsequent investigation has led much support to these ideas; the electrical condition of the colloid is undoubtedly a factor in the stability of its solutions. Billitzer,<sup>5</sup> however, has

<sup>1</sup> GRAHAM: *Loc. cit.*, p. 220.

<sup>2</sup> Cf. BREDIG: *Anorganische Fermente*, Leipzig, 1901, p. 28.

<sup>3</sup> HARDY: *Proceedings of the Royal Society*, 1900, lxvi, p. 110; *Journal of physiology*, 1899, xxiv, p. 288.

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

<sup>5</sup> Cf. BILLITZER: *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften*, Wien, math.-naturw. Klasse, 1904, cxiii, 7te Hft., p. 1159.

shown that isoelectric point and precipitation point need not necessarily coincide; and Pauli<sup>1</sup> has shown that in stable colloidal solutions of certain proteids after prolonged dialysis the particles appear uncharged. The charge, therefore, cannot be considered the essential feature in solutions of colloids any more than in those of crystalloids, where both electrolytes and non-electrolytes form equally stable solutions.

Hence also the osmotic pressure of colloidal solutions cannot be explained on the basis of the mutual repulsion of charged particles. It is possible that a small portion of the pressure may be thus accounted for, as pointed out by both Bredig<sup>2</sup> and Billitzer;<sup>3</sup> but the greater portion (apart from that due to free crystalloid impurities) must be due to some other condition, — possibly, as both the above authors suggest, to the adsorption of the solvent at the greatly developed surface of the colloid. Nevertheless, we still find attempts to explain the pressure as dependent on the electrified condition of the particles. Thus, in the important and interesting recent paper of Duclaux,<sup>4</sup> describing his extensive experiments with inorganic colloids, we find the state of solution or stability of the colloid to be ascribed to the so-called “active part” of the colloidal complex, that is, the portion of electrolyte associated with the colloidal substance (as  $\text{Fe}_2\text{Cl}_6$  with ferric hydroxide) which by its ionization imparts the charge to the particle and so conditions its electrical properties. He finds a decrease in stability with decrease in this “active” proportion of electrolyte, — *i. e.*; addition of less salt is then required for precipitation, — and correlatively a decrease in the maximum osmotic pressure which the solution can then exert (or its maximum concentration) without solidifying. Thus a ferric hydroxide solution in which the colloid has an approximate composition  $\text{Fe}_2\text{Cl}_6 + 40 \text{Fe}_2\text{O}_3$  can be brought to a concentration corresponding to an osmotic pressure of nearly 3 metres of water (*ca.* 220 mm. Hg) before solidifying; while, after removal of the “active part” to a proportion of 1  $\text{Fe}_2\text{Cl}_6 + 800 \text{Fe}_2\text{O}_3$ , this “limiting osmotic pressure” proved only 10 cm. of water; the corresponding concentrations were 15 per cent and 2 per cent. The

<sup>1</sup> PAULI: Beiträge zur chemischen Physiologie und Pathologie, 1906, vii, p. 531.  
Cf. also: Naturwissenschaftliche Rundschau, 1906, xxi, p. 3.

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

<sup>3</sup> BILLITZER: Zeitschrift für physikalische Chemie, 1903, xlv., p. 307.

<sup>4</sup> DUCLAUX: Journal de chimie physique, 1907, v, p. 29.

decrease of both stability and maximum osmotic pressure with removal of the electrolytic part of the colloid leads him to refer both properties to the electrical condition of the colloid particles or "micellæ." The osmotic pressure and the movement of the micellæ (Brownian movement) he ascribes to the electrostatic repulsions of the charged micellæ, "both mutual and with those of the intermicellar liquid." He finds that the charge on the particles increases with the "active part" of the colloid; hence their electrical repulsion and Brownian movement also increase, — thus being explained the fact that solutions of high osmotic pressure can be prepared only from colloids with a large "active part."

Duclaux's view is similar to that of Moore and Roaf, in that both ultimately refer the osmotic pressure of the colloid to the associated electrolytes. But, as already urged, certain colloidal solutions contain no electrolytes and others appear to have little or no charge; and on the above view it would be necessary to regard these two types of solution as quite distinct in nature from the usual charged type. The most natural means of avoiding this difficulty would seem to be the conclusion that there is no essential difference between the conditions of solution of crystalloids and colloids; that just as the former comprise both electrolytes and non-electrolytes with all gradations between the two, so also colloids may be stronger or weaker electrolytes (*i. e.*, may liberate ions in varying degree, and so acquire charges), or in some cases may be practically uncharged. The general relations between solute and solvent must, then, be referred to other conditions; and the suggestion that these relations are of the kind classed broadly under "adsorption phenomena" seems most worthy of consideration.

In view of the present tendency to regard the attraction between solute and solvent as of the same general nature as true chemical affinity,<sup>1</sup> it is interesting to note that solutions show certain analogies to the class of combinations known as "adsorption compounds." These last may vary continuously in composition, just as do solutions;<sup>2</sup> moreover, if there is an adsorption between solute and solvent, the distinctive characteristic of solutions becomes intelligible,

<sup>1</sup> Cf. the recent work of H. C. JONES and collaborators, "Hydrates in aqueous solution," the Carnegie Institution, Washington, 1907.

<sup>2</sup> Cf. v. BEMMELEN'S various papers on the hydrates of various metallic oxides in *Zeitschrift für anorganische Chemie*, especially iv *Abhandlung*, 1899, xx, p. 185, and succeeding papers.

namely, the fact that work is needed to separate the two, and hence that dissolved substances exert osmotic pressure and lessen the concentration of the vapor phase of the solvent. Certain similarities between the phenomena of osmotic pressure and those of simple swelling of colloids like gelatine or agar in water afford further indication that the two processes are fundamentally alike. Since the swelling of solid gelatine sheets in water is quite generally referred to adsorption or "imbibition" of the liquid by the colloid particles, it is difficult to imagine why such action should not be regarded as the effective factor causing absorption of water in the fluid mixtures of the two substances, *i. e.*, solutions; the typical absorption-pressure or osmotic pressure of solutions would thus be accounted for. In point of fact, the respective action of electrolytes on swelling processes of solid colloids, and on the osmotic pressure of solutions of the same colloids, are so nearly identical (as I shall show below) that the conclusion seems unavoidable that only one fundamental process is concerned in both. Finally, if solution is of the same nature as swelling action, and if the latter is due to an adsorption, we are led to infer, since no sharp distinction can be drawn between colloids and crystalloids, that osmotic effects in general are due to attractive or adsorptive relations between solute and solvent.<sup>1</sup>

## II. EXPERIMENTAL METHOD.

The following experiments have been performed chiefly on solutions of egg albumin and gelatine; a few inorganic colloids, as ferric hydroxide, arsenious sulphide, and shellac suspensions, have been used in some experiments. I have, throughout, made use of a very simple type of osmometer, consisting of a flask-shaped sac of nitro-cellulose (celloidin or gun-cotton) provided with a perforated rubber cork, through which passes a narrow vertical glass tube, and immersed in a fluid contained in a battery jar covered with a glass plate. The flask-shaped membrane is made by coating the interior of a 50 c.c. flask with a 10 per cent solution of nitro-cellulose in equal parts of alcohol and ether, draining off the superfluous solu-

<sup>1</sup> Compare ARMSTRONG: "The origin of osmotic effects," Proceedings of the Royal Society, Series A, 1906, lxxviii, p. 264. ARMSTRONG refers osmotic effects ultimately to an attraction between the molecules of solute and those of solvent similar to that which the water molecules themselves have for one another. He quotes v. LAAR (Amsterdam Royal Academy, 1906) as giving expression to similar views.

tion, and then removing the solvent by passing a current of air, and bathing in hot water. The membrane may then easily be withdrawn from the interior of the flask. It retains the shape of the flask perfectly, is tough, only slightly extensible, and, if of the proper thickness, almost impermeable to the proteids employed, while allowing extremely ready passage to all crystalloids. It thus makes an excellent dialyser. The procedure is briefly as follows: the membrane is washed in boiling distilled water; the colloidal solution is introduced to a level with the neck, and a well-fitting rubber cork, with glass manometer tube inserted, is placed in position and tied securely with a long elastic rubber band which encircles the neck a large number of times; an absolutely hermetical junction is thus secured. The band is so adjusted that the fluid within the membrane is forced part way up the manometer tube, preferably to a height corresponding to the expected pressure reading; the membrane is thus distended from the first, and entrance of water has an immediate effect in raising the level of the column of fluid. The membrane with manometer-tube attachment is immersed in a definite volume of the pure solvent (water or salt-solution, etc.) contained in a battery jar; the jar is covered with a glass plate perforated by a small hole through which passes the manometer tube; the latter is clamped in a vertical position. The purpose of the glass plate is simply to prevent evaporation.

The advantage of this osmometer is its simplicity and ease of preparation; by its use it is possible to perform twelve or fifteen experiments in a day without difficulty; numerous determinations can thus be made, — a matter of great importance in dealing with colloidal solutions, as will appear below. Direct reading of the height of the column of fluid has the advantage of giving more delicate pressure determinations than those possible with a mercury manometer; the readings can easily be reduced to millimetres of mercury when the specific gravity of the solution within the membrane is known. In most instances this may be considered as simple unity; in any case it can easily be determined. The error introduced by the slight dilution of the solution due to the entrance of a certain small quantity of solvent is usually so slight as to be negligible; if desired, the necessary correction may be made. The passage of water through celloidin membranes is very rapid; the actual upward movement of the column of fluid in the osmometer tube can easily be seen at first, if the osmotic pressure is high and

the fluid stands low in the tube. This ready permeability to water permits equilibrium to be reached within a few hours. The height of the column then remains stationary with constant temperature, — at least for several hours. Usually osmometers have been left overnight and readings made the next morning. By the end of the second day the height of the column, especially if the pressure is high, may have fallen a few millimetres; this is due to the fact that the membrane is seldom absolutely impermeable to the proteid. The column, however, remains stationary at its maximum height for some hours before showing perceptible decline.

I have not taken any especial precautions in the matter of stirring; in a few instances the contents of the osmometer have been stirred at intervals by slight compression and relaxation of the membrane; the results after such stirring were in no observable respect different from usual. With an osmometer having such a relatively large area of membrane in proportion to the volume of solution, stirring appears unnecessary, though it may hasten to a certain degree the attainment of an equilibrium.

Equilibrium, however, is reached very soon in this apparatus; and there can be no doubt that a maximum height, recorded after an immersion of eighteen or twenty-four hours, and remaining stationary for a number of hours thereafter at constant temperature, represents a true equilibrium, and the following records have been made with this understanding. All observations have been made at room temperature, and no attempt has been made to determine degree of variation with changes of temperature. Within the range of room temperatures such variations would be slight, as compared with those due to other factors considered below (as agitation, rate of admixture of electrolyte, etc.).

In the following experiments the attempt has been made to determine the comparative influence of various electrolytes and non-electrolytes on the osmotic pressure of two proteids, gelatine and egg albumin. The solutions of these colloids have not been especially purified; the egg albumin was partially freed from globulin by the dilution with distilled water and filtration; the gelatin solutions were prepared from commercial sheet-gelatine. So far as my experience has gone, dialysis does not affect the osmotic properties of these colloids to any noteworthy degree; if necessary, however, the following results may be held to apply to solutions of the above proteids *plus* a certain unknown and small crystalloid content.

Dialysis, at best, effects only an incomplete removal of crystalloids, and it has accordingly been omitted in the present series of experiments.

The procedure has been as follows: All experiments are performed in series; in any one series the same colloidal solution (*e. g.*, 1.5 per cent gelatine) is used. One solution serves as control; to the others are added definite quantities of the electrolyte (or non-electrolyte) whose action is to be tested. All solutions of the series are brought to equal volumes; the concentration of colloid throughout a series remains constant, the quantity and nature of the added electrolyte alone varying. To the distilled water outside the membrane is added the same electrolyte in the same concentration as within the membrane. The whole system within the osmometer — contents of membrane and surrounding fluid — thus contains the given electrolyte in uniform concentration. The pressure observed must, under these conditions, be due to the colloid and not to the substance added (which, moreover, always readily traverses the membrane). The effect of the substance on the osmotic properties of the colloid is seen on comparison with the control. The volume of the colloidal solution within the membrane has been always approximately 60 c.c.; that of the outer fluid, 420 c.c.; their relative volumes have thus been approximately constant throughout.

### III. EXPERIMENTAL RESULTS.

*A. Action of non-electrolytes on osmotic pressure.* — In agreement with their generally observed negative action in precipitating colloids, non-electrolytes are found to exhibit no definite influence on the osmotic pressure of proteid solutions. The following experiments illustrate (see Table I).

In each series the pressure readings show a general uniformity, with certain minor fluctuations. The greatest divergence from the control pressures is seen in the solutions containing urea; it will be noted that in the two series of gelatine solutions the pressures are approximately one fifth higher in the presence of urea than in the control. It is possible that this variation has some significance; the osmotic pressure of gelatine is perceptibly increased by the presence of a very small proportion of alkali (see Table II, below), and the above slight excess of pressure may indicate a partial action of the urea as a feeble base. In the solutions containing the other

more typical non-electrolytes, the variations from the osmotic pressure of the control are slighter, and no especial significance is to be attached to them. They are, I believe, to be referred chiefly to unavoidable variations in the manipulation of the solutions,—variable changes in the aggregation state being produced by this,

TABLE I.

EGG ALBUMIN + NON-ELECTROLYTES.					
Series I.			Series II.		
Exp.	Solution.	Pressure.	Exp.	Solution.	Pressure.
1	1.25 % egg albumin	mm. Hg. 22.4	1	1.6 % egg albumin	mm. Hg. 29.4
2	“ “ + <i>m</i> /6 sucrose	21.5	2	“ “ + <i>m</i> /6 glycerine	29.5
3	“ “ + <i>m</i> /6 dextrose	21.8	3	“ “ + <i>m</i> /6 urea	27.9
GELATINE + NON-ELECTROLYTES.					
Series III.			Series IV.		
Exp.	Solution.	Pressure.	Exp.	Solution.	Pressure.
1	1.25 % gelatine	mm. Hg. 6.2	1	1.25 % gelatine	mm. Hg. 5.5
2	“ “ + <i>m</i> 6 sucrose	6.6	2	“ “ + <i>m</i> /6 dextrose	5.7
3	“ “ + <i>m</i> /6 dextrose	5.8	3	“ “ + <i>m</i> /6 glycerine	5.6
4	“ “ + <i>m</i> /6 glycerine	5.9	4	“ “ + <i>m</i> /6 urea	6.6
5	“ “ + <i>m</i> /6 urea	7.3	....	....	....

and by the process of mixing itself. The number of uncontrolled variables is always considerable in work with colloidal solutions; all influences affecting the aggregation state, such as agitation, rate of admixture of electrolyte, etc. (see below, page 163), have a certain influence on the osmotic pressure, so that solutions prepared in as nearly as possible identical manner will often show slight but unmistakable differences in physical properties. This variability is seen to a still greater degree in the readings, given below, of experiments with electrolytes. The above determinations are sufficient to

show that non-electrolytes have — at least in comparison with electrolytes — little or no influence on the osmotic pressure of the above colloidal solutions.

*B. Action of electrolytes on osmotic pressure.* — In contradistinction to non-electrolytes, all classes of electrolytes produce marked alteration in the osmotic pressure of colloidal solutions, in some cases depressing, in others increasing, that of the original solution. In general, the principle holds that any electrolyte which, when added in sufficient quantity, precipitates the colloidal solution, depresses the osmotic pressure when added in concentrations less than those required for precipitation; while an electrolyte that promotes solution of a colloid increases the latter's osmotic pressure. There is, in short, a relation between the precipitating or anti-precipitating action of an electrolyte, and its depressant or augmentative influence on the pressure of the colloid. This principle, stated thus unequivocally, seems indeed almost self-evident when it is considered that the osmotic pressure is a direct measure of the work necessary to separate solute and solvent, and that the addition of a precipitating salt, even in quantity insufficient for precipitation, must decrease the work necessary to effect such separation, since less salt is then required to complete the process. The first evident effect is naturally a depression of osmotic pressure. On the other hand, the addition of acid to a solution of gelatine furthers solution, renders separation more difficult, and correspondingly increases osmotic pressure; and obviously the osmotic pressure of a globulin solution is directly dependent on the presence of salt, — probably increasing up to a certain maximum as the concentration of salt increases, after which it declines again; these latter relations, however, have not yet been investigated.

In the following account the effects of addition of acid and alkali and of various salts will be considered in order.

*C. Experiments with acid and alkali.* a. *Gelatine solutions.* — Addition of small quantities of either acid or alkali to solutions of gelatine produces a marked increase in osmotic pressure. Two preliminary experiments gave the following result: 1.5 per cent gelatine gave a pressure of 7 mm. Hg; the same solution after the addition of HCl to  $m/610$  concentration gave a pressure of 33.1 mm. Hg; with  $m/610$  KOH the pressure was 22.8 mm. Hg.

The following table gives the record of three series of experiments; 60 c.c. of the colloidal solution was added rapidly and with constant stirring to the measured quantity of  $n/10$  HCl or KOH

in a small beaker, and all members of a series were made to equal volume. The solutions were then introduced into the osmometers. The following pressures were obtained.

TABLE II.

1.5 % GELATINE + HCl AND KOH.					
Series I.					
Exp.	Electrolyte.	mm. Hg.	Exp.	Electrolyte.	mm. Hg.
1	0 (control)	8.2	5	<i>m</i> /1025 HCl	26.5
2	<i>m</i> /3100 HCl	6.8	6	<i>m</i> /770 HCl	32.4
3	<i>m</i> /2050 HCl	12.3	7	<i>m</i> /620 HCl	34.9
4	<i>m</i> , 1550 HCl	17.9	8	<i>m</i> /412 HCl	39.3
1.5 % GELATINE + HCl AND KOH.					
Series II.			Series III.		
Exp.	Electrolyte.	mm. Hg.	Exp.	Electrolyte.	mm. Hg.
1	0 (control)	6.2	1	0 (control)	7.9
2	<i>m</i> /3100 HCl	6.1	2	<i>m</i> /3100 KOH	14.1
3	<i>m</i> /1240 HCl	27.4	3	<i>m</i> /620 KOH	23.7
4	<i>m</i> /620 HCl	33.1	4	<i>m</i> /412 KOH	25.1
5	<i>m</i> /310 HCl	33.2	5	<i>m</i> /310 KOH	29.0

In the presence of either acid or alkali the osmotic pressure of gelatine thus shows a marked increase, which, within the above range of concentrations, exhibits a certain proportionality to the quantity of acid or alkali added. For equivalent concentrations, acid produces a somewhat greater increase than alkali.

The change in osmotic properties is to be attributed to a finer subdivision of the colloid particles and a consequent increase in the surface of interaction between colloid particles and medium. The change can hardly be attributed to an increase in the molecular concentration of the solution, due to hydrolytic splitting of the

gelatine molecule, for in the first place the quantity of acid or alkali is insufficient to produce, in so short a space of time and at room temperature, so extensive a chemical transformation as a fourfold increase in osmotic pressure would imply; again, the change in osmotic properties occurs in a strictly continuous manner, and is readily, though gradually, reversible when the acid is removed from the solution by dialysis. The increased pressure may safely be regarded as due to a simple change in aggregation state.

It is interesting to compare the above results with those described by Wolfgang Ostwald<sup>1</sup> on the influence of acids and alkalis on the swelling of solid gelatine plates immersed in water; acid in low concentration ( $m/210$ ) causes a slight diminution in the swelling capacity; higher concentrations increase the velocity and degree of swelling to several times the original; with progressively increasing concentration of acid the degree of swelling increases rapidly up to a certain optimum concentration ( $m/26$ ), after which there is a decline. Alkali produces similar effects, except that there is no initial decrease. The resemblance to the above results, is striking; there is even a slight diminution of the normal osmotic pressure in presence of low concentrations of acid. I have not yet performed experiments with higher concentrations of acid; the above figures, however, show that the increase of pressure is at first rapid, and then more gradual as the concentration of acid increases. The absolute concentrations of electrolyte for corresponding effects are much lower in my own than in Ostwald's experiments; this is, no doubt, simply due to the fact that the gelatine is in lower concentration in the solutions than in the solid or swollen form.

In the experiments with salts given below, it will be seen that in their respective actions on osmotic pressure, alkali salts with different anions form a series closely similar to that found by Hofmeister<sup>2</sup> for their action in swelling gelatine plates. The two processes — swelling in the solid condition of the colloid, and absorption of water due to the osmotic pressure of its solutions — are, in fact, influenced by ions in essentially the same manner; a clear indication that at bottom both processes are dependent on the same condition. This agreement confirms the view expressed above,

<sup>1</sup> OSTWALD, W.: *Archiv für die gesammte Physiologie*, 1905, cviii, p. 563, and cix, p. 277.

<sup>2</sup> Cf. HOFMEISTER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1891, xxviii, p. 210.

that the phenomena of osmotic pressure are due, like those of swelling, to an adsorption of the solvent by the particles of dissolved substance.

b. *Albumin solutions.* — Egg albumin differs from gelatine in exhibiting no increase of osmotic pressure in the presence of acid or alkali. The following three series of experiments, made at different times and with different preparations of albumin, indicate

TABLE III.  
EGG ALBUMIN + ACID AND ALKALI.

Series I. 1.5 % Albumin.					
Exp.	Electrolyte.	mm. Hg.	Exp.	Electrolyte.	mm. Hg.
1	0 (control)	25.6	7	<i>m</i> /3100 KOH	24.1
2	<i>m</i> /3100 HCl	20.7	8	<i>m</i> /1240 KOH	22.6
3	<i>m</i> /1240 HCl	11.5	9	<i>m</i> /620 KOH	20.2
4	<i>m</i> /620 HCl	14.1	10	<i>m</i> /412 KOH	18.0
5	<i>m</i> /412 HCl	20.4	11	<i>m</i> /310 KOH	17.9
6	<i>m</i> /310 HCl	22.2			
Series II. 1.5 % Albumin.					
1	0 (control)	23.7	7	<i>m</i> /3000 KOH	23.8
2	<i>m</i> /3000 HCl	5.1	8	<i>m</i> /1200 KOH	23.3
3	<i>m</i> /1200 HCl	16.2	9	<i>m</i> /600 KOH	21.6
4	<i>m</i> /600 HCl	14.1	10	<i>m</i> /400 KOH	20.7
5	<i>m</i> /400 HCl	20.4	11	<i>m</i> /300 KOH	18.4
6	<i>m</i> /300 HCl	22.6			
Series III. 1.25 % Albumin.					
1	0 (control)	18.4	5	<i>m</i> /120 KOH	11.5
2	<i>m</i> /120 HCl	13.1	6	<i>m</i> /80 KOH	10.1
3	<i>m</i> /80 HCl	10.9	7	<i>m</i> /60 KOH	9.4
4	<i>m</i> /60 HCl	8.6			

the general character of the results,—Series I and II with low concentrations of electrolyte, Series III with higher.

The osmotic pressure of albumin solutions is thus depressed by both acid and alkali, though to a less degree, within the range of concentrations employed, than by neutral salts (see below). The determinations with the lower concentrations of HCl (Series I and II) show a result that seems curious at first sight, — a maximum of depression with the two lowest concentrations of HCl ( $m/3000$  and  $m/1200$ ); this apparent anomaly is in all likelihood to be explained on the ground of the presence of a certain variable proportion of alkali albumin in the egg white employed (obtained from common shop eggs); solution 2 of Series II showed, in fact, a considerable clouding on first admixture of the acid; a removal from solution or approach in this direction, due to partial neutralization, would account for the initial marked decline in pressure. Addition of somewhat more acid ( $m/400$  to  $m/300$ ) apparently restores the original state of solution; further addition of acid produces a steady fall of pressure, as seen in Series III; such progressive decline would lead eventually to precipitation. The series with alkali show steady decline with increase in concentration of alkali.

*D. Reversibility of action of acid in gelatine solutions.* — As already mentioned, the withdrawal of the acid from gelatine solutions by dialysis is followed by a return of the osmotic pressure toward its original value. There is, however, a marked lagging or “hysteresis,” the solution tending to conserve the heightened osmotic pressure conferred on it by the acid. In other words, as the concentration of acid in the colloidal system diminishes, the osmotic pressure also falls, but at a much slower rate, so that at any time the pressure is greater than can be accounted for on the basis of the acid actually present at that time. The osmotic properties of the solution can then be explained only by reference to a previous stage in its history, when the concentration of acid was higher. Thus, in one experiment, the outer fluid of the osmometer of solution 8 of Series I ( $m/412$  HCl) was replaced by distilled water. In seven days, after several changes of water, the pressure had fallen to about half its original value, *i. e.*, to 18.9 mm. Hg, although nearly all the acid must by this time have been removed. Several days later the pressure had reached 9.5 mm., which approaches that of the control. The tendency to retain the aggregation state conferred on the colloidal particles by the acid, and with it the increased osmotic

pressure, constitutes an interesting case of hysteresis of a kind not previously described, so far as I am aware. Probably the pressure would have fallen more rapidly if the solution had been agitated or the temperature higher. The exact investigation of this class of phenomena, so interesting to the biologist, is, however, as yet in its early stages. It is necessary continually to take them into account in dealing with changes in colloidal solutions, as I shall endeavor to show in more detail later (see page 163).

*E. Experiments with salts.*—The addition of salts invariably produces a fall in the osmotic pressure of the above colloidal solutions. The degree of this depression varies with the concentration of the added salt, and with the nature of both anion and cation. In general, neutral salts of the alkali metals produce least depression, alkali earths somewhat more, and heavy metals most of all, these last showing wide variations among themselves. For salts of the same metal with different anions, chlorides depress to a greater degree than bromides, and these than iodides, while sulphocyanates have still less depressant action. The relative action of different anions will be considered at length below. The degree of hydrolysis is also a factor in the action of any salt, especially in the case of gelatine, which, as already seen, is especially sensitive to changes in the acidity or alkalinity of its solutions.

In the following determinations all experiments were made as follows: 50 c.c. of the colloidal solution—*e. g.*, 1.5 per cent egg albumin—was poured somewhat rapidly and with constant stirring into 10 c.c. of the salt solution—*e. g.*  $m/2$  NaCl—contained in a beaker; the resulting solution contains 1.25 per cent albumin and  $m/12$  NaCl. Care was taken to conduct this mixing process with the greatest possible uniformity throughout; the rate of admixture of the electrolyte appears to be a factor of no small importance in determining the physical properties of the colloidal system,<sup>1</sup> and part of the variability in the following determinations is undoubtedly to be ascribed to variations in the rate of mixture and in the other manipulation of the solutions before their introduction into the osmometer. Mechanical stirring or agitation, apart from its influence on the rate of mixture, has in itself an effect on the aggregation state of the colloid, and so on its osmotic pressure (see below, page 163). Hence, in experiments of the fol-

<sup>1</sup> Cf. FREUNDLICH: Zeitschrift für physikalische Chemie, 1903, xliv, p. 129.

TABLE IV.  
 1.25 % EGG ALBUMIN + SODIUM AND POTASSIUM SALTS.

Series I.							
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0	18.0		8	<i>m</i> /12 KCl	2.6	0.144
2	<i>m</i> /12 NaCl	3.25	0.180	9	<i>m</i> /12 KBr	3.2	0.177
3	<i>m</i> /12 NaBr	2.5	0.139	10	<i>m</i> /12 KI	4.4	0.244
4	<i>m</i> /12 NaI	3.3	0.183	11	<i>m</i> /12 KNO <sub>3</sub>	3.0	0.166
5	<i>m</i> /12 NaNO <sub>3</sub>	3.4	0.188	12	<i>m</i> /12 KCNS	3.8	0.211
6	<i>m</i> /12 NaCNS	4.1	0.288	13	<i>m</i> /12 K <sub>2</sub> SO <sub>4</sub>	2.1	0.117
7	<i>m</i> /12 Na <sub>2</sub> SO <sub>4</sub>				Aver. pressure	3.24	0.180
Series II.							
1	0	21.6		8	<i>m</i> /24 KCl	4.4	0.204
2	<i>m</i> /24 NaCl	5.0	0.231	9	<i>m</i> /24 KBr	4.8	0.222
3	<i>m</i> /24 NaBr	4.6	0.213	10	<i>m</i> /24 KI	5.3	0.245
4	<i>m</i> /24 NaI	4.9	0.226	11	<i>m</i> /24 KNO <sub>3</sub>	5.5	0.255
5	<i>m</i> /24 NaNO <sub>3</sub>	4.8	0.222	12	<i>m</i> /24 KCNS	5.7	0.264
6	<i>m</i> /24 NaCNS	5.3	0.245	13	<i>m</i> /24 K <sub>2</sub> SO <sub>4</sub>	3.9	0.180
7	<i>m</i> /24 Na <sub>2</sub> SO <sub>4</sub>	4.0	0.185		Aver. pressure	4.766	0.220
Series III.							
1	0	21.6		8	<i>m</i> /48 KCl	6.1	0.282
2	<i>m</i> /48 NaCl	5.7	0.264	9	<i>m</i> /48 KBr	6.2	0.287
3	<i>m</i> /48 NaBr	6.3	0.291	10	<i>m</i> /48 KI	6.3	0.291
4	<i>m</i> /48 NaI	6.25	0.289	11	<i>m</i> /48 KNO <sub>3</sub>	5.3	0.245
5	<i>m</i> /48 NaNO <sub>3</sub>	6.25	0.289	12	<i>m</i> /48 KCNS	6.4	0.296
6	<i>m</i> /48 NaCNS	6.3	0.291	13	<i>m</i> /48 K <sub>2</sub> SO <sub>4</sub>	5.6	0.259
7	<i>m</i> /48 Na <sub>2</sub> SO <sub>4</sub>	5.3	0.245		Aver. pressure	6.0	0.277
Series IV.							
1	0	18.0		8	<i>m</i> /96 KCl	6.8	0.377
2	<i>m</i> /96 NaCl	6.8	0.377	9	<i>m</i> /96 KBr	6.8	0.377
3	<i>m</i> /96 NaBr	6.8	0.377	10	<i>m</i> /96 KI	6.6	0.366
4	<i>m</i> /96 NaI	6.85	0.380	11	<i>m</i> /96 KNO <sub>3</sub>	7.3	0.405
5	<i>m</i> /96 NaNO <sub>3</sub>	6.8	0.377	12	<i>m</i> /96 KCNS	6.6	0.366
6	<i>m</i> /96 NaCNS	7.0	0.388	13	<i>m</i> /96 K <sub>2</sub> SO <sub>4</sub>	5.7	0.316
7	<i>m</i> /96 Na <sub>2</sub> SO <sub>4</sub>	5.5	0.305		Aver. pressure	6.63	0.366

lowing kind, uniformity in manipulation should be observed as far as possible.

In Tables IV to VIII I have given, in addition to the actual pressure in millimetres of mercury observed in each determination, the proportion which such pressure bears to that of the pure colloidal solution (the "control" pressure); this facilitates comparison between solutions belonging to different series. In Table IV I have also given, at the foot of each series, the average pressure for all the solutions of that series (exclusive of the control) and its proportion to the control pressure; the general manner in which the pressure varies with the concentration of salt is thus made clearer by neglecting the variations of the individual salts.

It is to be noted that with decrease in the concentration of salt, there is a decrease in the proportionate lowering of osmotic pressure, but at a much slower rate; there is also seen a decrease in the variability of the pressures in the different solutions of a series, the individual pressure determinations in a series showing progressively greater uniformity (*i. e.*, less average deviation from the average value for each series) as the concentration of salt decreases.

The following two series were made with egg albumin in more concentrated solution, using the same salts (with potassium tartrate substituted for the sulphate) as in Series II in  $m/24$  concentration.

TABLE V.

CA. 2.5 % EGG ALBUMIN +  $m/24$  SODIUM AND POTASSIUM SALTS.

Series I.				Series II.			
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. HG.	Ppn.
1	0 (control)	45.3		1	0 (control)	51.5	
2	$m/24$ NaCl	13.0	0.286	2	$m/24$ KCl	13.0	0.252
3	$m/24$ NaBr	13.0	0.286	3	$m/24$ KBr	13.6	0.266
4	$m/24$ NaI	13.6	0.300	4	$m/24$ KI	13.5	0.262
5	$m/24$ NaNO <sub>3</sub>	13.3	0.293	5	$m/24$ KNO <sub>3</sub>	13.5	0.262
6	$m/24$ NaCNS	13.9	0.307	6	$m/24$ KCNS	13.2	0.258
				7	$m/24$ K <sub>2</sub> C <sub>4</sub> H <sub>4</sub> O <sub>6</sub>	11.9	0.231

In these series osmotic pressure is proportionately less lowered by this concentration of salt than in the more dilute albumin solutions of Table IV, there being less electrolyte relatively to a given quantity of the colloid; and the readings with different salts vary less from an average. Otherwise, the general result is the same, and the salts range themselves in the same order, tartrate acting similarly to sulphate.

A number of similar determinations have been made with solutions of gelatine, and the following Tables (VI and VII) give the results of these.

On inspection of the several tables of pressure determinations, the following chief uniformities are apparent. There is, in all cases, a depression of the osmotic pressure of the pure colloidal solution, and the degree of depression increases with increase in the concentration of the added salt, but evidently at a much slower rate. Since ionization is almost complete in the salts of Table IV at the dilutions employed, it is evident that the ionic concentration decreases in the four series in a geometrical progression with a factor of approximately one half; taking the average depressions in the four series of  $m/12$ ,  $m/24$ ,  $m/48$ , and  $m/96$  salt content, we find these to be respectively about 82 per cent, 78 per cent, 72 per cent, and 64 per cent of the control pressures. So far as any deductions can be drawn from a series of data manifestly incomplete, the indications seem to be that doubling the ionic concentration produces an approximately constant absolute decrease in osmotic pressure, *i. e.*, that the effect would be found in general to vary with the logarithm of the ionic concentration. Measurements with colloidal solutions are, however, so variable that a very large number of determinations would be needed to establish definitely the existence of such a relation, and it is best to defer for the present any attempt at exact formulation.

Two chief differences are at once noticeable between the two proteids. The osmotic pressure of gelatine solutions is much lower than that of egg-albumin solutions of the same concentration, and a given concentration of salt produces uniformly a much greater proportionate depression with albumin than with gelatine. Otherwise the general effects observed are the same in the two series of determinations. The high osmotic pressure of egg-albumin solutions is a fact previously observed; in the experiments of Moore and Parker<sup>1</sup>

<sup>1</sup> MOORE and PARKER: This journal, 1902, vii, p. 261.

TABLE VI.  
1.25 % GELATINE + POTASSIUM SALTS.

Series I.							
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	7.9		7	<i>m</i> /24 KBrO <sub>3</sub>	3.6	0.455
2	<i>m</i> /24 KCl	3.3	0.417	8	<i>m</i> /24 KCNS	3.75	0.474
3	<i>m</i> /24 KBr	3.7	0.468	9	<i>m</i> /24 KCOOCH <sub>3</sub>	2.9	0.367
4	<i>m</i> /24 KI	3.7	0.468	10	<i>m</i> /24 K <sub>2</sub> SO <sub>4</sub>	3.4	0.430
5	<i>m</i> /24 KNO <sub>3</sub>	3.5	0.443	11	<i>m</i> /24 K <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	3.4	0.430
6	<i>m</i> /24 KClO <sub>3</sub>	3.7	0.468				
Series II.							
1	0 (control)	14.4		5	<i>m</i> /24 KNO <sub>3</sub>	7.8	0.541
2	<i>m</i> /24 KCl	5.6	0.389	6	<i>m</i> /24 KBrO <sub>3</sub>	8.3	0.576
3	<i>m</i> /24 KBr	9.2	0.639	7	<i>m</i> /24 KCNS	11.0	0.764
4	<i>m</i> /24 KI	9.2	0.639	8	<i>m</i> /24 K <sub>2</sub> SO <sub>4</sub>	8.1	0.562
Series III.							
I	0 (control)	4.6		5	<i>m</i> /48 KNO <sub>3</sub>	2.5	0.543
2	<i>m</i> /48 KCl	2.4	0.521	6	<i>m</i> /48 KClO <sub>3</sub>	2.2	0.478
3	<i>m</i> /48 KBr	2.9	0.630	7	<i>m</i> /48 KBrO <sub>3</sub>	2.4	0.521
4	<i>m</i> /48 KI	3.0	0.650	8	<i>m</i> /48 KCNS	3.2	0.693
Series IV.							
1	0 (control)	10.4		4	<i>m</i> /48 KClO <sub>3</sub>	5.1	0.490
2	<i>m</i> /48 KCl	3.7	0.355	5	<i>m</i> /48 KBrO <sub>3</sub>	3.8	0.365
3	<i>m</i> /48 KNO <sub>3</sub>	3.9	0.375	6	<i>m</i> /48 Na <sub>2</sub> CO <sub>3</sub> <sup>1</sup>	5.9	0.567
<sup>1</sup> For comparison with neutral salts.							

TABLE VII.  
1.25% GELATINE + ALKALI AND ALKALI-EARTH SALTS.

Series I.							
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	6.7		7	<i>m</i> /24 KBr	3.0	0.447
2	<i>m</i> /24 NaCl	2.9	0.431	8	<i>m</i> /24 KI	3.2	0.477
3	<i>m</i> /24 NaBr	3.7	0.552	9	<i>m</i> /24 KNO <sub>3</sub>	3.1	0.462
4	<i>m</i> /24 NaI	4.0	0.597	10	<i>m</i> /24 KCNS	3.7	0.552
5	<i>m</i> /24 NaNO <sub>3</sub>	3.3	0.492	11	<i>m</i> /24 K <sub>2</sub> SO <sub>4</sub>	2.6	0.388
6	<i>m</i> /24 Na <sub>2</sub> SO <sub>4</sub>	2.9	0.431		....	..	....
Series II.							
1	0 (control)	6.3		6	<i>m</i> /48 NaNO <sub>3</sub>	2.8	0.444
2	<i>m</i> /48 NaF	2.9	0.460	7	<i>m</i> /48 NACNS	3.5	0.555
3	<i>m</i> /48 NaCl	3.0	0.476	8	<i>m</i> /48 NaCOOCH <sub>3</sub>	3.3	0.524
4	<i>m</i> /48 NaBr	3.1	0.492	9	<i>m</i> /48 Na <sub>2</sub> SO <sub>4</sub>	3.1	0.492
5	<i>m</i> /48 NaI	3.2	0.508	10	<i>m</i> /48 Na <sub>3</sub> citrate	3.1	0.492
Series III.							
1	0 (control)	5.9		8	<i>m</i> /96 NaCOOCH <sub>3</sub>	3.7	0.607
2	<i>m</i> /96 NaF	2.8	0.474	9	<i>m</i> /96 Na <sub>2</sub> SO <sub>4</sub>	3.0	0.508
3	<i>m</i> /96 NaCl	2.8	0.474	10	<i>m</i> /96 Na <sub>3</sub> citrate	2.7	0.457
4	<i>m</i> /96 NaBr	3.1	0.525	11	<i>m</i> /96 MgCl <sub>2</sub>	2.4	0.406
5	<i>m</i> /96 NaI	3.4	0.576	12	<i>m</i> /96 CaCl <sub>2</sub>	2.0	0.338
6	<i>m</i> /96 NaNO <sub>3</sub>	3.0	0.508	13	<i>m</i> /96 SrCl <sub>2</sub>	2.3	0.389
7	<i>m</i> /96 NACNS	3.3	0.559	14	<i>m</i> /96 BaCl <sub>2</sub>	2.0	0.338

TABLE VIII.

1.25 % EGG ALBUMIN. SHOWING THE PROPORTIONATE LOWERING OF OSMOTIC PRESSURE FOR SODIUM AND POTASSIUM SALTS WITH VARIOUS ANIONS. FIGURES GIVE THE PROPORTION OF THE CONTROL PRESSURE.

Conc.	NaCl	NaBr	NaI	NaNO <sub>3</sub>	NaCNS	Na <sub>2</sub> SO <sub>4</sub>
<i>m</i> /12	0.180	0.139	0.183	0.188	0.228	(no determination)
<i>m</i> /24	0.231	0.213	0.226	0.222	0.245	0.185
<i>m</i> /48	{ (a) 0.264 (b) 0.269 (c) 0.286 Av. 0.280	0.291	0.289	0.289	0.291	0.245
<i>m</i> /96	{ (a) 0.377 (b) 0.305 Av. 0.341	0.377	0.380	0.377	0.388	0.305
Average for all conc.'s	0.258	0.255	0.269	0.269	0.288	0.230 <sup>1</sup>
Order for Na-salts: SO <sub>4</sub> > Cl and Br > NO <sub>3</sub> and I > CNS.						
Conc.	KCl	KBr	KI	KNO <sub>3</sub>	KCNS	K <sub>2</sub> SO <sub>4</sub>
<i>m</i> /12	0.144	0.177	0.244	0.166	0.211	0.117
<i>m</i> /24	0.204	0.222	0.245	0.255	0.264	0.180
<i>m</i> /48	{ (a) 0.282 (b) 0.283	0.287	0.291	0.245	0.296	0.259
<i>m</i> /96	{ (a) 0.377 (b) 0.305 Av. 0.341	0.377	0.366	0.405	0.366	0.316
Average for all conc.'s	0.243	0.266	0.286	0.268	0.284	0.218
Order for K-salts: SO <sub>4</sub> > Cl > NO <sub>3</sub> and Br > I > CNS.						
Other salts with plurivalent anions (citrate, tartrate, phosphate, ferrocyanide) all showed, in the few experiments tried, marked depressions comparable to or greater than that of sulphate.						
<sup>1</sup> Supposing <i>m</i> /12 Na <sub>2</sub> SO <sub>4</sub> to produce the same depression as <i>m</i> /24.						

TABLE IX.  
 1.25 % GELATINE. PROPORTIONATE LOWERING OF OSMOTIC PRESSURE FOR NA- AND K-SALTS WITH DIFFERENT ANIONS.  
 FIGURES GIVE PROPORTION OF CONTROL PRESSURE.

Conc.	KCl	KBr	KI	KNO <sub>3</sub>	KClO <sub>3</sub>	KBrO <sub>3</sub>	KCNS	KCOOCH <sub>3</sub>	K <sub>2</sub> SO <sub>4</sub>	K <sub>2</sub> C <sub>2</sub> O <sub>4</sub>
<i>m</i> /24	( <i>a</i> ) 0.416 ( <i>b</i> ) 0.389	( <i>a</i> ) 0.468 ( <i>b</i> ) 0.639 ( <i>c</i> ) 0.447	( <i>a</i> ) 0.468 ( <i>b</i> ) 0.639 ( <i>c</i> ) 0.477	( <i>a</i> ) 0.443 ( <i>b</i> ) 0.541 ( <i>c</i> ) 0.462	0.468	( <i>a</i> ) 0.455 ( <i>b</i> ) 0.576	( <i>a</i> ) 0.474 ( <i>b</i> ) 0.764 ( <i>c</i> ) 0.552	0.367	( <i>a</i> ) 0.430 ( <i>b</i> ) 0.562 ( <i>c</i> ) 0.388	0.430
Average	0.403	0.518	0.528	0.482	0.468	0.515	0.597	0.367	0.460	0.430

Order: COOCH <sub>3</sub> > Cl > SO <sub>4</sub> > ClO <sub>3</sub> > NO <sub>3</sub> > BrO <sub>3</sub> > Br > I > CNS.										
Conc.	KCl	KBr	KI	KNO <sub>3</sub>	KClO <sub>3</sub>	KBrO <sub>3</sub>	KCNS	K <sub>4</sub> Fe(CN) <sub>6</sub>		
<i>m</i> /48	( <i>a</i> ) 0.521 ( <i>b</i> ) 0.355 ( <i>c</i> ) 0.444 ( <i>d</i> ) 0.387 ( <i>e</i> ) 0.434	0.630	0.650	( <i>a</i> ) 0.543 ( <i>b</i> ) 0.375	( <i>a</i> ) 0.478 ( <i>b</i> ) 0.490	0.365	0.693	( <i>a</i> ) 0.516 ( <i>b</i> ) 0.536		
Average	0.428	0.630	0.650	0.459	0.484	0.365	0.693	0.526		

Order: BrO<sub>3</sub> > Cl > NO<sub>3</sub> > ClO<sub>3</sub> > Br > I > CNS

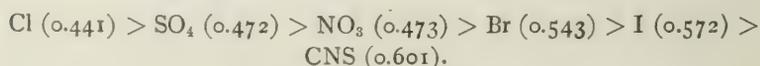
Conc.	NaCl	NaBr	NaI	NaNO <sub>3</sub>	NaCNS	NaCOOCH <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>	Na <sub>3</sub> Citrate	Na <sub>2</sub> HPO <sub>4</sub>	
m/24	0.431	0.552	0.597	0.492	....	....	0.431	....	....	
Order: SO <sub>4</sub> and Cl > NO <sub>3</sub> > Br > I.										
Conc.	NaF	NaCl	NaBr	NaI	NaNO <sub>3</sub>	NaCNS	NaCOOCH <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>	Na <sub>3</sub> Citrate	Na <sub>2</sub> HPO <sub>4</sub>
m/48	0.460	(a) 0.476 (b) 0.481 (c) 0.451 (d) 0.463	0.492	0.508	0.444	0.555	0.524	0.492	0.492	(a) 0.565 (b) 0.419
Average	0.460	0.468	0.492	0.508	0.444	0.555	0.524	0.492	0.492	0.492
Order: NO <sub>3</sub> > F > Cl > PO <sub>4</sub> , citrate, SO <sub>4</sub> , and Br > I > COOCH <sub>3</sub> > CNS.										
Conc.	NaF	NaCl	NaBr	NaI	NaNO <sub>3</sub>	NaCNS	NaCOOCH <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>	Na <sub>3</sub> Citrate	
m/96	0.474	0.474	0.525	0.576	0.508	0.559	0.627	0.508	0.457	
Order: PO <sub>4</sub> > F and Cl > SO <sub>4</sub> and NO <sub>3</sub> > Br > CNS > I > COOCH <sub>3</sub> .										
Average of all the above values for the salts used also in Table VIII: Cl, 0.441; SO <sub>4</sub> , 0.472; NO <sub>3</sub> , 0.473; Br, 0.543; I, 0.572; CNS, 0.601.										

its pressure for a given concentration proved from three to five times greater than that of the serum proteids. The relatively slight depressions produced by addition of salt to gelatine solutions indicate that its state of solution is a more stable one than that of egg albumin. This is in accord with the general observation of the relative difficulty of separating gelatine from solution by addition of salts, or otherwise.

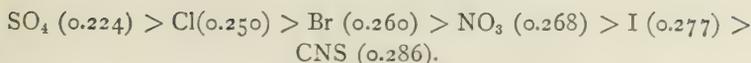
*Specific action of different ions.*—There is also evident a certain specificity in the action of each salt of a given metal, indicating inequality of influence on the part of the different anions. Thus, in general, chlorides depress more than bromides or nitrates, and these to a somewhat greater degree than iodides; sulphocyanates have relatively slight depressant action, while sulphates have more than any of the salts just named.

In order to gain a more exact and objective estimate of the relative depressant powers of the various anions, I have tabulated in Tables VIII and IX the proportionate pressures observed in all the determinations with salts of sodium and potassium in various concentrations, and 1.25 per cent solutions of both albumin and gelatine. Individual variability is so strongly marked in colloidal solutions that little reliance can be placed on the order of action observed in a single series of determinations; thus occasionally a determination with a bromide may show a distinctly greater depression than the corresponding one with a chloride (as in Series I of Table IV), although the reverse is the true order. The errors introduced by uncontrolled fluctuations of this kind are best avoided by taking the average of a number of determinations.

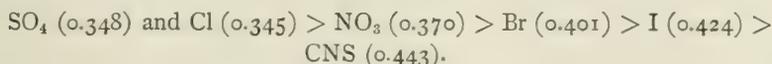
From the above tables it appears that when the salts of any given series are arranged in the order of their depressant power, a certain constancy is seen, which becomes better defined on taking the averages of a considerable number of independent determinations. Thus, in the series of experiments with gelatine, the averages of all values for salts with the following anions (for which the greatest number of determinations were made) give the following order:



The similar series of averages for the albumin series is for Na- and K-salts taken together:



The chief difference between the two proteids is that the determinations with gelatine give sulphate a somewhat less depressant action than chloride.<sup>1</sup> The mean of the results with the two colloids gives the following order:



Sodium fluoride showed, in the two experiments with gelatine solution, a depression somewhat greater than that of the chloride. Chlorate appears similar to nitrate. In the case of albumin solutions other salts with plurivalent anions — tartrate, phosphate, citrate, and ferrocyanide — agreed in the few experiments tried, in showing well-marked depressant action similar to that of sulphate. In the case of gelatine the influence of such salts on the neutrality of the solution is apparently a factor, for phosphate and ferrocyanide produced less depression than would otherwise have been expected. Potassium oxalate, in the one experiment tried, gave the same depression as the corresponding solution of potassium sulphate.

So far as can be judged from data at present insufficient for purposes of very accurate comparison, the anions range themselves, according to their relative influence on the osmotic pressure of proteid solutions, in somewhat the following order: sulphate (with other plurivalent salts: tartrate, citrate, phosphate, ferrocyanide) > (fluoride and) chloride > nitrate (and chlorate) > bromide > iodide > sulphocyanate. This order is essentially identical with that found by Hofmeister and Pauli for the general influence of anions on the aggregation state of colloids; and the above results afford, in this sense, a decided confirmation of theirs. They also indicate the closeness of the relation existing between osmotic pressure and aggregation state in colloidal solutions.

Several series of determinations were made with the aim of ascertaining the relative influences of lithium, sodium, potassium, and ammonium on osmotic pressure, the chlorides of these four metals and in addition the sulphate and sulphocyanate of ammonium being

<sup>1</sup> In the experiments of Table XI below with ammonium salts the sulphate showed uniformly a decidedly greater depressant action than the chloride for solutions of albumin; in gelatine solutions the difference was less decided.

used. The result shows an approximate equality of action for all four cations; the anions showed the typical order of depressant action seen above:  $\text{SO}_4 > \text{Cl} > \text{CNS}$ . For purposes of comparison,

TABLE X.

1.25 % GELATINE + SALTS OF ALKALI AND BIVALENT METALS.

Series I.							
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	5.4	....	6	$m/48 \text{NH}_4\text{CNS}$	2.8	0.518
2	$m/48 \text{LiCl}$	2.9	0.537	7	$m/48 (\text{NH}_4)_2\text{SO}_4$	2.3	0.426
3	$m/48 \text{NaCl}$	2.6	0.481	8	$m/192 \text{CaCl}_2$	2.0	0.370
4	$m/48 \text{KCl}$	2.4	0.444	9	$m/192 \text{CoCl}_2$	2.0	0.370
5	$m/48 \text{NH}_4\text{Cl}$	2.6	0.481	10	$m/192 \text{CuCl}_2$	3.3	0.611
Series II.							
1	0 (control)	6.2	....	6	$m/48 \text{NH}_4\text{CNS}$	3.3	0.532
2	$m/48 \text{LiCl}$	2.4	0.387	7	$m/48 (\text{NH}_4)_2\text{SO}_4$	2.5	0.403
3	$m/48 \text{NaCl}$	2.8	0.451	8	$m/48 \text{Na}_2\text{HPO}_4$	2.6	0.419
4	$m/48 \text{KCl}$	2.4	0.387	9	$m/48 \text{K}_4\text{Fe}(\text{CN})_6$	3.2	0.516
5	$m/48 \text{NH}_4\text{Cl}$	2.4	0.387	....	....	....	....
Series III.							
1	0 (control)	6.9	....	6	$m/48 \text{NH}_4\text{CNS}$	4.6	0.666
2	$m/48 \text{LiCl}$	3.8	0.550	7	$m/48 (\text{NH}_4)_2\text{SO}_4$	3.2	0.463
3	$m/48 \text{NaCl}$	3.2	0.463	8	$m/48 \text{Na}_2\text{HPO}_4$	3.9	0.565
4	$m/48 \text{KCl}$	3.0	0.434	9	$m/48 \text{K}_4\text{Fe}(\text{CN})_6$	3.7	0.536
5	$m/48 \text{NH}_4\text{Cl}$	3.3	0.478	....	....	....	....

several experiments with salts of bivalent metals, and alkali metals with plurivalent anions were introduced into this series.

The results of these determinations show no noteworthy differences between the four alkali chlorides; lithium chloride shows on

the average a somewhat less depressant action than the other three, but the difference is not sufficiently marked to be decisive. Lithium, according to its general precipitating powers, should depress more

TABLE XI.  
EGG ALBUMIN + SALTS OF ALKALI METALS.

Series I. 1.6 % Albumin.				Series II. 1.25 % Albumin.			
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	29.6	....	1	0 (control)	20.8	....
2	<i>m</i> /48 LiCl	8.3	0.280	2	<i>m</i> /48 LiCl	5.4	0.259
3	<i>m</i> /48 NaCl	7.2	0.243	3	<i>m</i> /48 NaCl	5.6	0.269
4	<i>m</i> /48 KCl	6.8	0.229	4	<i>m</i> /48 KCl	5.9	0.283
5	<i>m</i> /48 NH <sub>4</sub> Cl	6.2	0.209	5	<i>m</i> /48 NH <sub>4</sub> Cl	4.5	0.216
6	<i>m</i> /48 NH <sub>4</sub> CNS	5.4	0.182	6	<i>m</i> /48 NH <sub>4</sub> CNS	5.5	0.264
7	<i>m</i> /48 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	4.8	0.162	7	<i>m</i> /48(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2.7	0.129
Series III. 1.25 % Albumin.				Series IV. 1.25 % Albumin.			
1	0 (control)	21.0	....	1	0 (control)	19.9	....
2	<i>m</i> /96 LiCl	6.9	0.324	2	<i>m</i> /48 NaCl	5.7	0.286
3	<i>m</i> /96 NaCl	6.4	0.305	3	<i>m</i> /48 Na <sub>2</sub> Citrate	4.0	0.201
4	<i>m</i> /96 KCl	6.4	0.305	4	<i>m</i> /48 Na <sub>2</sub> HPO <sub>4</sub>	3.7	0.186
5	<i>m</i> /96 NH <sub>4</sub> Cl	6.0	0.286	5	<i>m</i> /48 K <sub>4</sub> Fe(CN) <sub>6</sub>	3.7	0.186
6	<i>m</i> /96 NH <sub>4</sub> CNS	6.0	0.286	..	....	....	....
7	<i>m</i> /96(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	4.6	0.219	..	....	....	....

strongly than sodium. With respect to sodium and potassium, the result has appeared, on averaging all the determinations made with salts of the two metals under like conditions, that sodium has a slightly greater depressant action than potassium; the former is somewhat more active as precipitant, and this result should be expected. The average depressant power of ammonium, as estimated from the above relatively few determinations, appears almost the same as

that of potassium. The three salts of this base show the usual order of relative activity:



The three experiments with bivalent metals in Series I of Table X show, in the case of calcium and cobalt, a strongly marked depressant action on gelatine solutions; copper, on the contrary, depresses

TABLE XII.  
1.25 % GELATINE + ALKALI EARTH CHLORIDES.

Series I.				Series II.			
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	5.9		1	0 (control)	4.4	
2	<i>m</i> /96 MgCl <sub>2</sub>	3.2	0.542	2	<i>m</i> /192 MgCl <sub>2</sub>	2.0	0.455
3	<i>m</i> /96 CaCl <sub>2</sub>	2.7	0.457	3	<i>m</i> /192 CaCl <sub>2</sub>	2.0	0.455
4	<i>m</i> /96 SrCl <sub>2</sub>	3.1	0.525	4	<i>m</i> /192 SrCl <sub>2</sub>	2.0	0.455
5	<i>m</i> /96 BaCl <sub>2</sub> <sup>1</sup>	2.7	0.457	5	<i>m</i> /192 BaCl <sub>2</sub> <sup>1</sup>	1.8	0.409
<sup>1</sup> Considerable precipitate of BaSO <sub>4</sub> .				<sup>1</sup> Precipitate of BaSO <sub>4</sub> .			

relatively slightly, a peculiarity to be ascribed in all probability to the acidity of its solutions, which counteracts to a certain degree the depressant action of the Cu-cations. The relatively slight depressant action, as compared with other alkali salts, shown by phosphate and ferrocyanide in the same table, may also be referred to the hydrolysis of the salts, and the influence of the slight alkalinity in checking the depression. The case of Na<sub>2</sub>CO<sub>3</sub> (Series IV of Table VI) is similar. Salts like citrate and oxalate, which form more nearly neutral solutions, have a similar depressant action to sulphates (*cf.* Table IX).

With albumin solutions, the alkali salts of plurivalent acids show in all cases marked depressant action similar to, or greater than, that of sulphates. This is distinctly seen in the determinations with citrate, phosphate, and ferrocyanide in Table XI, and tartrate in Table V. Similarly with salts of plurivalent metals, these all act as powerful depressants, and in the case of many heavy metals begin in very low concentrations to precipitate the albumin.

The results of a number of determinations with salts of the alkali earths and heavy metals are given in Tables XII and XIII.

TABLE XIII.

EGG ALBUMIN + SALTS OF BIVALENT METALS.

Series I. 1.6 % egg albumin.							
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	29.4	....	3	<i>m</i> /192 MnCl <sub>2</sub>	4.1	0.139
2	<i>m</i> /192CaCl <sub>2</sub>	5.6	0.189	4	<i>m</i> /192 CoCl <sub>2</sub> <sup>1</sup>	5.6	0.189
Series II. 1.25 % albumin.							
Exp.	Salt.	mm. Hg.	Ppn.	Exp.	Salt.	mm. Hg.	Ppn.
1	0 (control)	21.5	....	6	<i>m</i> /960 MnCl <sub>2</sub>	6.9	0.321
2	<i>m</i> /960 MgCl <sub>2</sub>	7.3	0.339	7	<i>m</i> /960 CoCl <sub>2</sub> <sup>2</sup>	5.6	0.260
3	<i>m</i> /960 CaCl <sub>2</sub>	7.6	0.353	8	<i>m</i> /960 CdCl <sub>2</sub> <sup>3</sup>	4.1	0.190
4	<i>m</i> /960 SrCl <sub>2</sub>	7.2	0.335	9	<i>m</i> /960 Pb(NO <sub>3</sub> ) <sub>2</sub> <sup>3</sup>	2.8	0.130
5	<i>m</i> /960 BaCl <sub>2</sub>	7.6	0.353	10	<i>m</i> /960 CuCl <sub>2</sub> <sup>4</sup>	1.6	0.074
<sup>1</sup> A little precipitation of albumin. Other salts — CuCl <sub>2</sub> , Pb(NO <sub>3</sub> ) <sub>2</sub> , CdCl <sub>2</sub> , AlCl <sub>3</sub> — produced marked precipitation when added in the above proportion, and the pressure readings obtained were uncertain. <sup>2</sup> Solution cloudy. <sup>3</sup> Considerable precipitation. <sup>4</sup> Largely precipitated.							

It is evident from these tables that the above salts have more energetic depressant action than those of the alkali metals. The series with egg albumin show that heavy metal cations act more energetically than those of alkali earths; and also that the salts most effective as precipitants, are those having greatest depressant action. It is interesting to note that Series II of Table XIII shows a close parallelism between the decomposition tensions of the various cations and the action of the respective salts, the degree of depression increasing with decrease of decomposition tension.<sup>1</sup>

<sup>1</sup> Compare MATHEWS' experiments on comparative precipitating powers of the heavy metal cations: This journal, 1905, xiv, p. 203.

There is again seen a decided difference between the two proteids, with respect to the degree of depression produced by a given concentration of any salt; alkali-earth salts, when added in  $m/960$  concentration to albumin solutions, produce fully as great depressions as they do when added in five times this concentration to the equivalent gelatine solutions, — another indication of how much more readily the state of solution is altered in the former than in the latter of these two colloids.

#### IV. GENERAL INFLUENCE OF SALTS ON THE STATE OF SOLUTION OF PROTEIDS.

Moore and Roaf<sup>1</sup> describe several experiments in which sodium chloride and magnesium sulphate were added to serum with the effect of lowering the osmotic pressure; the addition of 1 per cent NaCl brought the pressure from 28.2 mm Hg (the value with distilled water as outer fluid) to 19.3 mm. Hg; using 20 per cent  $MgSO_4$  as outer fluid, the pressure first rose and gradually declined to 18 mm. Hg. The depressions obtained are relatively slight as compared with those described above. The authors' aim is given as follows: "Since a large amount of neutral salt added to a proteid-containing solution has the effect of salting out the proteid in many cases, the present experiment was designed to determine whether, at a point short of precipitation, any increased aggregation occurred as shown by diminished osmotic pressure." The experiments described in the present paper appear to justify the conclusion that precipitation by addition of salt to a colloidal solution represents merely the end stage of a process which in itself is perfectly continuous. The state of solution of the colloid, so far from remaining unchanged until a certain quantity of salt has been added, changes continually as the concentration of salt increases; the osmotic pressure appears steadily to decline, with increasing concentration of salt, until a certain critical point is reached; this is probably the point at which the surface energy of the colloidal particles begins to exceed in value the energy keeping the colloid in solution (or solution energy), of which the osmotic pressure is a measure. At this stage equilibrium can no longer persist, and the colloid begins to separate from solution; until this point is reached no visible change may occur in the solution, though its relative

<sup>1</sup> MOORE and ROAF: *Loc. cit.*

instability may be made evident in a variety of ways. The salts most effective in "salting out" are naturally those combining a well-marked specific depressant action on osmotic pressure with a high solubility — like ammonium sulphate.

#### V. VARIABILITY IN OSMOTIC PRESSURE OF COLLOIDAL SOLUTIONS.

It is evident, at a glance, that the above proportionate pressure values vary widely, even for solutions of the same composition, and the impression might hence arise that the experimental error is unduly large, and the method untrustworthy for exact determinations. I believe that the true explanation of this variability is of quite a different nature, and that the variable values observed correspond more or less accurately to an actual variability in the solutions themselves. It is noteworthy that all the hitherto published determinations of the osmotic pressure of proteids show the same wide variation in values, even in cases where the most careful and exact methods have been employed. This is exemplified especially by the work of Reid. Taking his determinations of the osmotic pressure of hæmoglobin solutions,<sup>1</sup> — which in this instance he attributes to the proteid itself and not to the associated crystalloid impurities, — we find, for solutions of crystallized hæmoglobin varying in concentration from 2.76 per cent to 6.05 per cent, a widely varying osmotic pressure per unit concentration of colloid, ranging from 3.51 to 4.35 mm. Hg. for each 1 per cent of hæmoglobin. It is noticeable that the lowest relative pressures are found in the more concentrated solutions, — a circumstance suggesting that in these the colloid has a somewhat coarser state of aggregation than in the more dilute solutions. The measurements of Starling,<sup>2</sup> and especially of Moore and Parker,<sup>3</sup> and Moore and Roaf,<sup>4</sup> also show marked variations of pressure in solutions of the same composition.

It appears necessary to recognize that the conditions influencing the osmotic pressure of a colloidal solution are more numerous than in the case of a crystalloid solution, where concentration and temperature together suffice to fix the value definitely. A great variety of conditions enter in determining the osmotic pressure of a colloidal solution, of which temperature and concentration are only two.

<sup>1</sup> REID: *Journal of physiology*, 1905, xxxiii, p. 12.

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

<sup>3</sup> MOORE and PARKER: *Loc. cit.*

<sup>4</sup> MOORE and ROAF: *Loc. cit.*

The state of aggregation of the colloid is of primary importance in this relation; and this is influenced by numerous conditions — thermal, mechanical, and those dependent on the previous individual history of the colloid. The individual particles of solute are no longer monomolecular (as is typical, though not universal, in solutions of crystalloids), but consist of more or less complex aggregates of molecules; obviously the variability of these aggregates in dimensions and physical properties introduces a further complexity, which must be considered in dealing with these solutions. To the two variables, concentration and temperature, must be added a third, the aggregation state, with whatever other conditions are involved by this; this third variable, which is largely independent of the other two (though it is influenced by both) must have a known value before the properties of the solution can be predicted.

It is natural to assume that each "solution aggregate" or colloidal particle has the same osmotic effect as a molecule or ion in a crystalloidal solution, though this has not been proved. The osmotic pressure, on this assumption, depends on the number of particles in unit volume and on the temperature. It seems also *a priori* probable that the colloidal particles must offer a certain resistance to subdivision or fusion, and hence that a given aggregation state, once attained, will be only slowly altered by a change of conditions. Hence, if in the preparation of a particular colloidal solution a certain state of aggregation is produced, this will tend to be conserved, and the osmotic properties appropriate to this state will tend to persist, for some time after the original conditions have been changed. We have, in fact, evidence that this is the case in the characteristic "hysteresis" of colloidal solutions, — a peculiarity to which I shall now refer more particularly, since it accounts for much of the otherwise apparently anomalous behavior of these solutions.

Given a colloidal system in equilibrium under a given set of conditions, it is usually found that when the external conditions are altered, the resultant change in the physical properties of the system proceeds at a much slower rate, the change in properties tending to *lag behind* the change in conditions, sometimes to a very considerable degree. This peculiarity has been especially studied by van Bemmelen.<sup>1</sup> As one of its consequences, he found that the

<sup>1</sup> VAN BEMMELEN: *Zeitschrift für anorganische Chemie*, 1896, xiii, p. 232, 1898, xviii, 1898, *et loc. cit.* above.

hydration and dehydration curves of inorganic gels (silicic acid, metallic oxides) obtained by plotting the relation between vapor tension and water content at various temperatures, showed quite different courses, so that a gel has different properties for the same water content according to whether it has reached the particular condition by dehydration from a more swollen condition or by addition of water from one less swollen. In general, owing to this peculiarity, the physical properties of the colloid system are dependent not only on the conditions of temperature, concentration, and chemical composition existing at the time of the determination, but also, to a variable and often considerable degree, on the special conditions to which the system has previously been exposed. Hence two gels of colloidal silicic acid, identical in composition, may exhibit different vapor tensions; also, under certain conditions, a more dilute solution may have a lower vapor tension than one more concentrated, so that water may pass from the more concentrated to the more dilute solution, — a result that seems paradoxical and would be impossible in crystalloid solutions.

Similar conditions exist with respect to the osmotic pressure of colloidal solutions; the pressure for a given composition may vary according to the previous history of the particular solution, and under certain conditions a less concentrated solution may have a greater osmotic pressure than one more concentrated, involving a possible transfer of water from the more concentrated to the more dilute solution. Such possibilities should be taken into account in considering the conditions of absorption, secretion, and other processes involving transfer of fluid in the organism.

In considering the factors of the above variability, we must also take into account the tendency to "spontaneous" change in aggregation state so frequently observed in colloids. Van Bemmelen concludes that in a colloidal substance no state of rest exists,<sup>1</sup> but that there is a continual and apparently spontaneous alteration in the molecular condition, and hence in the physical properties of the colloidal substance. This alteration is very slow at normal temperature and is accelerated by heat, — a peculiarity which would seem to indicate that the underlying condition is simply extreme slowness in reaching a state of true chemical equilibrium. For instance, the vapor tension — and with it the correlated power of absorbing or giving off water — of a gel of given percentage composition changes

<sup>1</sup> Cf. VAN BEMMELEN: *Loc. cit.*, 1899, xx, p. 206.

slowly under the influence apparently of time alone. In consequence, the water content of a hydrogel of silicic acid or ferric hydrate depends not only on the concentration of the gas phase and the temperature (as do the properties of a typical crystalloid solution), but also on the simple lapse of time since its formation.

In general, according to van Bemmelen, the properties of such a colloidal system depend not only on its percentage composition, but also on the molecular structure acquired in its preparation,<sup>1</sup> together with the modifications it has undergone in the process of dehydration, or through time, action of water, heating, mechanical treatment, etc., — that is, on special peculiarities due to influences acting on the colloid at various times during its previous history; these have produced modifications that remain more or less permanently fixed in its structure by virtue of its typical hysteresis. Similar considerations apply to solutions of proteids, and must be taken into account in considering the conditions of the above variability. It is impossible to subject any two solutions to exactly the same treatment, and variations in the aggregation state due to inequalities of treatment, combined with inertia in changing an aggregation state once acquired, are, in all probability, the chief reasons for the above variability.

It may not be out of place here briefly to suggest that what applies to colloids in simple solution must also apply *a fortiori* to the colloids composing protoplasmic structures. The possibilities of such differentiation are here necessarily much greater, and modifications of the above kind must play an important part in determining the properties of "living" systems at given periods of their history. The suggestion has been made by Pauli and others that it is to this property of hysteresis that we must look as possibly affording a physical substratum for one of the most distinctively adaptive of the properties of organisms; namely, their possession of peculiarities of action and reaction that can be explained only by reference to conditions and stimuli that have acted at previous periods in their individual history, — in a word, those processes collectively designated in conscious organisms as memory. The chief value of such a hypothesis consists in its suggestions of exact research, and it ought not to be

<sup>1</sup> GIOLITTI, F.: *Gazetta chimica Italiana*, 1906, xxxvi, pp. 157 and 433, gives a number of striking instances of how the properties of colloidal solutions of ferric hydrate and tungstic acid can vary according to their method of preparation. See also the paper of DUCLAUX, *loc. cit.* above.

impracticable to subject it to the direct experimental test.<sup>1</sup> At all events, the colloids composing living substance demonstrably possess this property; and the inference can scarcely be avoided that it must play some rôle, and possibly the above all-important one, in the processes of the organism. This remains for future experiment to determine.

#### VI. INFLUENCE OF MECHANICAL AGITATION AND OF CHANGES OF TEMPERATURE ON OSMOTIC PRESSURE.

Inequalities in the rate of admixture of the electrolyte and in the mechanical treatment of the solutions have been already suggested as possible sources of the observed variability of pressure. Differences in temperature conditions in different series of experiments also probably play some part. I have as yet made no study of the influence of the first named of these factors; but the results of several experiments in which solutions were subjected, respectively, to violent shaking and to temporary warming before measuring the pressures, seem significant and are accordingly presented here.

In the first of the two series of the following table (with 1.25 per cent gelatine) the solutions were mixed with as little agitation as possible; one solution of each of the six pairs was then shaken violently in a small flask for a short time; the other remained unshaken. The solutions were then introduced into the osmometers under as nearly as possible identical conditions. In the series with 1.6 per cent albumin solution the procedure was similar; one solution of each pair was shaken by hand as violently as possible for thirty seconds.

In the gelatine series there is relatively slight difference between the shaken and the unshaken solutions; the former, however, in every case show a slightly higher osmotic pressure. In the albumin series little difference is seen between Solutions 1 and 1*a*; in all the others the shaken solution shows a distinctly lower pressure. Several conditions must be considered in accounting for this effect; the shaking process transforms a considerable part of the solution into the condition of a finely divided foam, and it is probable that

<sup>1</sup> Possibly by determining the temperature coefficient for the establishment of associations in animals, *e. g.*, the relative number of trials required to fix an association or habit in any given cold-blooded animal at different temperatures. Agreement with the temperature coefficient of hysteretic changes would confirm the hypothesis. So far as I am aware, no exact investigation has hitherto been made of the influence of temperature conditions on either one of these processes.

in the foamwork of thin films forming this portion of the solution the concentration of proteid is somewhat higher than elsewhere, since, according to Gibbs and J. J. Thomson,<sup>1</sup> substances that lower the surface tension of the solvent, tend to accumulate in the surface

TABLE XIV.

Series I. 1.25 % Gelatine.			Series II. 1.6 % Egg Albumin.		
Exp.	Solution.	mm. Hg.	Exp.	Solution.	mm. Hg.
1	Pure gelatine	4.2	1	Pure albumin	32.1
1a	" " (shaken)	5.3	1a	" " (shaken)	31.3
2	Gelatine + $m/48$ NaCl	2.6	2	Albumin + $m/48$ NaCl	9.0
2a	" " " (shaken)	2.9	2a	" " " (shaken)	6.8
3	Gelatine + $m/48$ NaBr	2.8	3	Albumin + $m/48$ NaBr	9.2
3a	" " " (shaken)	2.9	3a	" " " (shaken)	7.8
4	Gelatine + $m/48$ NaI	2.6	4	Albumin + $m/48$ NaI	8.9
4a	" " " (shaken)	2.8	4a	" " " (shaken)	6.6
5	Gelatine + $m/48$ NaCNS	2.9	5	Albumin + $m/48$ NaCNS	8.45
5a	" " " (shaken)	3.1	5a	" " " (shaken)	7.2
6	Gelatine + $m/48$ Na <sub>2</sub> SO <sub>4</sub>	2.4	..	....	....
6a	" " " (shaken)	2.6	..	....	....

film in somewhat greater concentration than in the other regions of the solution. On account of the large surface extent of the above foam, this effect would be multiplied in that part of the solution, and the concentration of the remainder would be somewhat lowered. It seems hardly possible, however, that this effect is sufficient to account for the differences of pressure observed in the series of albumin solutions. From the 60 c.c. of solution, it is easy after shaking to pour off 50 c.c. of a clear fluid; the volume of liquid contained in the foam is thus certainly less than 10 c.c., and it would be necessary to suppose that the concentration of albumin in this portion of the solution was approximately doubled to account for the average difference between shaken and unshaken in the

<sup>1</sup> Cf. HOEBER: *Physikalische Chemie der Zelle und der Gewebe*, 2te Auflage, Leipzig, 1907, p. 209.

series of solutions containing salt. The true explanation is, in all probability, that a certain aggregation of colloid particles occurs in consequence of the shaking, and that this condition of increased aggregation tends to persist, and is responsible for the lowering of pressure. Ramsden's<sup>1</sup> work has shown that mechanical shaking may produce coagulative changes in colloidal solutions. The fact that the change is well marked only in those solutions to which salt was added, and is seen in all of these, tends to support this view; it is to be expected that agitation will more readily induce aggregation changes in solutions whose stability has already been lowered by the addition of salt, than in those where the state of subdivision is relatively fine, and the osmotic pressure correspondingly high. It may be concluded that the osmotic pressure and the other physical properties of a colloidal solution are affected to no slight degree by the mechanical conditions acting on it during or after its formation. The change in the gelatine solutions is less marked, and is in the opposite direction. What the meaning of this difference may be is difficult to say; it is probably related to the above-mentioned difference between the two colloids in regard to the ease with which their respective states of solution are altered.

Temperature conditions also influence the osmotic pressure of the solution. Moore and Roaf found in the case of gelatine solutions that the pressure increased with rise of temperature at a more rapid rate than the absolute temperature; and that the increased osmotic pressure persisted, by a kind of hysteresis, for several days after normal temperature was restored, gradually returning to its original value. They ascribed the effect to a finer subdivision of colloidal particles at high temperatures. In the following four experiments a similar condition was found. A 1 per cent gelatine solution was used; this was kept in a flask surrounded by chopped ice for a certain period (twenty-four to forty-eight hours); a portion was then transferred to a small, tightly corked flask which was kept immersed in a water bath at 65° to 70° for a period of three to four hours. The contents of the flask were then cooled; 60 c.c. of the solution was then placed in a beaker, an equal quantity of the still cold solution was taken; the temperatures of both were then equalized by bringing to room temperature, and each was placed in an osmometer.

The next morning (*ca.* eighteen hours later) readings were taken. The following results were gained in four experiments.

<sup>1</sup> RAMSDEN: *Zeitschrift für physikalische Chemie*, 1904, xlvii, p. 336.

Warming the solution thus produces a considerable increase in its osmotic pressure, and this increase persists for some time after the solution has cooled. The difference between the warmed and the unwarmed portions of the solution gradually diminishes; in Experiment 1, four days later, the cooled portion still gave *ca.* 5.0 mm. Hg pressure; that of the warmed had declined to 5.3 mm. Hg. The heightened pressure is thus only temporary.

TABLE XV.

Time of experiment.		Pressure in mm. Hg.	
		A. Unwarmed.	B. Warmed.
1	May 2-3	5.0	6.4
	May 2-4 <sup>1</sup>	4.9	6.0
2			
3	May 7-8	5.7	6.2
4	May 13-14	5.6	6.0

<sup>1</sup> Same solution as in Experiment 1, but cooled twice as long.

I hope before long to make a more extended study of this and similar changes, and of the rate at which they proceed under different conditions of temperature and concentration, the influence of mechanical factors, etc. The slight permeability of the above pure celloidin membranes to gelatine introduces a difficulty; it will be necessary in experiments extending over prolonged periods of time, such as would be necessary in an investigation of this kind, to use membranes that are not only unaltered by the action of the solution, but are in addition absolutely impermeable to the colloid. Several other questions remain for examination, among them the rate of change of osmotic pressure under influence of time, the influence of the rate of addition of the electrolyte to the colloidal solution, the influence of withdrawal of the electrolyte after having allowed it to act for varying periods, the general part played by purely mechanical factors, etc. It will be fruitless to form any detailed theory regarding the physico-chemical condition of the colloids in living cells, and the nature of the changes undergone by the colloidal constituents of protoplasm, until we are in possession of a more exact and extended series of data bearing on the above questions.

VII. INFLUENCE OF THE OSMOTIC PRESSURE OF THE COLLOIDS  
ON THE ABSORPTION PHENOMENA OF CELLS.

The absorption of water by cells has hitherto been referred rather to the osmotic pressure of the crystalloids than to that of the colloids of protoplasm. In plant cells, where the phenomena admit of readiest study, the osmotic pressure or turgor is usually ascribed to the crystalloid substances contained in the central vacuole or vacuoles, the protoplasmic portion of the cell being regarded as merely a semi-permeable membrane. In the appearance of such vacuoles,<sup>1</sup> however, osmotic relations between the colloid-rich portions or phases of the protoplasmic system and the more fluid portions must play a part. In brief, the osmotic properties of the protoplasmic colloids must also be considered. The possibility that this factor may be of importance in determining the water content of the cell appears to have been little considered by biologists, although, as Duclaux expresses it,<sup>2</sup> "it is evident that if the colloids of living cells can develop pressures of the same order" (as those observed in artificial colloidal solutions) "we have no right to neglect them, and to attribute to crystalloids alone the observed osmotic phenomena."

The most distinctive characteristic of the osmotic pressure of the cell colloids is that it is not a constant varying with concentration and temperature alone, but that it changes with the state of aggregation, which must constantly vary according to the conditions affecting the cell. Thus the osmotic pressure may at one period increase, with resultant absorption of fluid from the surroundings; a change may then follow in the reverse direction, due to the colloids' entering a state of increased aggregation, with a consequent fall of osmotic pressure and separation or secretion of fluid.

The facts which I have recently described in considering the influence of various salts in promoting water absorption or swelling in ciliated epithelial cells,<sup>3</sup> receive a ready explanation on the basis of the above theory. It is observed that cells swell more or less rapidly in pure isotonic solutions of sodium or potassium salts; the rapidity of swelling with salts of different anions increases in the order: plurivalent anions < Cl < NO<sub>3</sub> < Br < I < CNS, an order

<sup>1</sup> VAN BEMMELLEN describes the formation of hollow spaces in various inorganic gels, a phenomenon apparently analogous to vacuole formation in protoplasm.

<sup>2</sup> DUCLAUX: *Loc. cit.*, p. 41.

<sup>3</sup> R. LILLIE: This journal, 1906, xvii, p. 91. Cf. especially p. 122 *et seq.*

identical with that found above. The explanation of these facts is simple: the electrolyte content of the cell in its normal medium, sea water, imparts to the colloids a state of aggregation, and hence of osmotic pressure, such that the water content remains — so far as can be seen — approximately constant. When such a cell is immersed in (*e. g.*) a pure isotonic NaCl solution, a tendency arises for the ions of the protoplasm other than Na and Cl to diffuse outward and to be replaced by sodium and chlorine, leaving the protoplasm with an excess of these ions and a relative deficiency in Mg, Ca, and  $\text{SO}_4$  ions; in the presence of these last-named ions the colloids have a lower pressure than in their absence; hence their removal results in a heightening of osmotic pressure and absorption of water. If isotonic NaBr is used, the swelling is more rapid in consequence of the greater osmotic pressure imparted by the Br ions. Similarly, iodides induce greater swelling than bromides, and so on with the other salts. The above order of effectiveness is thus readily accounted for; and the dependence of the rate of absorption on the osmotic pressure of the cell colloids is clearly indicated.

In conclusion, it affords me much pleasure to express to Dr. Howell and the members of the physiological staff of Johns Hopkins University my best thanks for the many courtesies I enjoyed during my stay in their laboratory. To Dr. Abel and the members of the department of Physiological Chemistry I am also under many obligations — including the use of a room in the Pharmacological Laboratory — which it is a pleasure for me to acknowledge. My thanks are due also to Dr. Mall for the use of certain materials from the Anatomical Laboratory.

#### SUMMARY.

1. The osmotic pressure of solutions of gelatine and of egg albumin were measured directly, using osmometers with membranes of nitro-cellulose (celloidin). The following pressure determinations were made: (1) of the approximately pure colloidal solution; (2) of the same with the addition of various electrolytes and non-electrolytes in definite concentrations; and (3) of the colloidal solution (or mixtures of colloidal solution and electrolyte) after subjection to mechanical agitation or temporary elevation of temperature.

2. The osmotic pressure of the colloids remains unaffected after

the addition of non-electrolytes (sucrose, dextrose, glycerine, urea) to the solution. All electrolytes alter the osmotic pressure, in some cases decreasing, in others diminishing, the pressure of the original solution.

3. Acid and alkali increase the osmotic pressure of gelatine solutions; in general these substances affect the osmotic pressure of gelatine solutions in the same manner as they do the rate of swelling of solid gelatine plates immersed in water.

4. Addition of salts depresses the osmotic pressure of both colloids; the degree of depression is a function of the nature of both the anion and the cation of the salt. It increases in the order: alkali metals < alkali earths < heavy metals (for cations); and CNS < I < Br < NO<sub>3</sub> < Cl < F < plurivalent anions — SO<sub>4</sub>, tartrate, citrate, phosphate (for anions).

5. The hysteresis of the colloidal solution plays an important part in determining its osmotic properties; temporary elevation of temperature and mechanical shaking are shown to produce more or less persistent changes in the osmotic pressure of the solutions. Similarly with other conditions affecting the aggregation state of the colloid (mode of preparation, age, rate of admixture of electrolyte, etc.). The individual history of the particular solution is thus a factor; hence solutions of the same composition may show quite different osmotic properties.

6. In their respective actions in promoting the absorption of water by cells, the series of alkali metal salts show the same general order of effectiveness as in their action on the osmotic pressure of the above colloids. The absorption of water by cells under these conditions is thus to be referred to the osmotic pressure of the cell colloids. This last is not a constant quantity, but varies according to the state of aggregation of the colloids.

## THE ACTION OF NORMAL FATIGUE SUBSTANCES ON MUSCLE.

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THERE exists in the abundant literature of fatigue, strangely enough, no account of an adequate investigation of the physiological action of fatigue substances. The most comprehensive study is that which was chronologically the first, namely, Ranke's, whose account was published in 1865; and to it we must still refer most of our knowledge of this subject. Although invading a new field, it was, and continues to be, a most valuable contribution. But at the time of its appearance there was wanting the most important single method of investigating muscular activity, the graphic method with its modern refinements, and without this there were necessary limitations to Ranke's research. He did, however, demonstrate the depressing or fatiguing action on skeletal muscle, first, of an extract of muscle supposedly containing all the products of muscular activity; and then of two of those products, lactic acid and kreatin; and he gave to these two the name "fatigue substances," — a term which has ever since remained in good usage, though with a varying domain. The depressing action of these substances when injected into a muscle was exhibited in a lowering of the irritability of the muscle, as measured by the intensity of the electrical stimulus required to elicit a minimal contraction; and in a lessened distance over which a pointer attached to the muscle was swung when the latter was stimulated by a tetanizing series of stimuli constant in intensity. Decreased irritability and working power are two of the prominent, but not the only, characteristics of the fatigued muscle. Of the materials originally designated fatigue substances, Ranke himself later rejected kreatin, because of an error discovered in his earlier experiments, and furthermore accepted carbon dioxide and acid

potassium phosphate. In recent years there has been a pretty general acceptance of Ranke's later classification. Weichardt is, I think, the only one who has attempted to add specifically to the list of substances whose presence is responsible for normal fatigue. He believes that he has demonstrated the existence in fatigued muscles of a distinct toxin allied to bacterial toxins, and capable, when injected into fresh animals, of producing of itself the phenomena of fatigue. He has gone even further, and obtained, he believes, by accepted bacteriological methods, an antitoxin, which possesses the power of nullifying the effects of the toxin and restoring the fatigued organism to its previous condition. So sweeping a claim, and one of such interest, demands careful examination, and I hope at a future date to review Weichardt's work experimentally. It is not improbable that future research will discover other substances that are causative of normal fatigue. It seems scarcely credible that the few named shall prove to be, of all the links in the long metabolic chain, the only substances that are depressant to protoplasmic activity. It is especially the intermediary metabolic products that must be examined from this standpoint.

But whether any of these prove to be efficacious, and whether a fatigue toxin be a reality or not, one fact must be accepted, namely, that of acidification of the muscle in fatigue. That the working muscle produces acid has been known since du Bois Reymond's demonstration in 1859 of an acid reaction in this tissue after activity. Many and conflicting attempts have been made to determine the nature of the substance that is responsible for this acid reaction. By various investigators it has been ascribed to one or all of the three recognized fatigued substances, and it seems not improbable that they all share in it. Carbon dioxide is apparently a factor; monopotassium phosphate is probably so; paralactic acid is surely present, as is now clearly demonstrated by the excellent work of Fletcher and Hopkins. Whether the last-named substance occurs free or in combination with potassium or other elements as a neutral salt, is not quite clear.

Before proceeding further in our search for fatigue substances, however, it is desirable to know how the three that are recognized affect the muscle in which they occur. The present research deals with this phase of the general problem, and leaves untouched the question of possible fatigue substances other than these.

It may be stated at the outset that I find these substances to act

on skeletal muscle in ways essentially identical. Such action is, however, of two directly opposite modes, the appearance of the one or the other mode being dependent upon the quantity of the substance that is used, and the duration of its activity. If used in what we may term moderate or large quantity, or in smaller quantity over a longer time, each substance is distinctly fatiguing, its action being characterized by a decrease in the irritability and the working power of the muscle; a lessened height to which the load is lifted; a decrease in the total amount of work that is performed; in warm-blooded animals a diminished duration of the single contraction; and in cold-blooded animals a slowing or increased duration of the single contraction, a slowing which affects chiefly the phase of relaxation and is followed by a quickening. The opposite action of the same substances is seen when they are employed in small quantity, or in moderate quantity for a brief time. Instead of a diminution of activity there is an augmentation, which is characterized by an increase in irritability and working power, an increase in the height to which the load is lifted, and an increase in the total amount of work performed. This latter mode of action is, I believe, responsible for the progressive augmentation of activity that is customarily present in the early stages of a series of successive muscular contractions, and is known as the *treppe*. I have already considered this in a separate paper. The present contribution is confined to the depressing action of the fatigue substances in question.

My experiments were performed between the months of October and June inclusive, on both frogs and cats. The methods that were used are the same that were employed in the study of the cause of the *treppe*, and for an account of them the reader is referred to the former article. It may be stated here, briefly, that in the majority of cases, after killing the animal, irrigation of two corresponding muscles of opposite legs was employed, physiological salt solution or whipped blood being used for the one, or control, muscle, and for the other the same liquid containing a certain quantity of the particular fatigue substance to be tested. The muscles were then stimulated at regular intervals by break induction shocks applied directly until fatigue was pronounced, and graphic records of the contractions were made. A delicate isotonic system was employed, and great care was exercised to eliminate errors of technique. A very large number of experiments have been made, and the results have been desirably uniform.

RESULTS.

Some of the results that have been obtained have been presented in various preliminary papers, especially in a lecture before the Harvey Society. The reader is also referred to the article on the cause of the *treppe*, in some of the illustrations of which may be found graphic records of typical experiments demonstrating not only the augmenting, but also the fatiguing, action of fatigue substances. In considering the results it should be clearly borne in mind that all three fatigue substances act in ways that qualitatively are essentially identical. The action of any one substance should not therefore be considered as specific for that substance in distinction from the others. Results obtained from each of them will, however, be presented.

Their fatiguing action on frog's muscle is illustrated by Figs. 1 and 3 of the article on the *treppe* and by Fig. 1 of the present paper, which is the graphic record of a typical experiment on the action of mono-potassium phosphate.

*December 22, 1905.* Frog, weight 42 gm., pithed. One thigh was ligated temporarily. 25 c.c. of 0.75 per cent solution of sodium chloride were injected into the bulbus arteriosus during a period of eight minutes. After thirty minutes more had elapsed the irrigated gastrocnemius was excised and prepared for direct stimulation. Maximal stimuli, 30 per minute. Weight actually lifted 5 gm. Every fiftieth contraction was recorded. After the record was completed the temporary ligature on the opposite leg was removed, and 25 c.c. of 0.75 per cent solution of sodium chloride containing  $\frac{20}{100}$   $\text{KH}_2\text{PO}_4$  were injected through the bulbus into the opposite leg. After a wait of thirty minutes the gastrocnemius was removed, and a record as above was made, the muscle curves rising from the same abscissas as those of the first muscle. The tracing shows every fiftieth curve of each muscle from the 1st to the 651st inclusive. The longer, or, in the later contractions, the lower, curves are those of the muscle under the influence of mono-potassium phosphate.

Contrary to what appears in many experiments with mono-potassium phosphate, no preliminary augmentation is here present. From the 51st contraction onward, however, the fatiguing action of the drug is manifest. This is seen first in a greater lengthening of the muscle curves as compared with the normal curves — in other words, a greater slowing of the contraction process, which is confined almost

wholly to the phase of relaxation. This excessive slowing in duration of the contraction is apparent during the first 400 contractions, after which time, in harmony with the diminished lifting power, the duration becomes relatively lessened. The diminished lifting power is exhibited from contraction 151 onward in a constantly progressive degree. When the experiment ends with contraction 651, the poisoned muscle is performing about one half the work of the other. Increased length and diminished height are thus the striking features of the contraction curves of the poisoned, as compared with those of the normal, muscle. Curve 51 of the poisoned muscle is almost the exact counterpart, in both height and length, of curve 151 of the normal muscle. In other words, the poisoned muscle at its 51st contraction is already fatigued to the same extent as the normal muscle at its 151st. The same comparison may be made between curves 101 of the poisoned and 201 of the normal muscle. The pronounced fatiguing action of mono-potassium phosphate is evident. In the present experiment a fairly strong solution of the drug was employed. A similar effect may be obtained when a weaker solution acts for a longer time.

Figure 2 demonstrates the fatiguing action of mono-potassium phosphate on cat's muscle.

*March 18, 1907.* A cat was killed by decapitation at 2.20 P. M. A cannula was placed at once in each femoral artery, and at 2.27 irrigation of the two legs was begun simultaneously under a constant pressure of 150 mm. Hg and a temperature of 40° C. The right leg was irrigated with the whipped blood of a bullock, the left leg with similar blood to which had been added pure mono-potassium phosphate in quantity to equal 1/25 of a grammolecular solution. The irrigation continued for fifteen minutes. After its cessation the corresponding extensor longus digitorum muscles were rapidly excised, placed in moist chambers at room temperature, attached to exactly similar levers, weighted so as to lift 5 gm. each, and stimulated simultaneously by the same series of maximal break induction shocks at the rate of 29 in the minute, every fiftieth contraction being recorded on a rapidly moving drum. The record began at 2.48. The left-hand figure shows the tracing of the normal muscle, the right-hand figure that of the muscle under the influence of the mono-potassium phosphate.

The normal muscle of this experiment becomes fatigued in the manner customary to the muscles of warm-blooded animals. There is no lengthening of the duration of the contraction, as fatigue proceeds, but there is a progressive diminution in the extent of the lift.

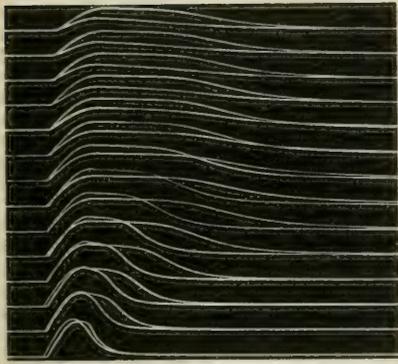


FIG. 1.

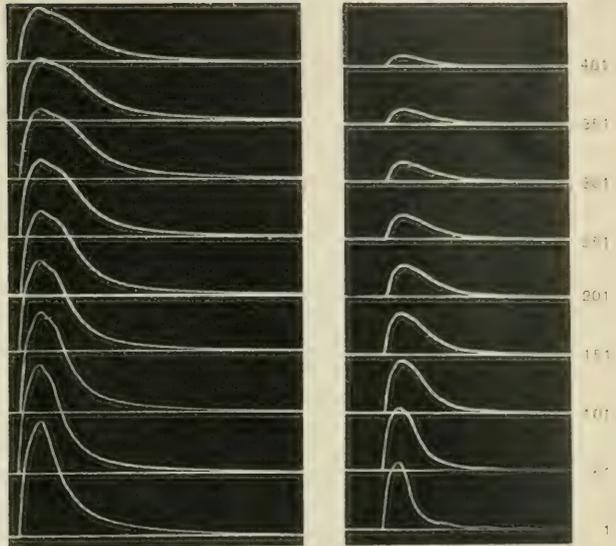


FIG. 2.



FIG. 3.



FIG. 4.

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ACTION OF FAT-SUBSTANCES.



(For a discussion of this subject the reader is referred to the author's articles, "The fatigue of cold-blooded compared with that of warm-blooded muscle," and "Temperatur und Muskelermüdung.") The muscle that is under the influence of the added fatigue substance shows this phenomenon to a more marked degree than the normal muscle. Beginning with the first contraction, the curves of the former are much lower than those of the latter. The 401st contraction reveals pronounced exhaustion, while the normal muscle at this time is still performing nearly one half the amount of work that it performed at first. Beginning with the first contraction also, the poisoned muscle exhibits the shortness of curve that is characteristic of warm-blooded muscle in the later stages of fatigue. The physiological action on warm-blooded muscle of  $\text{KH}_2\text{PO}_4$  in such quantity as is here employed — and the same may be said of the other fatigue substances — thus differs in one sense qualitatively from its action on cold-blooded muscle. This is a striking and important fact. In each case, however, the substance serves simply to hasten the appearance of, and thus accentuate the phenomena of, normal fatigue, which are characteristic of the type of muscle involved. In this sense the action is qualitatively the same with the two types. The qualitative difference may be cited in support of the accepted theory of fatigue as due to products of metabolism. In such an experiment as the above we artificially introduce into the muscle a store of such products, and in consequence the muscle, when put into action, exhibits already in its customary manner a certain degree of fatigue.

Figure 3 shows the characteristic result of an experiment in which  $\text{CO}_2$  is employed in considerable quantity.

*October 18, 1906.* A cat was killed by decapitation at 10.10 A. M. A cannula was placed at once in each femoral artery, and at 10.15 irrigation of the two legs was begun simultaneously under a constant pressure, and a temperature of  $36.5^\circ \text{C}$ . The right leg was irrigated with 0.9 per cent solution of  $\text{NaCl}$ , the left leg with similar solution which had been charged with  $\text{CO}_2$ . The irrigation continued for three minutes. After its cessation the corresponding extensor longus digitorum muscles were rapidly excised, placed in moist chambers at room temperature, attached to exactly similar levers, weighted so as to lift 5 gm. each, and stimulated simultaneously by the same series of maximal break induction shocks at the rate of 29 in the minute, the contractions being recorded on a slowly moving drum. The record began at 10.23. In the figure the upper tracing represents the normal muscle, the lower the muscle under the influence of  $\text{CO}_2$ .

Here we have a typical case of fatigue superinduced by an excessive amount of a fatigue substance. In the muscle charged with  $\text{CO}_2$  there was no preliminary augmentation. There was lessened lifting power from the beginning, and an earlier onset of exhaustion, than in the companion muscle. Figure 1 of the article on the cause of the *treppe* illustrates the same phenomena together with the additional slowing of relaxation peculiar to cold-blooded muscle. These phenomena which are exhibited by the striated muscle of the frog under the influence of  $\text{CO}_2$ , have been previously observed by several investigators, especially Waller and Sowton, Lahousse, Lhotak de Lhota and Spada.

Lastly, Fig. 3 of the article on the *treppe* represents the fatiguing action on cold-blooded muscle of sarcolactic acid, and Fig. 4 of the present article that of lactic acid on warm-blooded muscle. In the latter experiment I employed the ordinary lactic acid of the chemist (Merck) instead of sarcolactic acid, because of the fact that my supply of the latter substance had become exhausted. This experiment was performed in the customary manner as follows:

*April 22, 1907.*—A cat was killed by decapitation at 12.26 P. M. A cannula was placed in each popliteal artery, and at 12.33 irrigation of the two legs was begun simultaneously under a constant pressure, and a temperature of  $39^\circ \text{C}$ . The right leg was irrigated with the whipped blood of a bullock, the left leg with similar blood to which had been added lactic acid in quantity to equal  $1/20$  of a grammolecular solution. The irrigation continued for ten minutes. After its cessation the corresponding extensor longus digitorum muscles were rapidly excised, placed in moist chambers at room temperature, attached to exactly similar levers, weighted so as to lift 5 gm. each, and stimulated simultaneously by the same series of maximal break induction shocks at the rate of 29 in the minute, the contractions being recorded on a slowly moving drum. The record began at 12.49. In the figure the upper tracing represents the normal muscle, the lower the muscle under the influence of lactic acid.

There is here the customary picture of preponderating fatigue in the muscle placed artificially under the influence of a fatigue substance.

In dealing with the cause of the *treppe* the question was raised as to whether the fatigue substances act through their acid character solely. The same problem presents itself here. The physiological actions of the three substances are essentially identical qualitatively, and free hydrogen ions are present in the solutions of all. The same

facts are true of other substances with which I have experimented, namely, oxybutyric acid, hydrochloric acid, sulphuric acid, and mono-sodium phosphate. It thus appears to be a fair inference that the fatiguing action of all of these substances is associated, in part at least, with free hydrogen ions. But our knowledge of the physiological action of potassium salts makes it certain that in solutions of  $\text{KH}_2\text{PO}_4$  K ions are also responsible for a part of the results. With regard to lactic acid I have tried to obtain light by comparing the action of the free acid with that of its salts. I have employed sodium, potassium, and ammonium lactates in solutions that are neutral to litmus, alkaline to Congo red, and only faintly acid to phenolphthalein, and in each case I have obtained qualitatively the same fatiguing effect as with the free acid. In equimolecular solutions ( $\frac{m}{20}$ ), acting for the same number of minutes, potassium lactate proved to be the most fatiguing; sodium lactate seemed to rank next; while ammonium lactate exerted at first an augmenting action, which was followed by depression. These facts seem to justify the conclusion that both anions and cations share in the physiological action of lactic acid. The actual state of the latter in muscle, whether free or combined, has not yet been elucidated. We seem to be warranted, however, in deducing the general conclusion that normal fatigue substances exert their fatiguing action partly through their acid character, that is, their hydrogen ions, and partly through other portions of their molecules.

Another obvious question is whether fatigue substances act directly on the living substance of the muscle cells or on their nerve supply. I find that the effects occur similarly with both curarized and non-curarized frog's muscle, and with cat's muscle long after its excision, when its nerves have long ceased to respond to stimuli and presumably all nerve substance is dead. There is no doubt, therefore, that fatigue substances act directly on the protoplasm of the muscle cells. This, however, leaves still open the question as to whether they may not also depress the substance of both nerve fibres and intramuscular nerve-endings. Probably they do this, but we have no direct experimental evidence regarding it. Nevertheless, it is probable that the fatigue substances of muscle are general fatigue substances, depressing not only the cells in which they arise, but distant ones belonging to both similar and dissimilar tissues.

To what extent in the intact body fatigue of muscle shares in general bodily fatigue is not yet fully known. Following Wundt, James,

Münsterberg, and Baldwin, we must believe, however, that both the feeling of effort which accompanies muscular action, and the sensation of fatigue, are chiefly of peripheral origin. Moreover, the long current belief that in continued labor the central nervous system becomes fatigued before the peripheral tissues is now rendered extremely doubtful by the work of Kraepelin, G. E. Müller, Henri, R. Müller, Hough, Woodworth, Storey, and Joteyko. Their results make it even probable that the brain and spinal cord are, like the nerve fibre, resistant, and they throw a certain measure of doubt on all supposed proofs of central fatigue. For a more specific discussion of this subject and references to the literature, the reader is referred to my Harvey Lecture. I hope also to be able to take up the matter experimentally at an early date.

#### CONCLUSIONS.

1. The physiological action on skeletal muscle of each of the commonly recognized fatigue substances, namely, carbon dioxide, paralactic acid, and mono-potassium phosphate, is of two opposite modes, the appearance of the one or the other mode being dependent upon the quantity of the substance that is present and the duration of its activity.

If present in small quantity, or moderate quantity for a brief time, each substance causes an augmentation of activity of the muscle, which is characterized by an increase in irritability and working power, an increase in the height to which the load is lifted, and an increase in the total amount of work performed.

If present in moderate or large quantity, or in smaller quantity for a longer time, each substance causes a depression of activity, or fatigue, of the muscle, which is characterized by a decrease in irritability and working power; a lessened height to which the load is lifted; a decrease in the total amount of work performed; in warm-blooded animals a diminished duration of the single contraction; and in cold-blooded animals an increased duration of the single contraction, which affects chiefly the phase of relaxation and is followed by a quickening.

2. The depressing action of fatigue substances is due in part, but not wholly, to free acid, since it is shown by neutral solutions of sodium, potassium, and ammonium lactate.

3. The depressing action of fatigue substances occurs in both

curarized and non-curarized frog's muscle and in cat's muscle in which the nerve has long since died. Hence the action is exerted upon the muscle protoplasm itself.

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THE RELATION BETWEEN THE BLOOD SUPPLY TO  
THE SUBMAXILLARY GLAND AND THE CHARACTER  
OF THE CHORDA AND THE SYMPATHETIC SALIVA  
IN THE DOG AND THE CAT.

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I. INTRODUCTORY.

**T**HE literature. — In the dog stimulation of the chorda tympani nerve produces vaso-dilation in the submaxillary gland *pari passu* with secretion of saliva, and stimulation of the cervical sympathetic causes vaso-constriction in the gland simultaneously with a scanty secretion of relatively concentrated saliva. The natural inference is that the difference in the character of the chorda and the sympathetic saliva is due to this difference in the blood supply of the gland. Heidenhain<sup>1</sup> investigated this question in the dog and the rabbit, reaching the conclusion that the percentage composition of the saliva does not vary with the vascular condition of the gland. Heidenhain reports one experiment on the dog's parotid, in which he tied both subclavians and both carotids and stimulated Jacobson's nerve. By this procedure the blood supply to the gland is not completely shut off, but it is greatly diminished. He found no increase in the percentage composition of the chorda saliva collected during the diminished blood supply. Heidenhain also reports two experiments on the rabbit's parotid. In the rabbit the ligation of both subclavians and both carotids shuts off all the blood from the parotid gland, according to Heidenhain. He found no increase in the solids of the saliva following the stimulation of Jacobson's nerve with the gland completely deprived of blood. Heidenhain concludes, therefore, that the difference in the vascular conditions of the salivary glands on stimulation of the sympathetic and the cranial secretory

<sup>1</sup> HEIDENHAIN: *Archiv für die gesammte Physiologie*, 1878, xvii, p. 1; HERMANN'S *Handbuch*, v, p. 46.

nerves is not a factor in determining the difference between chorda and sympathetic saliva. This conclusion is generally accepted by physiologists.

Even prior to Heidenhain's observations Eckhard<sup>1</sup> reports that compression of the submaxillary vein in the dog does not increase the solids in the chorda saliva. It does not appear from the published accounts that Eckhard actually made quantitative determinations. If such determinations were made, the figures are not reported.

Zerner<sup>2</sup> diminished the blood supply to the dog's submaxillary gland by lowering the general blood pressure by transection of the spinal cord in the cervical region. He reports two such experiments, finding in each case the chorda saliva richer in organic constituents than normally.

Langley and Fletcher<sup>3</sup> found that diminution of the oxygen supply by dyspnoea, as well as diminution of the arterial blood supply by partial bleeding, increases the percentage of solids in the dog's chorda saliva from the submaxillary gland. The bleeding experiments consisted in two trials on one dog only, and the secretion was obtained by pilocarpin injection. No statement is made as to whether the increase in the percentage composition of the chorda saliva under these conditions approximates the usual concentration of sympathetic saliva. Langley and Fletcher point out that their results suggest that the dog's sympathetic saliva may be more concentrated than the chorda saliva because of the diminished blood supply to the gland on the sympathetic stimulation. But they do not conclude that such is actually the case, especially in view of the fact that in the cat the submaxillary sympathetic saliva is normally more dilute than the chorda saliva, — a fact discovered by Langley himself. In his article on the salivary glands in Schäfer's Textbook, Langley does not emphasize these results in accounting for the difference between chorda and sympathetic saliva. In fact, he practically ignores them by stating (p. 539) that "it has not been shown that the diminution of the blood supply causes any marked increase in the organic constituents of the saliva."

The usual difference in the percentage composition of chorda and sympathetic saliva constitutes the main support of Heidenhain's

<sup>1</sup> ECKHARD: Beiträge, 1860, ii, p. 212.

<sup>2</sup> ZERNER: Medizinische Jahrbücher, 1887, p. 534.

<sup>3</sup> LANGLEY and FLETCHER: Philosophical transactions, 1888, clxxx, p. 109.

theory of trophic secretory fibres to the salivary glands. If this difference can be shown to be due to the difference in the blood and oxygen supply to the gland, the theory becomes superfluous, if not untenable; and in view of the conflicting and meagre data on this point in the literature a re-examination of the whole subject seemed to us very desirable.

2. **Experimental methods.**— Our experiments were confined to the submaxillary salivary gland of the cat and the dog, most of the work being done on the latter. The arterial blood supply to the gland was diminished to any degree desired by compression of the submaxillary artery. This method has the disadvantage that after isolation of the artery in the dog the stimulation of the cervical sympathetic is usually without any effect on the submaxillary gland, as it is difficult to isolate the gland artery without severing the sympathetic branches that pass from the superior cervical ganglion to the gland. When sympathetic saliva is desired for comparisons, this must, therefore, be obtained either before isolation of the gland artery or by stimulation of the sympathetic fibres passing along the artery to the gland. In some experiments the blood supply to the gland was controlled by tying off both the vertebrals and one carotid and compressing the other carotid to the degree desired. But in the dog it is sometimes not possible by this method to diminish the blood supply to the submaxillary gland to the same extent that takes place on stimulation of the sympathetic.

In all of our experiments on the effect of diminished blood supply on the percentage composition of the chorda saliva, our aim was to reduce the volume of blood passing through the active gland to that which passes through the gland on stimulation of the sympathetic. This can be done only approximately, as the degree of diminution of the blood supply to the gland on sympathetic stimulation is variable. In general, the stronger stimulation causes the greater vaso-constriction in the gland, and consequently the greater diminution of arterial blood. But the same strength and character of stimulus does not produce the same degree of vaso-constriction in all animals, nor in the same animals at different periods of the same experiment. These differences are, no doubt, due to the varying excitability of the sympathetic fibres, the conditions of the gland itself, the general blood pressure, and the degree of anæsthesia,— factors that cannot be made to run a uniformly parallel course. In our experience stimulation of the sympathetic in the dog reduces the out-

put of blood from the submaxillary veins to from one quarter to one eighth of that in the resting gland.

In order to be sure of the relative amount of blood passing through the gland at any period, we invariably measured the outflow of blood from the main gland veins that empty into the external maxillary, the facial, or, in the dog, directly into the external jugular vein. In the dog a small vein leaves the hilus of the submaxillary gland alongside the salivary duct. This vein was not taken into account in our experiments. It can readily be tied off without injury to the chorda tympani, but this was not necessary for our purpose, as we only desired to reduce the arterial blood supply to that on stimulation of the cervical sympathetic, and in all of our determinations of the latter that hilus vein was not taken into account in measuring the blood output. The output of blood from the gland veins was recorded in drops on the kymograph by a magnetic signal. In the experiments on the effects of tying the gland veins, the small vein leaving the gland alongside the salivary duct was also usually left unobstructed.

The saliva was measured, dried, weighed, and finally incinerated in porcelain crucibles. By this method some of the volatile salts are lost, so that the percentage of organic material probably appears as slightly greater than the actual. But this error is of no consequence in our work, as only comparative figures are sought, and it would seem probable that approximately the same relative amounts of volatile salts should be lost in each sample of saliva.

Since the great work of Heidenhain on salivary secretion, it is well known that the percentage of inorganic constituents in the saliva is in part dependent on the rate of secretion and the strength of the stimulus applied to the secretory nerves. Because of the nature of our experiments these two factors could not be controlled so as to render them uniform, and for that reason but little significance can be attached to the variations in percentage composition of the inorganic constituents that appear in our results.

Ether anæsthesia was used exclusively in all of our experiments.

## II. THE CERVICAL SYMPATHETIC IN THE DOG AND THE CAT CONTAINS SECRETORY FIBRES TO SUBMAXILLARY SALIVARY GLAND.

1. It is well established that the vaso-dilation following the stimulation of the chorda tympani has no causal connection with the secretion of saliva by the gland in consequence of the stimulation.

The secretion is caused by the action of the secretory fibres on the gland cells. The vaso-dilation is necessary for the secretion, to be sure, but only in the way of supplying oxygen, water, and organic pabulum to the gland cells. The old conception that the flow of saliva from the submaxillary gland following the stimulation of the cervical sympathetic is not a true secretory process, but simply forcing out of the gland all or part of the saliva remaining from previous activity by the contraction of muscular elements in the gland, has also been quite generally abandoned by physiologists in favor of the view that the sympathetic contains true secretory fibres. But the existence in the cervical sympathetic of true secretory fibres to the salivary glands is not so conclusively established as that of the cranial secretory fibres. Mathews,<sup>1</sup> in particular, has within recent years urged the contraction hypothesis of the formation of sympathetic saliva, however, without bringing any decisive experiment to bear on it. The contraction hypothesis, which may be partly right, is certainly wrong in its main contentions as far as regards the submaxillary gland of the dog and the cat, because in these animals sympathetic secretory fibres to the gland can be conclusively demonstrated.

The main evidence so far advanced for the existence of sympathetic secretory fibres to the salivary glands is (1) the changes produced in the gland cells on sympathetic stimulation; (2) the difference in the character of chorda and sympathetic saliva; (3) the effect on the composition of chorda saliva of a previous sympathetic stimulation; and (4) the paralysis of the secretory action of the cranial fibres by atropin before the paralysis of the sympathetic secretory action. This last evidence is, of course, indirect. If all the actions of the sympathetic on the submaxillary gland can be shown not to be due to secretory nerves, the supposed greater resistance of the sympathetic secretory fibres to the action of atropin means simply that atropin paralyzes the chorda secretory fibres or the gland cells before it paralyzes the sympathetic fibres innervating the muscular elements in the gland. It will be shown in a subsequent part of this paper that the effect of sympathetic stimulation on the subsequent chorda saliva in the dog, as well as the effect on the composition of the saliva secured by simultaneous stimulation of the chorda and the sympathetic, can be accounted for by the vaso-constrictor action of the

<sup>1</sup> MATHEWS: *Annals of the New York Academy of Science*, 1898, xi, p. 293; this journal, 1901, iv, p. 482.

sympathetic. The argument based on the changes in the gland cells on sympathetic stimulation remains so far unanswered. No one has shown that similar changes can be produced by the supposed compression of the cells on contraction of muscular elements in the gland. The gland cells can, of course, be subjected to any degree and persistence of pressure by massage of the gland.

That mere mechanical tension or pressure on the gland cells is the cause of the histological changes in the cells produced by the stimulation of the sympathetic seems so highly improbable that it is hardly worth the while to stop to determine experimentally. However, this has now been done. Mr. F. C. McLean has carried through two series of experiments on the cat's submaxillary gland. In each experiment the gland on one side was left quiescent, while the other gland was massaged or kneaded directly for twenty-five to thirty minutes. The resting and the massaged gland were then carried through the same fixing and staining processes. The results were negative. The cells of the massaged gland exhibited the same appearance as those of the resting gland. Pressure or tension on the cells does not bring about the cell changes caused by sympathetic stimulation. The massage did not start any secretion in the gland.

The main arguments advanced in support of the contraction theory are: (1) the presence of muscle cells and myo-epithelial cells in the wall of the salivary ducts and about the secreting tubules in some salivary glands; (2) the small amount and relatively rapid cessation of the saliva flow on stimulation of the sympathetic; and (3) the back flow of saliva into the glands in some animals on cessation of the sympathetic stimulation.

It is well known that the musculature of the gland arterioles takes no part in the supposed squeezing process, as we may have pronounced vaso-constriction in the glands without any flow of saliva from the ducts. Furthermore, Kolossow,<sup>1</sup> who described myo-epithelial cells in many glands, including some of the salivary glands, states specifically that he did not find these elements in the submaxillary salivary gland. Nothing is known of the innervation of the muscle cells in the walls of the salivary ducts.

The back flow of saliva into the gland on cessation of the sympathetic stimulation is said to be very marked in the sheep's parotid. There is no back flow into the dog's and the cat's submaxillary on

<sup>1</sup> KOLLOSSOW : *Archiv für mikroskopische Anatomie*, 1898, lii, p. 25.

discontinuing the sympathetic stimulation, according to our observations, even when the tension in the duct amounts to from 10 to 20 mm. of saliva. The only explanation of a back flow into the gland, on the secretory nerve theory, is this, that it is brought about by the reabsorption of water from the saliva by the secreting cells, and possibly the walls of the salivary ducts. If such a back flow is very rapid and takes place in the absence of pressure, it is probably not entirely due to the reabsorption of water, although we have as yet no means of knowing how rapidly the secreting cells may take up water from a hypotonic solution like saliva when the stimuli enabling them to eliminate this dilute solution cease. Nothing can be concluded from the fact that liquid may be forced into the gland through the salivary duct under greater or less pressure. Such pressure will necessarily distend the tissues in the gland, and perhaps by ruptures cause some of the liquid to escape by way of the lymph spaces and efferent lymph ducts.

2. **The difference in the percentage composition of chorda and sympathetic saliva cannot be explained by the contraction hypothesis.**—

(1) What are the conditions in the cat? Langley showed long ago that the submaxillary sympathetic saliva in the cat is more dilute than the chorda saliva, and that the greater dilution is mainly due to the smaller amount of organic constituents present. We have confirmed Langley on this point, as will be shown in a later section of this paper. The sympathetic submaxillary saliva in the cat is usually more dilute than the chorda saliva. On the contraction hypothesis the sympathetic saliva is, of course, nothing but chorda saliva forced out of the gland. This renders necessary the improbable assumption that the normal chorda saliva has been rendered more dilute on standing in the gland by the reabsorption of some of the organic constituents by the gland cells or by the cells of the salivary ducts. But, as a matter of fact, this dilute sympathetic saliva in the cat is obtained even when the chorda stimulation is immediately followed by stimulation of the sympathetic, so that there is not even a cessation of the saliva flow while the electrodes are being shifted from the chorda to the sympathetic. Consequently there is no time for reabsorption. Nor could such reabsorption go on during the stimulation of the sympathetic, as the sympathetic saliva flow from the cat's submaxillary gland is relatively rapid.

(2) How does the contraction hypothesis account for the concentrated saliva in the case of the dog's submaxillary gland? It is well

known that under certain conditions the submaxillary sympathetic saliva in the dog may be as dilute as the chorda saliva, but ordinarily the former is much richer in organic constituents than the latter. The contraction hypothesis demands that sympathetic saliva following immediately after chorda stimulation be of the same character and concentration as the chorda saliva. A more concentrated saliva could only be obtained after a period of rest of the gland by assuming that during rest water and salts are taken up from the saliva by the gland cells. Neither of these demands stands the test of experiment.

It is true, however, that after fifteen to thirty minutes' quiescence of the dog's submaxillary gland the first 0.5 c.c. of saliva following chorda stimulation is more concentrated than the subsequent portion of saliva secured during the same period of stimulation. The greater concentration of the initial portion is practically wholly due to the greater amount of organic matter present. We made six experiments on this point. Our results are recorded in Table I. The data of only five of these experiments are given, because, through an accident in manipulation, one series yielded reliable figures for the organic constituents only. But so far as these figures go they are in complete agreement with those given in Table I. And inspection of this table shows that out of the seventeen comparisons made fourteen cases show a much greater concentration of the organic solids in the initial portion of the saliva, in two cases this relation is reversed, while in one case there is practically no difference. The four comparisons of the experiment not tabulated all show a greater concentration of the organic constituents.

There is no constant or preponderant difference in the inorganic salts. The variations in the concentrations of the salts are probably due mainly to the fact that their percentage content depends on the rate of the secretion, and the rate is not constant during any one period of stimulation.

These figures demonstrate the further fact that the first 0.5 c.c. of chorda saliva from the dog's submaxillary gland after a period of rest is in most instances as rich in organic constituents as the sympathetic saliva of the same gland.

What is the cause of this greater concentration of the organic solids in the initial portion of chorda saliva after gland rest? Nothing seems more natural than to suppose that the greater part of the initial 0.5 c.c. of saliva represents that portion remaining in the gland

from the previous period of activity, and that this has suffered concentration by the reabsorption of water and salts to the exclusion of the organic constituents. If this is the correct explanation, there should be a back flow of chorda saliva into the gland during the period of rest, but so far as we know no such back flow has been recorded for the chorda saliva of the dog's submaxillary gland, and we failed to find any indication of it in our experiments.

But even assuming that the concentration is due to the reabsorption of water and salts in the manner just stated, our experiments show conclusively that this does not account for the concentrated sympathetic saliva so as to render the secretory nerve theory superfluous. If the sympathetic saliva is simply chorda saliva forced out of the gland by muscular contraction, the sympathetic saliva taken immediately after cessation of chorda stimulation, so as to give no time for the assumed reabsorption, should be as dilute as the chorda saliva immediately preceding it. This is not the case. If on cessation of the chorda stimulation the cannula is quickly emptied of chorda saliva, the stimulation of the sympathetic immediately begun, and the first two or three drops of saliva flowing from the cannula discarded as being undoubtedly chorda saliva, the sample obtained is typical concentrated sympathetic saliva. The figures proving this point do not stand out so clearly in Table I, as most of the data in that table bear on a different question. They are therefore restated in Table II. The sample of sympathetic saliva is in every case taken immediately after cessation of the chorda stimulation so as not to allow time for the assumed reabsorption.

In the absence of evidence of any considerable reabsorption of water and salts from the chorda saliva, the course of the concentration of the initial chorda saliva after a period of gland rest is probably the following. It is well known that the dog's submaxillary chorda saliva becomes gradually poorer in organic constituents on repeated stimulations. The same relation probably holds good for even single periods of activity, the first part of the saliva being more concentrated than the later portion. It is also probable that on chorda stimulation in the dog the first 0.5 c.c. of saliva is secreted under condition of insufficient amount of oxygen. The transfer of oxygen from the capillary walls to the gland cells must take place through the lymph of the tissue spaces, and is probably a process of diffusion. The increased oxygen supply to the cells will therefore lag behind the time of action of the secretory nervous impulses on the gland cells. And it will be

shown in a subsequent part of this paper that deficiency in the oxygen supply to the active gland increases the organic solids in the saliva, or rather decreases the rate of secretion of water and salts.

3. The contraction hypothesis is rendered untenable by the amount of saliva that may be obtained from the dog's submaxillary gland in a single period of uninterrupted stimulation of the cervical sympathetic.—Muscular contraction cannot force out any more saliva than what is stationary in the secretory tubules and in the salivary ducts, and it is practically certain that it could not even force out all of that. It is obvious, from the anatomical structure of the submaxillary gland, that this stationary saliva can never reach the amount of one fourth the total volume of the gland. In the dog stimulation of the sympathetic usually yields only a small quantity of saliva from the submaxillary gland, and the advocates of the contraction hypothesis point to this as an evidence in support of their contention. It will be shown later that one cause, and probably the main one, of the usual quick cessation of the flow of sympathetic saliva in the dog is the vasoconstriction accompanying the secretion. If the arterial blood supply to the gland is greatly diminished during chorda stimulation, the chorda saliva is scarcely any more abundant than sympathetic saliva.

In some cases, however, a single period of continuous stimulation of the cervical sympathetic with the weak interrupted current will yield from 1 to 3 c.c. of saliva. The flow of the saliva is slow, to be sure, but constant, and in order to obtain the stated amounts the stimulation must be kept up for from ten to fifteen minutes. The greatest quantity of sympathetic saliva obtained during a single period of stimulation was 5.5 c.c. The period of stimulation was eighteen minutes. The stimulation was discontinued, not because the flow of saliva had stopped, but because we thought we had already secured a quantity of saliva equal to the volume of the gland. But that was not the case. The gland, freed from connective tissue, had a volume of 6.5 c.c. But even so, this experiment amounts to a *reductio ad absurdum* of the contraction hypothesis, for by a single period of stimulation more water was "squeezed" out of the gland than was contained in the whole gland tissue, and at the end of the stimulation the same quantity of water was still left in the gland! The gland was quiescent at the beginning of the stimulation, and the saliva flow ceased immediately on discontinuing the stimulation.

On the contraction hypothesis we must assume that the contractile

TABLE I.

Submaxillary saliva of dog. Percentage composition of the first 0.5 c.c. of chorda following stimulation of the cervical sympathetic. The numbers

Number of experiment.	Saliva.	Solids per 100 c.c.		
		Total.	Inorganic.	Organic.
I	1. Chorda, reflex secretion . . .	1.14	0.32	0.72
	2. Chorda, after 18 minutes' rest .	1.51	0.45	1.06
	3. Chorda, immediately following 2	1.40	0.21	1.19
	4. Chorda, after 27 minutes' rest .	1.68	0.56	1.12
	5. Chorda, immediately following 4	1.16	0.22	0.94
	6. Sympathetic, after 5 minutes' rest	2.11	0.31	1.80
	7. Chorda, immediately following 6	1.17	0.26	0.91
	8. Chorda, after 18 minutes' rest .	1.43	0.23	1.20
	9. Chorda, immediately following 8	1.42	0.34	1.08
	10. Chorda, after 24 minutes' rest .	1.82	0.26	1.56
	11. Chorda, immediately following 10	1.34	0.20	1.14
	12. Sympathetic, immediately after 11	1.92	0.34	1.58
II	1. Chorda, reflex secretion . . .	0.85	0.21	0.64
	2. Chorda, after 18 minutes' rest .	1.65	0.42	1.23
	3. Chorda, immediately after 2 . .	1.24	0.33	0.91
	4. Chorda, after 25 minutes' rest .	1.51	0.40	1.11
	5. Chorda, immediately after 4 . .	1.46	0.22	1.24
	6. Sympathetic . . . . .	1.92	0.28	1.64
	7. Chorda, immediately after 6 . .	1.30	0.27	1.03
	8. Chorda, after 20 minutes' rest .	1.73	0.26	1.47
	9. Chorda, immediately after 8 . .	1.14	0.41	0.73
	10. Sympathetic, immediately after 9	1.92	0.34	1.58
III	1. Chorda, after rest . . . . .	1.44	0.28	1.16
	2. Chorda, immediately after 1 . .	1.16	0.28	0.88
	3. Chorda, after 15 minutes' rest .	1.80	0.26	1.54
	4. Chorda, immediately after 3 . .	1.40	0.33	1.07

TABLE I.

saliva after periods of rest of the gland with that of succeeding saliva, and with the saliva indicate the sequence of taking the samples in each experiment.

Number of experiment.	Saliva.	Solids per 100 c.c.		
		Total.	Inorganic.	Organic.
III	5. Chorda, after 15 minutes' rest .	2.18	0.25	1.93
	6. Chorda, immediately after 5 . .	1.72	0.30	1.42
	7. Chorda, after 15 minutes' rest .	1.58	0.26	1.32
	8. Chorda, immediately after 7 . .	1.31	0.26	1.05
	9. Chorda, after 15 minutes' rest .	1.72	0.23	1.49
	10. Chorda, immediately after 9 . .	1.08	0.24	0.84
	11. Sympathetic, immediately after 10	1.84	0.28	1.56
	12. Chorda, immediately after 11 .	1.15	0.26	0.89
IV	13. Sympathetic, immediately after 12	1.70	0.21	1.49
	1. Chorda, after 20 minutes' rest .	1.95	0.41	1.54
	2. Chorda, immediately after 1 . .	2.09	0.41	1.68
	3. Chorda, after 20 minutes' rest .	3.51	0.46	2.05
	4. Chorda, immediately after 3 . .	1.54	0.51	1.00
	5. Sympathetic, immediately after 4	2.52	0.56	1.96
	6. Chorda, immediately after 5 . .	1.67	0.43	1.24
	7. Sympathetic, immediately after 6	1.88	0.59	1.29
V	8. Chorda, immediately after 7 . .	1.12	0.53	0.59
	1. Chorda, after 20 minutes' rest .	3.26	0.52	2.74
	2. Chorda, immediately after 1 . .	2.33	0.33	2.00
	3. Chorda, after 15 minutes' rest .	2.39	0.49	1.90
	4. Chorda, immediately after 3 . .	1.66	0.55	1.11
	5. Sympathetic, immediately after 4	3.06	0.66	2.40
	6. Chorda, immediately after 5 . .	1.45	0.74	0.71
	7. Sympathetic, immediately after 6	1.82	0.39	1.43
	8. Chorda, after 20 minutes' rest .	1.71	0.49	1.22
9. Chorda, immediately after 8 . .	1.36	0.50	0.76	

elements in the gland may require from ten to twenty minutes to reach their maximum degree of shortening on stimulation of their motor nerves, for the saliva may flow from the duct for that length of time on continuous stimulation of the sympathetic. And with the salivary duct open the flow of saliva will cease practically with the end of the muscular contraction, that is, when the muscles have reached the maximum degree of contraction. We know of no muscular elements that require that length of time to reach the maximal degree of shortening.

The existence of sympathetic secretory fibres to the submaxillary gland of the dog and the cat is therefore certain. Contraction of muscular elements in the gland *may* play a part at the beginning of the expulsion both of chorda and sympathetic saliva, but the only real evidence for this is the existence of muscular elements in the walls of the salivary ducts and between the glandular tissues.

### III. DIMINUTION OF THE BLOOD SUPPLY TO THE SUBMAXILLARY GLAND DURING ACTIVITY BY COMPRESSION OF THE GLAND ARTERY GREATLY INCREASES THE PERCENTAGE OF ORGANIC CONSTITUENTS IN THE SALIVA.

I. The results of our nine experiments, seven on dogs and two on cats, are recorded in Table III. The two experiments on the cat were made for us by Mr. G. Ryan and Mr. F. C. McLean. These results show that when the arterial blood supply to the gland during chorda stimulation is reduced approximately to that on stimulation of the sympathetic, the chorda saliva becomes just as concentrated as sympathetic saliva. Within limits, the greater the diminution of the blood supply, the greater the percentage increase in the solids. These figures show, furthermore, that the greater concentration of this chorda saliva is due practically entirely to the organic constituents. Even to the naked eye this chorda saliva appears thick and viscid and in every way similar to the dogs' sympathetic saliva, and the quantitative determinations show that this is actually the case.

No conclusions can be drawn touching the concentration of the salts in these different salivas, as our results show that these may be practically the same (Nos. 2, 7), a little greater (Nos. 1, 3, 6), or a little less than in the normal chorda saliva. These differences in the salt content of the saliva obtained under these conditions are probably in the main due to differences in the rate of secretion and in the strength of the stimulus, but we have no data by which to decide the point at present.

2. The chorda saliva secreted under greatly diminished blood supply to the gland also resembles sympathetic saliva in the quantity and the rate of secretion. It was noted by Langley and Fletcher that on partial bleeding of the dog the quantity of saliva obtained on chorda stimulation was less than the normal. Our observations on this point confirm those of Langley and Fletcher. With the

TABLE II.

Organic constituents of dog's submaxillary chorda saliva compared with that of the sympathetic saliva immediately following it.

Experiment.	Saliva.	Organic solids per 100 c.c.	Experiment.	Saliva.	Organic solids per 100 c.c.
1	<sup>1</sup> Chorda	1.14	5	<sup>1</sup> Chorda	1.00
	<sup>2</sup> Sympathetic	1.58		<sup>2</sup> Sympathetic	1.96
2	<sup>1</sup> Chorda	0.73	6	<sup>1</sup> Chorda	1.24
	<sup>2</sup> Sympathetic	1.58		<sup>2</sup> Sympathetic	1.29
3	<sup>1</sup> Chorda	0.84	7	<sup>1</sup> Chorda	1.11
	<sup>2</sup> Sympathetic	1.56		<sup>2</sup> Sympathetic	2.40
4	<sup>1</sup> Chorda	0.89	8	<sup>1</sup> Chorda	0.71
	<sup>2</sup> Sympathetic	1.49		<sup>2</sup> Sympathetic	1.43

arterial blood supply to the gland almost shut off in the dog, stimulation of the chorda produces only a few drops of saliva, and after periods of rest subsequent stimulation of the nerve yields gradually less saliva. We have here, apparently, a duplication of the usual rapid failure of the secretory nervous mechanism typical for the sympathetic secretory fibres in the dog.

3. On greatly diminishing the blood supply to the dog's submaxillary gland, stimulation of the chorda diminishes for a time the output of blood from the gland veins. In some of our experiments the output at the beginning of the stimulation was reduced to one third that preceding the stimulation. Two factors contribute to this anomalous condition, namely, the quantity of water that leaves the blood to enter the lymph and ultimately the saliva, and the active dilation of the arterioles due to the stimulation of the vaso-dilators in the chorda. The first factor plays in all probability only a small part, inasmuch as the chorda stimulation produces only a scanty secretion

when the blood supply is greatly diminished. The main factor is the vaso-dilation, as shown by the fact that the diminution in the venous output from the gland may be nearly as great after paralysis

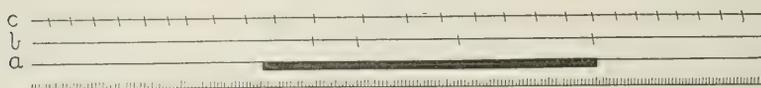


FIGURE 1.—About two thirds the original size.. Dog. Record of blood flow through submaxillary gland and of salivary secretion on stimulation of the chorda tympani nerve during partial occlusion of the gland artery. *a*, stimulation of chorda. *b*, record of saliva flow in drops. *c*, record of blood flow (in drops) from the submaxillary vein, showing diminution of flow of blood from the gland on stimulation of the chorda. Time, seconds.

of the secretory mechanism by atropin as before. When the bore of the gland artery is greatly diminished, the greater quantity of blood entering the gland on chorda stimulation, owing to diminished resistance in the arterioles, is not great enough to counterbalance the space

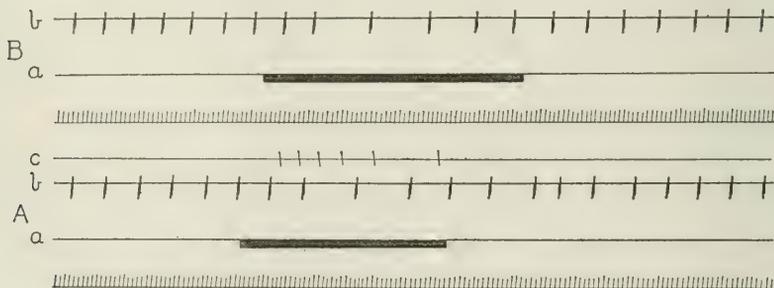


FIGURE 2.—About one half the original size. Dog. Record of blood flow from submaxillary gland vein on stimulation of the chorda tympani nerve during partial occlusion of the gland artery. *A*, before, *B*, after, paralysis of the secretory fibres by atropin. *a*, stimulation of chorda. *b*, record of blood flow in drops from submaxillary vein. *c*, record of saliva flow in drops. Showing the diminution in the venous output on chorda stimulation after paralysis of the secretion by atropin. Time, seconds.

volume yielded by the dilation of the arterioles; and until that space is filled up there will necessarily be a lessened flow through the capillaries and the vein. When the dilated arterioles are filled, we have a return to the original rate of output from the gland veins, or it may even be slightly increased. Records illustrating these conditions are reproduced in Figs. 1 and 2.

When the stimulation of the chorda reduces the blood flow from the gland vein, this condition is necessarily accompanied by dimin-

TABLE III.

The effect on the percentage composition of the dog's submaxillary chorda saliva of greatly reducing the arterial blood supply to the gland by partial occlusion of the gland artery.

No. of experiment.	Saliva, submaxillary gland.	Solids, per 100 c.c.		
		Total.	Inorganic.	Organic.
I. Dog	1. Sympathetic . . . . .	1.91	0.40	1.51
	2. Chorda . . . . .	1.16	0.40	0.76
	3. Chorda, art. occl. . . . .	2.28	0.50	1.78
	4. Chorda . . . . .	1.13	0.31	0.82
II. Dog	1. Sympathetic . . . . .	2.26	0.59	1.67
	2. Chorda . . . . .	1.10	0.44	0.66
	3. Chorda, art. occl. . . . .	2.31	0.43	1.88
	4. Chorda . . . . .	1.20	0.31	0.89
III. Dog	1. Reflex (ether) . . . . .	0.89	0.24	0.65
	2. Sympathetic . . . . .	1.23	0.33	0.90
	3. Chorda . . . . .	1.32	0.61	0.71
	4. Chorda, partial occl. art. .	1.73	0.77	0.95
	5. Chorda, greater occl. art. .	2.03	0.64	1.38
IV. Dog	1. Reflex (ether) . . . . .	0.89	0.24	0.65
	2. Sympathetic . . . . .	1.88	0.40	1.48
	3. Chorda . . . . .	1.73	0.79	0.93
	4. Chorda, partial occl. art. .	1.67	0.69	0.98
	5. Chorda, greater occl. art. .	2.05	0.64	1.45
V. Dog	1. Chorda . . . . .	1.62	0.56	1.05
	2. Sympathetic . . . . .	1.80	0.49	1.30
	3. Chorda, art. occl. . . . .	2.11	0.51	1.60
	4. Chorda . . . . .	1.61	0.43	1.21
VI. Dog	1. Sympathetic . . . . .	1.93	0.40	1.52
	2. Chorda . . . . .	1.36	0.44	0.91
	3. Chorda, art. occl. . . . .	2.77	0.59	2.18
	4. Chorda . . . . .	1.84	0.49	1.34
VII. Dog	1. Chorda . . . . .	0.94	0.19	0.75
	2. Chorda, art. occl. . . . .	1.68	0.21	1.48
	3. Chorda . . . . .	1.22	0.26	0.96
VIII. Cat	1. Chorda . . . . .	1.51	....	....
	2. Chorda, art. occl. . . . .	2.25	....	....
	3. Chorda . . . . .	1.61	....	....
IX. Cat	1. Chorda . . . . .	1.76	....	....
	2. Chorda, art. occl. . . . .	2.41	....	....
	3. Chorda . . . . .	1.39	....	....

ished tension and rate of flow of the blood through the capillaries. Consequently we have here a condition of glandular secretion *pari passu*, with reduced rate of flow and pressure of the blood in the gland capillaries.

4. The effect of a period of diminished blood supply on the percentage composition of the chorda saliva obtained after restoration of the normal circulation is not conclusively determined by our experiments, as will be seen on examination of Table III. In four experiments (2, 6, 7, 8) the chorda saliva obtained after re-establishing the normal circulation is more concentrated than the normal, and the main factor is the greater amount of the organic constituents. In two experiments (1, 5) there is practically no difference, while in one case (9) the chorda saliva subsequent to readmitting the blood is more dilute. When the blood is readmitted to the dog's submaxillary gland, after a few minutes' complete occlusion the gland secretes spontaneously for a time, as noted by Mathews,<sup>1</sup> and this spontaneous saliva is very dilute, as compared to normal chorda saliva. Quantitative determinations in two experiments gave the following figures per 100 c.c. of saliva:

1.	{	1. Chorda saliva before occlusion — solids: 1.16	{	1. Inorganic: 0.40
				2. Organic: 0.76
		2. Spontaneous saliva after occlusion — solids: 0.69	{	1. Inorganic: 0.20
				2. Organic: 0.49
2.	{	1. Chorda saliva before occlusion — solids: 1.10	{	1. Inorganic: 0.44
				2. Organic: 0.66
		2. Spontaneous saliva after occlusion — solids: 0.63	{	1. Inorganic: 0.25
				2. Organic: 0.38

The spontaneous saliva following the re-establishment of the gland circulation after anemia is therefore very poor in both organic and inorganic constituents. The mechanism of this saliva formation is not known. It is not reflex. It may be due to substances accumulated in the gland cells or the surrounding lymph during the anemia, in which case the secretion would be analogous to the transient heightened excitability and stimulation of muscle cells and nerve centres on readmitting the blood after anemia. The ganglion in the hilus of the gland as well as the secretory nerve fibres themselves may also be involved in this secretion.

The work of Heidenhain and Langley seems to show that stimulation of the sympathetic increases the organic constituent in the sub-

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

sequent chorda saliva. In our experiments this is not invariably the case. Before all the differences between chorda and sympathetic stimulations as regards the saliva can be conclusively ascribed to the differences in the blood supply, it must be shown that diminished blood supply to the gland concentrates the subsequent chorda saliva. Our data indicate that such is the case in some of the experiments. There is only an apparent discrepancy between this fact and the fact that the spontaneous saliva following anemia is so abnormally dilute, as the condition of the gland is necessarily very different in these cases, and the mechanism of the secretion may be entirely different.

#### IV. DIMINUTION OF THE OXYGEN SUPPLY TO THE GLAND BY OCCLUSION OF THE GLAND VEINS INCREASES THE ORGANIC CONSTITUENTS IN THE CHORDA SALIVA.

I. The data of our seven experiments on this point are recorded in Table IV. The vein occlusion increases the percentage of solids in the chorda saliva, and this increase is mainly due to the organic constituents. In only one case (No. 3) does this chorda saliva attain a concentration equal or exceeding that of the sympathetic saliva from the same gland.

In the dog and the cat the chorda tympani remains functional for over an hour with the main gland veins occluded. Such occlusion does not completely stop the gland circulation. Some oxygen reaches the gland by mere diffusion. And the fact that venous occlusion greatly increases the output of lymph also increases the arterial blood supply to the gland. In the dog, moreover, the small vein alongside the salivary duct was not occluded, and owing to the greater pressure in the gland veins, the output of blood from this vein was probably as great as the total venous output from the gland on sympathetic stimulation. The condition in the cat was not closely investigated, but on tying the main gland veins the chorda remains functional for a relatively longer time than in the dog.

There can be no question, however, but that with the main gland veins occluded the oxygen supply on chorda stimulation is much less than on chorda stimulation with the circulation unimpeded. It is probably even less than that of the resting gland. The capillary pressure is at the same time greatly increased. The gland cells have a supply of water, salts, and organic pabulum even greater than under

normal conditions, the only deficiency being oxygen. And this deficiency makes the chorda saliva alter its percentage composition in the direction of that of sympathetic saliva.

2. Occlusion of the gland veins diminishes the quantity of the chorda saliva, and hastens the fatigue or failure of the chorda secretory fibres, just as we found in the case of compression of the gland artery. The longer the period of venous occlusion, the greater the diminution in the quantity of chorda saliva obtainable. This relation appears also to hold for the concentration of the saliva; that is, the longer the period of occlusion, the greater the percentage of organic constituents (Table IV, No. 7).

3. Our experiments were not directed with the view of determining whether the chorda saliva obtained after a period of venous occlusion is more concentrated than the chorda saliva prior to the occlusion. Our data are indecisive on this point. The first three samples of chorda saliva in Experiment 1, Table IV, are not complicated by sympathetic stimulation, and in that particular case the chorda saliva following the venous occlusion is much richer in organic solids than that obtained prior to the occlusion, in fact, even more so than the saliva collected during the occlusion. This point needs further investigation.

#### V. DOES THE SYMPATHETIC SALIVA IN THE DOG BECOME AS DILUTE AS THE CHORDA SALIVA ON CONDITION OF INCREASE IN THE OXYGEN SUPPLY TO THE GLAND?

1. We have shown that diminution in the oxygen supply to the submaxillary gland by arterial compression and venous occlusion renders chorda saliva of the same composition as that of the sympathetic saliva. This furnishes strong presumptive evidence that the cause of the greater concentration of the submaxillary sympathetic saliva in the dog is the attendant vaso-constriction. Our data do not constitute a proof of this point, however. This hypothesis cannot be considered proved until it has been shown that, given an ample blood supply, the sympathetic saliva becomes as dilute as normal chorda saliva. So far as we know, no experiments have been reported on this phase of the question.

We attempted, in the first place, to separate the secretory and the vaso-motor fibres in the cervical sympathetic by the degeneration method. Dogs were used for these experiments. The cervical sym-

TABLE IV.

The effect on the composition of the submaxillary chorda saliva of the occlusion of the submaxillary veins.

No. of experiment.	Saliva, submaxillary gland.	Solids, per 100 c.c.		
		Total.	Inorganic.	Organic.
I. Dog	1. Chorda . . . . .	1.02	0.38	0.64
	2. Chorda, veins occl. . . . .	1.74	0.45	1.29
	3. Chorda . . . . .	1.85	0.43	1.42
	4. Sympathetic . . . . .	2.46	0.53	1.93
	5. Chorda . . . . .	1.63	0.40	1.23
	6. Chorda, veins occl. . . . .	1.74	0.38	1.36
II. Dog	1. Chorda . . . . .	1.10	0.32	0.78
	2. Chorda, veins occl. . . . .	1.47	0.35	1.12
	3. Sympathetic . . . . .	2.46	0.53	1.93
	4. Chorda . . . . .	1.69	0.39	1.30
III. Dog	1. Chorda . . . . .	2.21	0.52	1.69
	2. Sympathetic . . . . .	2.05	0.55	1.49
	3. Chorda . . . . .	2.01	0.55	1.45
	4. Chorda, veins occl. . . . .	3.04	0.49	2.55
IV. Dog	1. Chorda . . . . .	1.21	0.25	0.93
	2. Chorda, veins occl. . . . .	1.61	0.28	1.33
V. Dog	1. Chorda . . . . .	1.22	0.26	0.96
	2. Chorda, veins occl. . . . .	1.77	0.36	1.41
	3. Sympathetic . . . . .	2.12	0.29	1.83
VI. Cat	1. Chorda . . . . .	1.10	0.10	1.00
	2. Sympathetic, slight vaso-constr.	1.08	0.12	0.96
	3. Chorda, veins occl. . . . .	1.30	0.15	1.15
VII. Cat	1. Sympathetic, slight vaso-constr.	0.90	0.10	0.80
	2. Chorda . . . . .	0.90	0.18	0.72
	3. Chorda, veins occl. . . . .	1.22	0.12	1.10
	4. Chorda, after 60 minutes' occl.	1.68	0.26	1.39

pathetic was transected in the neck, and its secretory and vaso-motor action on the submaxillary gland investigated two to four days after the operation. The cut nerve ceases to act on the submaxillary gland on the third day after the section. Furthermore, the vaso-motor and the secretory fibres lose their function practically at the same time. This experiment was repeated on four animals with the same results, and the method, not promising to yield any result, was abandoned. If the secretory fibres should have ceased to function sooner than the vaso-motor, the method would have been equally useless for our purpose.

We next endeavored to overcome the vaso-constrictor action of the sympathetic by simultaneous stimulation of the chorda after paralysis of the chorda secretory fibres by atropin. It is well known that the constrictor action of the sympathetic can be partly overcome in this way. In the three experiments made according to this method, the saliva obtained was less concentrated than normal sympathetic saliva. But the atropin diminishes the activity of the sympathetic secretory fibres also, so that the total quantity of saliva obtained in each case was small. It has been shown by Bancroft,<sup>1</sup> moreover, that while atropin paralyzes the secretory activity of the chorda, it does not stop all of its action on the gland cells, because the stimulation of the chorda after atropin paralysis greatly increases the CO<sub>2</sub> output of the gland. It is obvious that the chorda influences the chemical processes in the gland, although it does not produce any secretion. After atropin injection, the gland is not under normal physiological conditions. The data obtained by this method would therefore not be conclusive.

A method of more promise than the foregoing ones is to perfuse defibrinated blood through the gland artery under sufficient pressure to overcome the vaso-constrictor action of the sympathetic. This is easily accomplished; defibrinated blood may be forced through the gland artery under mercury or air pressure, regulated to completely counteract the sympathetic action. But under this degree of pressure the gland becomes œdematous very quickly.

As perfusing liquids, we made use of defibrinated dog blood, defibrinated ox blood, Locke's solution under oxygen pressure, and Locke's solution plus oxygenated dog corpuscles. The artificial solutions are useless for this experiment, because, when forced through the gland under sufficient pressure to overcome the vaso-motor action of the sympathetic, they produce œdema in the gland almost imme-

<sup>1</sup> BANCROFT: *Journal of physiology*, 1901, xxvii, p. 37.

diately. The gland is therefore in a very abnormal condition, and the results obtained from such a gland cannot be made use of in explaining the mechanisms of the processes of the normal gland. Both the chorda and the sympathetic secretory fibres remain functional for some minutes with the gland in maximum œdema from perfusion of a corpuscle suspension in Locke's solution, but the quantity of both the chorda and the sympathetic saliva is greatly reduced.

Defibrinated and oxygenated dog and ox blood is the most satisfactory perfusing medium. But even the dog blood perfused under this pressure renders the gland œdematous in a few minutes. The normal mechanism of the capillary endothelium is evidently disarranged under this pressure, so that more fluid gets into the lymph spaces than can escape through the lymphatics leading from the gland. In all, three trials were made, using defibrinated dog blood as perfusing medium. Small quantities of saliva were obtained by sympathetic stimulation, but this saliva was not identical with normal chorda saliva. It was more concentrated and viscid, thus resembling ordinary sympathetic saliva. Actual quantitative determinations were not made, for the reason that the data would not be conclusive in any event.

It might seem that these results indicate that the sympathetic submaxillary saliva in the dog does not change to the composition of chorda saliva on the blood and oxygen supply to the gland being ample. But they do not constitute a proof of this, for the following reasons. Although the gland is amply supplied with blood and oxygen, it cannot be said to work under normal conditions, because of the œdema rapidly developed by the excessive pressure in the capillaries. The gland is, furthermore, rendered anemic for the time required to make the necessary connections with the gland artery for the perfusion. And we have seen that anemia of the gland may increase the concentration of the chorda saliva obtained on subsequent stimulation. The method was abandoned, as there seemed to be no way of eliminating these two objections.

Some experiments were tried with the circulation in the dog's submaxillary gland reversed. The carotid was united with the external jugular vein by means of a cannula, and all the vein branches tied off, save the main submaxillary vein. A cannula was also placed in the submaxillary artery so as to measure the rate of the blood flow through the gland in the reversed direction. We reasoned that if the circulatory bed in the gland offers no resistance to the reversed flow

in the way of valves or other obstructions, the constriction of the arterioles by sympathetic stimulation would not cut down the blood supply as much as under normal conditions, because of the wider bed and therefore less resistance on the venous side of the capillaries. The tension in the capillaries on chorda and sympathetic stimulation with the circulation reversed is, of course, the reverse of the normal, the sympathetic stimulation increasing, the chorda stimulation decreasing it.

The net result of these experiments is this, that only a very small amount of blood can be made to flow through the dog's submaxillary gland in the reversed direction. This is true for the first two hours of reversal. We did not continue any of our experiments beyond that time. Although the full pressure of the carotid blood is exerted on the blood in the submaxillary vein, only a few drops per minute flow from the cannula in the submaxillary artery. In fact, the gland becomes rapidly asphyxiated.

The cause of this great resistance to the reversed blood flow is not obvious. Thrombi in the gland vessels are probably a factor in some cases, as a slightly greater flow was secured in the two experiments in which we rendered the blood non-coagulable by intravenous injection of hirudin. But even with the coagulation factor removed the best that we could secure was 5 to 6 drops per minute. And this was in relatively large dogs. This amount of blood is even less than that normally passing through the gland on stimulation of the sympathetic.

Both the chorda and the sympathetic remain functional for some time after reversing the circulation, but the saliva obtained is very scanty and very concentrated. This is to be expected, as the reversal gradually asphyxiates the gland, and we have shown that diminution in the oxygen supply to the gland greatly increases the organic solids in the saliva. The following is the analysis of the saliva samples from one of the experiments in which hirudin was used. The percentage of the organic solids was not determined, but it was evident on mere inspection of the saliva that the great concentration was due to the organic constituents.

1. Normal sympathetic saliva :	2.49	} Total solids per 100 c.c. saliva.
2. Normal chorda saliva :	1.80	
3. Sympathetic saliva, circulation reversed :	3.24	
4. Chorda saliva, circulation reversed :	5.60	

The chorda saliva on reversal of the circulation is nearly twice as concentrated as the sympathetic saliva. The reason for this is obvious. The chorda saliva is collected after the sympathetic sample. The condition of diminished blood supply to the gland has consequently lasted longer, and we have shown that the greater the degree of asphyxia, the greater the concentration of chorda saliva.

TABLE V.

Comparison of the composition of chorda and sympathetic submaxillary saliva in the cat, and the effect of diminished blood supply to the gland on the percentage of solids in the sympathetic saliva.

No. of experiment.	Saliva from submaxillary gland.	Solids per 100 c.c.		
		Total.	Inorganic.	Organic.
I.	1. Chorda . . . . .	0.72	....	....
	2. Sympathetic (in neck) . .	0.62	....	....
	3. Chorda . . . . .	0.82	....	....
	4. Sympathetic (in neck) . .	0.79	....	....
II.	1. Chorda . . . . .	0.60	0.13	0.47
	2. Sympathetic, compression of subm. artery	0.89	0.16	0.73
	3. Sympathetic, compression of subm. artery	0.89	0.24	0.65
	4. Sympathetic, great vaso-constriction	1.03	0.27	0.76
	5. Chorda . . . . .	0.84	0.20	0.64
III.	1. Chorda . . . . .	0.97	0.24	0.73
	2. Sympathetic (on gland artery) vaso-constriction	1.24	0.24	1.00
	3. Chorda . . . . .	1.20	0.20	1.00

2. None of the foregoing methods yielded conclusive results. But in the cat nature has apparently furnished conditions for deciding this question without resorting to any artificial contrivance. It was discovered by Langley<sup>1</sup> that the submaxillary sympathetic saliva in the cat is usually even more dilute than the chorda saliva. This we can confirm. When the sympathetic is stimulated in the neck with a weak interrupted current, the saliva obtained is usually more dilute than that obtained on chorda stimulation. At times, however, the

<sup>1</sup> LANGLEY: *Journal of physiology*, 1879, i, p. 96.

sympathetic saliva obtained in this way is of the same concentration or of a slightly greater concentration than the chorda saliva. Langley finds that the deficiency in solids is in the organic constituents. We did not make determinations beyond the total solids. One typical experiment is recorded in Table V, No. 1. In this case the gland vein was not isolated, so that we had no means of knowing the vascular condition in the gland on stimulation of the sympathetic.

Langley apparently assumes that this dilute sympathetic saliva from the cat's submaxillary gland is secreted under the same vascular conditions as is the concentrated sympathetic saliva in the dog. It has been shown, however, that the cervical sympathetic in the cat contains both vaso-dilator and vaso-constrictor fibres to the submaxillary gland, and that on simultaneous stimulation of both with the weak interrupted current, the dilators usually overpower the constrictors, so that we have an increased instead of a decreased flow of blood through the gland.<sup>1</sup> This fact naturally suggests that one of the causes of the cat's submaxillary sympathetic saliva being usually so dilute is the absence of vaso-constriction and the presence of vaso-dilation during the secretion. That the vascular condition of the gland is not the sole cause of the sympathetic saliva being dilute is shown by the fact that it is usually more dilute than the chorda saliva, although the vaso-dilatation on sympathetic stimulation rarely equals that on chorda stimulation.

When the submaxillary sympathetic saliva in the cat is secreted under conditions of diminished blood supply, it becomes more concentrated in organic constituents than the chorda saliva. The blood supply may be diminished by compression of the artery, or the determinations may be made on the individuals in which strong stimulation of the sympathetic branch from the superior cervical ganglion to the gland gives primary vaso-constriction. The data from two experiments demonstrating this point are given in Table V, Nos. 2 and 3. Given the same vascular conditions in the submaxillary gland during the secretion the cat's sympathetic saliva exhibits the same difference, in comparison with the chorda saliva, as is normally shown by the sympathetic saliva in the dog. But because of the difference in the submaxillary gland cells of the dog and the cat, the sympathetic saliva of the latter will never reach the absolute concentration of that of the former.

<sup>1</sup> CARLSON: This journal, 1907, xix, p. 408.

SUMMARY.

1. The cervical sympathetic contains secretory fibres to the submaxillary salivary gland.

2. Diminishing the oxygen supply to the active gland by occlusion of the gland veins or compression of the gland arteries diminishes the quantity of chorda saliva and increases its percentage of organic solids. The organic constituents of this chorda saliva in the dog may equal or exceed that of the sympathetic saliva.

3. At least in some cases the chorda saliva secreted after a period of diminished oxygen supply to the gland is richer in organic solids than the normal chorda saliva.

4. The normal oxygen supply to the gland on chorda stimulation must be reduced considerably before any marked change in the rate of secretion and the character of the saliva appears, but, in general, the greater the oxygen deficiency, the greater the decrease in rate of secretion and the total quantity of saliva, and the greater the increase in organic solids.

5. In the cat the sympathetic submaxillary saliva secreted under diminished oxygen supply is more concentrated as regards the organic constituents than normal sympathetic or chorda saliva.

6. There is probably no actual increase in the rate and quantity of secretion of the organic salivary constituents in gland anemia. This concentration is the result of diminution in the rate of secretion and the quantity of water and salts. The processes of secretion of water and inorganic salts are therefore more directly dependent on free oxygen than is the secretion of the organic matter in the saliva.

7. The differences between sympathetic and chorda submaxillary saliva can be accounted for by the difference in the distribution of the two sets of fibres in the gland and by the difference in the oxygen supply to the gland on chorda and sympathetic stimulation. Heidenhain's theory of trophic secretory nerve fibres is therefore superfluous, at least for the submaxillary gland of the cat and the dog.

# THE INNERVATION OF THE CEREBRAL VESSELS AS INDICATED BY THE ACTION OF DRUGS.

By CARL J. WIGGERS.

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

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## I. INTRODUCTION.

FOR some years past there has existed among physiologists a feeling of uncertainty in regard to active vaso-motor changes in the cerebral vessels. This was created by the histological discovery of fibres to these vessels<sup>1</sup> combined with an absolute lack of corroborative physiological evidence. For this reason Professor Lombard,

<sup>1</sup> GULLAND: *British medical journal*, Sept. 17, 1897, p. 78. OBERSTEINER: *Arbeiten aus dem Institut für Anatomie und Physiologie des Centralnervensystem an der Wiener Universität*, 1897, v, p. 215. HUBER: *Journal of comparative neurology*, 1899, ix, p. 1. HUNTER: *Journal of physiology*, 1902, xxvi, p. 465.

not willing to accept as final the negative results of active vaso-motor changes, suggested, in 1903, that the cerebral circulation might be studied by simpler and more direct physiological means than had been employed. In 1905 I published a method of studying the cerebral circulation in a direct manner.<sup>1</sup> The chief advantage claimed for the method was that it allowed a study of the brain circulation uninfluenced by the general blood pressure, — a factor which has given rise to no little misinterpretation in the study of brain reactions. The method consisted, in brief, in decapitating a dog, ligating intracranially all communications with the Circle of Willis, and then inserting a cannula into the basilar artery. The isolated brain was thus alone perfused at a constant pressure and temperature with a pulsating stream of Locke's solution. Thus prepared, the brain, still within the skull, was suspended in a warm chamber above a drip-registering apparatus, into which fluid dropped from the most dependent portion of the preparation. Thus the entire outflow with changes in its rate was constantly recorded. Since that report the apparatus, continuously recording the outflow, has been much improved upon. It is shown in Fig. 1.

The curve written by this apparatus on an evenly moving drum is a straight oblique line if the outflow be constant. It becomes curved as soon as the outflow rate is changed. Fig. 2 shows this diagrammatically. This means of showing slight and temporary changes in outflow immediately and accurately is not shared by a large number of devices used to study the rate of flow from veins. The crude and often fallacious method of counting or recording the number of drops flowing from a vein cannot do this, and is, moreover, not applicable to continuous outflow streams. Comparing by direct measurement or graphic registration the number of cubic centimetres flowing through an organ in equal time intervals,

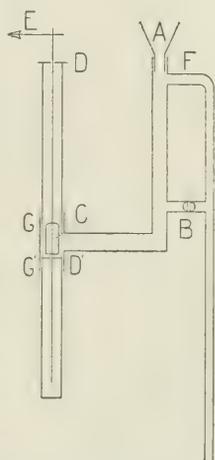
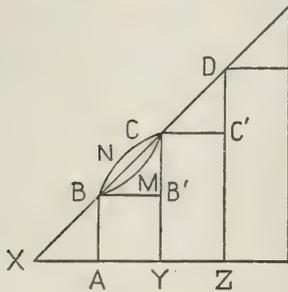


FIGURE 1. — *Drip registering apparatus.* — Fluid from preparation drips into funnel *A*. With stopcock *B* closed, fluid rises in both limbs, carrying up a bell float (*C*) with vaselined steel rod. *D* and *D'* are two guides; the latter is of platinum. The rapidity of rise is recorded on the drum by a straw pointer (*E*). *F* is an overflow tube. The apparatus dis-joints for cleaning at *G* and *G'*.

<sup>1</sup> For a more detailed description of the apparatus used, my former report may be consulted (*This journal*, 1905, XIV, p. 452).

or determining the time required for definite quantities to flow through an organ, are methods which give no indication of the nature of the flow between measurements. In Fig. 2 let the vertical distances  $AB$ ,  $B'C$ , and  $C'D$  each represent an outflow of 10 c.c., and the equal distances  $XA$ ,  $AY$ , and  $YZ$  on the abscissæ one-minute intervals. Measurements of the distances  $AB$ ,  $B'C$ , and  $C'D$  at the end of each



minute will then show that 10 c.c. have passed through the organ in that time. It gives no indication of what occurred during that minute. The outflow may have been constant, as shown by the straight line  $BC$ ; it may have been slowed at first and accelerated later, as shown by the curved line  $M$ ; or it may have been accelerated at first to be

slowed later, as shown by the curved line  $N$ . *Important changes are thus often lost if a continuous outflow record is not taken in perfusion experiments.*

As constriction between the arterial and venous systems in an organ ought certainly to diminish the outflow, it was thought that such continuous records would give evidence of the slightest vascular change in the organ. The results of some eighty perfusion experiments on the brain and kidneys, however, have shown that changes in the vessels may occur without causing changes in the outflow record which differ appreciably from those frequently occurring in normal outflow records. This occurs:

1. In cases in which the vaso-motor reaction is very slight or temporary and the flow through the organ is large. The time is then too short to cause an appreciable change in the outflow rate.

2. In cases in which opposite vaso-motor changes follow each other very rapidly. In these cases the one reaction tends to neutralize the other. I have obtained curves in which a dilation of a few seconds duration, followed by a temporary constriction, showed no change in outflow; also those showing an increase, others a decrease. The predominant changes alone were recorded.

For these reasons changes in the rate of outflow cannot be utilized in these cases, and a more delicate criterion must be selected. Such a one is found in the characteristic pressure variations in the tubes supplying the organ. The pressure for the perfusion fluid was created by allowing the water, kept at a constant level by the apparatus pic-

tured in Fig. 3, to flow into an oxygen-containing bottle, thus displacing the oxygen into the bottle containing the Locke's solution, the latter being driven into the cannula by the pressure created. Between the oxygen bottle and cannula a stopcock, regularly opened and closed by a cam and motor, is interposed, and the oscillating lateral pressure between the cannula and stopcock is recorded by a mercury manometer. The curve written on the drum resembles a blood-pressure curve with its cardiac oscillations. In some experiments these oscillations were further magnified by an aluminum lever actuated by the manometer float.

The changes which take place in this curve on increasing the peripheral resistance are quite different from those that we are familiar with in a blood-pressure record, for the lowest or diastolic portion of the oscillations feels the peripheral change much more than the upper or systolic portion. This is shown in Fig. 4, in which an increased peripheral resistance was artificially produced by gradually tightening a clamp on the outflow tube. The record shows that, while the systolic portion of the oscillations always rises, the change may be so slight as to be almost unmeasurable, for example, from *A* to *B* or from *C* to *D*. The diastolic change, on the other hand, is always evident. The reason that the systolic portion of the oscillations should be so little affected by slight peripheral changes is found in the arrangement of the apparatus. The manometer as arranged would only record the pressure created by the pressure-regulating device when the outflow from the cannula had entirely stopped. As a portion of this pressure is converted into velocity pressure when fluid leaves the system, it follows that the highest pressure recorded by this apparatus, when an organ is perfused, depends, not only on the pressure created, but also on the size of the outflow tube, as compared with the size of the tube carrying water under pressure into the oxygen bottle. The recorded

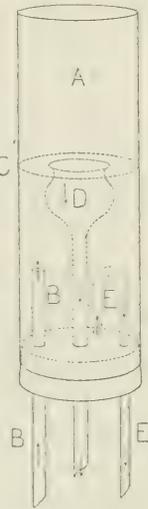


FIGURE 3. *Pressure-regulating apparatus.* — *A*, cylindrical lamp chimney with cork in bottom. Water from tap flows in through tube *B*, and reaching level *C*, flows off by thistle tube *D*. This insures a constant pressure level. Tube *E*, bent in U-form to prevent entrance of air, communicates with oxygen bottle. The whole apparatus is raised and lowered by pulley arrangement.<sup>1</sup>

<sup>1</sup> The principle of this apparatus was copied after a similar apparatus used by Professor Cushny.

pressure, which is referred to as systolic in this paper, is altered only by such marked changes in the vessels as cause a pronounced difference between the rate of outflow from the cannula and the rate of inflow into the oxygen bottle. Slight constriction causes little rise, because the slight change in outflow is evenly counteracted by an opposite change in inflow, so that their relative proportion is not disturbed.

The diastolic pressure, on the other hand, is being recorded when the moving stopcock entirely closes off the manometer from the central pressure. Whatever changes of pressure occur during this interval can only be attributed to changes in the calibre of the perfused vessels. As these drain the tubes distal to the stopcock, the extent to which the pressure falls, as well as the rate of fall, depends entirely on the degree of constriction or dilation present.

In this research both the changes in the rate of outflow and the changes in the diastolic pressure were utilized to study vaso-motor changes in the organs, and the one method served as a check on the other.

## II. THE ACTION OF DRUGS ON THE CEREBRAL VESSELS.

The use of drugs constitutes at present a much employed and convenient means of studying the physiological reactions of blood vessels. In this investigation their use was at first restricted to the determination of the existence of an active vaso-motor response in the cerebral vessels. Such a response was proved by the experiments reported in my former paper.<sup>1</sup> When, through the experiments of Lewandowsky,<sup>2</sup> Langley,<sup>3</sup> Brodie and Dixon,<sup>4</sup> Elliott,<sup>5</sup> and Dale,<sup>6</sup> the idea became prevalent that the presence of a vaso-motor innervation could be proved by the use of certain drugs, further experiments were performed to determine whether the reaction of the cerebral vessels to these drugs necessitated the assumption that they possessed such an innervation. To this end the actions of those drugs to which a neural or nerve end action has been assigned were compared with others supposed to affect the calibre of vessels by a direct muscular action.

<sup>1</sup> WIGGERS: This journal, 1905, xiv, p. 452.

<sup>2</sup> LEWANDOWSKY: *Archiv für Anatomie und Physiologie*, 1899, p. 360.

<sup>3</sup> LANGLEY: *Journal of physiology*, 1901, xxvi, p. 237.

<sup>4</sup> BRODIE and DIXON: *Journal of physiology*, 1904, xxx, p. 476.

<sup>5</sup> ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 401.

<sup>6</sup> DALE: *Journal of physiology*, 1906, xxxiv, p. 163.

The ability of one drug to modify the action of another was also retested, and all the results were then compared with controls on the renal vessels.

During the course of this investigation a number of points irrelevant to the conclusions sought were brought out, and these are included for their general pharmacological interest.

I. **The action of adrenalin.**<sup>1</sup> *Solution used.*—In order to obtain a solution of adrenalin of calculated strength, small quantities of the crystalline product (P. D. & Co.) were weighed out into capsules. Just before use one of these capsules was dissolved by heating with a small amount of Locke's solution, and then immediately cooled by the addition of cold Locke's solution to the required dilution. This method yielded a relatively little oxidized solution.

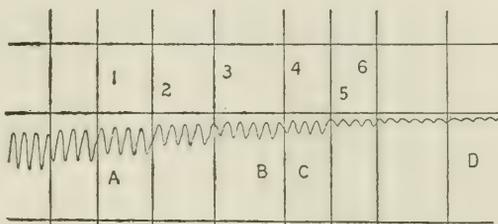


FIGURE 4.—Shows the effect of increasing the resistance to the outflow from the cannula by means of a screw clamp. The diastolic pressure is affected more than the systolic, and the descending limb of each wave becomes more gradual as the resistance is increased.

*Delicacy of response.*—From twenty-five experiments on the cerebral vessels, controlled by a like number on the kidney, it was found that the cerebral vessels constricted when doses of adrenalin ranging from 3 c.c. of a 1 : 250 solution to 3 c.c. of a 1 : 20,000 solution were introduced.<sup>2</sup> Greater dilutions proved negative. In some cases, however, the vessels failed to react to such weak solutions. Thus, in one experiment, 2 c.c. of a 1 : 5000 solution failed to cause a reaction. Two and one-half cubic centimetres caused a slight, and 3 c.c. a marked, reaction (Table I). The kidney vessels were constricted with 1 : 100,000 solutions, but not by weaker ones, as discernible by this method. These results demonstrate that, although the kidney vessels reacted to far weaker solutions than those of the brain, the latter, far from having no reaction, reacted even beyond expectation when the paucity of muscular elements in their wall is considered.

<sup>1</sup> The literature on the action of adrenalin was reviewed in my former paper (*loc. cit.*), and discussion of the relative value of these researches was made there.

<sup>2</sup> The drugs in all these experiments were introduced without changing the pressure, temperature, or composition of the perfusion fluid, by the method described in my former paper (*loc. cit.*).

TABLE I.  
ACTION OF ADRENALIN ON CEREBRAL VESSELS.

Number of experiment.	Strength of solution used.	Latent period in sec.	Maximal constrict. in min.	Outflow per 30 sec. before.	Outflow per 30 sec. after.	Percentage <sup>4</sup> outflow difference.	Extent of recovery.	Recovery in minutes.	Diastolic pressure change.
11	3 c.c., 1:250	0	1.5	c.c. 2.8	c.c. 0.9	68.0	c.c. none	..	R. <sup>5</sup>
2	3 c.c., 1:500	38 <sup>1</sup>	3.0	5.0	1.1	78.0	2.3	10.5	R = 3 mm.
5	3 c.c., 1:500	..	1.0	2.3?	1.1	52.0	none	none	R = 1 mm.
21	3 c.c., 1:500	0	3.5	4.7-	1.1	76.0	2.6	5.75	R = 3 mm.
36	3 c.c., 1:500	38 <sup>1</sup>	1.5	4.0	2.8	30.0	none	none	R.
	3 c.c., 1:500	..	2.0	10.0	8.3	17.0	none	none	....
30	3 c.c., 1:500	..	0.5	8.3	7.4	10.0	..	..	....
	3 c.c., 1:750	0	..	..	..	..	..	..	R = 2 mm.
3	c.c., 1:750	4 <sup>2</sup> , 10 <sup>3</sup>	0.66	5.2	4.4	11.0	5.2	5.0	R = 1.5 mm.
3	c.c., 1:750	0	1.0	5.7	4.4	22.5	5.7	2.0	R = 1 mm.
3	c.c., 1:500	6 <sup>2</sup> , 12 <sup>3</sup>	..	4.7	4.2	10.0	..	..	....
51	3 c.c., 1:1,000	..	1.0	..	less	..	complete	2.0	R = 0.5 mm.
45	1 c.c., 1:2,000	0	1.5	3.4	2.7	20.0	3.1	2.0	R = 1 mm.
1	c.c., 1:2,000	0	0.75	3.8	2.1	44.0	2.4	2.0	R = 1 mm.
56	3 c.c., 1:2,000	..	0.17	4.1	3.3	20.0	4.1	3.5	R = 0.5 mm.
46	2 c.c., 1:5,000	..	..	..	No reaction.	..	..	..	..
	2.5 c.c., 1:5,000	0	1.0	4.0	3.1	22.5	4.0	..	R = 0.5 mm.
3	c.c., 1:5,000	0	1.0	3.4	2.6	21.5	3.4	3.0	R = 0.5 mm.
85	3 c.c., 1:20,000	..	1.0	4.4	3.6	18.0	4.1	3.0	R = 0.5 mm.

ACTION OF ADRENALIN ON RENAL VESSELS.

35	3 c.c., 1:500	0	0.5	7.0	5.3	24.0	7.0	2.0	R = 8 mm.
	3 c.c., 1:500	0	0.33	13.0	11.5	11.5	13.0	1.0	R = 14 mm.
31	3 c.c., 1:750	0	0.25	7.5	4.6	38.0	5.9	6.0	R = 1 mm.
	3 c.c., 1:750	0	1.0	5.8	3.8	34.0	complete	..	R = 1 mm.
37	3 c.c., 1:1,000	0	1.0	8.1	5.3	34.5	8.0	1.0	R = 1 mm.
	3 c.c., 1:10,000	..	1.0	7.0	5.8	17.0	7.0	1.5	R = slight.
40	3 c.c., 1:2,000	0	..	4.4	1.1?	80.?	4.4	..	....
	3 c.c., 1:2,000	0	0.25	5.8	5.3	8.6	5.8	1.5	R = 0.5 mm.
53	3 c.c., 1:4,000	..	..	19.0	10.7	43.0	19.0	1.4	R = 26 mm.
	3 c.c., 1:8,000	..	..	18.2	10.0	45.0	18.2	1.4	R = 22.5 mm.
	3 c.c., 1:12,000	..	..	16.6	10.6	36.0	16.6	1.3	R = 23 mm.
	3 c.c., 1:20,000	..	..	18.0	10.8	40.0	18.0	1.16	R = 22.5 mm.
	3 c.c., 1:40,000	..	..	20.0	13.1	34.5	20.0	1.05	R = 22 mm.
	3 c.c., 1:60,000	..	..	15.0	11.1	26.0	15.0	1.0	R = 18 mm.
	3 c.c., 1:80,000	..	..	..	..	..	complete	0.66	R = 18.5 mm.
	3 c.c., 1:100,000	..	..	20.4	16.0	17.0	complete	0.5	R = 8.5 mm.
	3 c.c., 1:200,000	..	..	..	No reaction	..	..	..	..
	3 c.c., 1:200,000	..	..	..	No reaction	..	..	..	..
	3 c.c., 1:10,000	..	..	20.5	11.5	43.5	complete	1.5	R = 24 mm.

<sup>1</sup> Sodium nitrite tested before adrenalin in this experiment.

<sup>2</sup> Result calculated.

<sup>4</sup> Percentage outflow difference represents the results obtained by making the proportion, Outflow difference : Flow before drug

= X : 100.

<sup>6</sup> The letter R in last column means "rise."

<sup>3</sup> Result obtained by color method.

It is to be noticed that the degree of dilution to which the vessels reacted was never found to approximate the figure given by Meyer.<sup>1</sup> He showed that a strip of artery, if immersed in adrenalin solution, would react to dilutions as great as 1 : 1,000,000. Attention should be directed to the fact that a greater quantity of adrenalin was available for his immersed strip than would be available for the vessels of an organ perfused with the same quantity of solution; for in the latter case it must be distributed to a much larger surface of vessel wall, and remains in contact but temporarily. Moreover, the magnification of the contraction used by Meyer undoubtedly enabled the recognition of slighter reactions than could be hoped for by ordinary methods.

*The latent period.* — Oliver and Schäfer,<sup>2</sup> in their study of suprarenal extract, reported that the rise of pressure always occurred after an interval of latency. Regarding it, they say, "No doubt this interval is mainly occupied by the passage of the extract injected from the peripheral vein toward the heart, and thence to the arterial system." The "latent period" results of most investigators do not deduct the time required for the injected drug to pass through the animal or apparatus to its point of intended action. Such results are variable and misleading in comparisons. I fell into this error in my former paper when I showed that my latent periods with adrenalin were in harmony with those of S. J. and C. Meltzer.<sup>3</sup> These authors had observed that the cutting of nerves to the ear vessels appreciably lengthened the latent period of adrenalin, — an observation from which it must be inferred, either that the cutting of nerves affected the ability of vessels to react as readily, or that the first constriction resulting is of central origin. My substantiation of their long latent-period results must be retracted, for the fact is that the latent-period results of one worker cannot be compared with those of another if the latent period of the animal or apparatus be included, or if an average latent period for the apparatus be deducted. The reason is that, even were the quantity of fluid ahead of the drug always constant, the time taken to displace it would depend on the rate of flow through the organ, and must therefore vary in and during each experiment. For this reason the term "latent period" is employed in this paper to denote the time elapsing between the entrance of the

<sup>1</sup> MEYER: *Zeitschrift für Biologie*, 1906, *xlvi*, p. 3.

<sup>2</sup> OLIVER and SCHÄFER: *Journal of physiology*, 1896, *xviii*, p. 230.

<sup>3</sup> MELTZER, S. J. and C.: *This journal*, 1903, *ix*, p. 147.

drug into the blood vessels and the time a reaction occurs. Knowing the total latent period, the total amount of fluid passing through the organ during this time, and the amount that must be displaced in the apparatus before fluid reached the vessels, a little mathematical calculation can approximately determine this actual latent period. The results obtained by this calculation are not infrequently slightly negative, due, probably, to unequal onward diffusion of the drug in the different experiments. For this reason this method is less exact than another procedure employed, namely, to add to the injected drug, if this was not itself colored, some inert color, and mark with a time signal the time of its appearance in the end of the cannula.

Results obtained from such procedures can lay claim to no minute degree of accuracy, but are sufficiently correct to warrant the conclusion that adrenalin produces its reaction very shortly after the drug is in contact with the vessels, and that separation from the central nervous system does not actually lengthen the latent period.<sup>1</sup> These results are confirmed by those reported by Meyer. He showed that a strip of artery removed from the body reacted a few seconds after it was in contact with the drug.

*Course of the reaction.* — The results recorded in Table I show that the maximal degree of constriction, measured from the time a reaction began, was somewhat more rapidly reached in the kidney than in the brain vessels. In neither organ could any relation between the size of the dose and the rapidity of onset be determined. As regards the degree of reaction, the results show that a great variation exists in differences in outflow before and after the injection of the drug. To compare degree of outflow in the various experiments, however, such actual differences in the outflow, measured for thirty-second intervals before and after the use of the drug, must first be converted into percentage figures of the total flow through the organ at that time. These figures may conveniently be termed the *percentage outflow differences*. A glance at this column of figures shows that the degree to which the vessels reacted was but little dependent on the strength of the adrenalin solution used. In the cerebral vessels, for example, a 1 : 5000 solution could cause either a greater or smaller constriction than a 1 : 500 solution.<sup>2</sup> The same was true in the ves-

<sup>1</sup> In the two experiments shown in Table I in which longer latent periods were obtained, sodium nitrite had previously been used. For this reason they cannot be looked on as exceptions to this statement.

<sup>2</sup> Cf. Experiments 2, 36, and 46.

sels of the kidney. We may conclude, then, that a weak dose of adrenalin, the actual size of which must vary with the number and size of the vessels in the organ as well as their state of irritability at the time of application, supplies a stimulus to the vessel walls sufficient to cause a maximal response. The 1 : 40,000 solution in Experiment 53 may be looked on as such a threshold dose, just capable of producing a maximal constriction. Any smaller dose (1 : 60,000) caused a slighter degree of constriction. Any larger one, on the other hand, had no appreciably greater effect.

It cannot be said, however, that strong doses of adrenalin react in every respect like such a threshold dose, for the table shows that, though the degree may not have been affected, the duration of the reaction, as well as the completeness of recovery, vary directly with the strength of the dose. This is shown not only by successive applications of weaker solutions to the same preparation, in which the duration became less and less, as in Experiment 53; but also by applications of varying strengths to different preparations.

As regards the completeness and rapidity of recovery the cerebral vessels differed from those of the kidney, in that strong solutions caused a much more prolonged reaction, the recovery was slower and more imperfect, or at times failed altogether. This, however, cannot be regarded as a property peculiar to the cerebral vessels. The explanation is a mechanical one. The less spacious vascular channels of the brain allow fluid to pass through them less rapidly than do those of the kidney, so that the brain vessels, arteriole for arteriole, are exposed more thoroughly to the adrenalin than those of the kidney, which allow a greater portion to pass through unused. This explanation is based on the easily demonstrated principle that, if the time that the vessels of the perfused organ are exposed to a drug is altered, the duration of the reaction is changed. This was experimentally shown in several ways:

1. By augmenting the flow through an organ by raising the perfusion pressure. This was done in Experiment 35 on the kidney. Two successive doses of a 1 : 500 solution of adrenalin were applied. After the first dose the reaction passed off in two minutes. Before the second dose was applied the pressure was raised so as to almost double the flow through the organ, and the action of the second passed off in one minute.

2. By perfusing a part of an organ. If only one half of the kidney was perfused by ligating one of the two main branches of the

renal artery, the kidney vessels recovered as slowly and imperfectly as those of the brain.<sup>1</sup>

3. By utilizing the development of œdema which probably diminished the calibre of the vessels. It was found that when such œdema occurred, the adrenalin caused a much more prolonged reaction than when it was not present. In Experiment 53, for example, a 1 : 10,000 solution at the end of the experiment, when œdema was present, caused a more prolonged reaction than a 1 : 4000 solution at the beginning.

2. **The action of barium chloride.** *Literature.*—There seems to be but little literature on the peripheral action of barium chloride, and, as far as the cerebral circulation is concerned, no reference has come to my notice. In 1875 Boehm and Mickwitz<sup>2</sup> observed that an intravenous injection of barium chloride caused a constriction of the blood vessels in many parts of the body. Ringer and Sainsbury,<sup>3</sup> in 1883, came to the conclusion that the cause of the constriction was due to a direct muscular action. Ringer,<sup>4</sup> in 1886, showed that a 2 per cent solution caused a contraction of perfused vessels. Brodie and Dixon,<sup>5</sup> in 1904, described the course of action of barium chloride. They found that after a long latent period a constriction of slow onset but of persistent character was obtained with moderate doses. Larger doses caused a more rapid constriction, and this was preceded by a preliminary dilation.

*Solution used.*—Barium chloride was dissolved in Locke's solution to the per cent desired, and the slight precipitate forming allowed to settle. Only the supernatant fluid, which still contained barium as tested, though in somewhat smaller proportion than calculated, was used. The solutions used varied in dilution from 1 : 1000 to 1 : 10.

*Course of reaction.*—Barium chloride was applied to the normal vessels of the brain in only three experiments, and two controls on the vessels of the normal kidney were made; but it was applied in a dozen other experiments after other drugs had acted, which did not alter the general character of the reaction. In stronger concentrations barium chloride caused, sometimes after a slight preliminary dilation, a marked and rapid constriction of the cerebral vessels,

<sup>1</sup> Cf. Experiment 5 with Experiment 31, Table I.

<sup>2</sup> BOEHM and MICKWITZ: Archiv für experimentelle Pathologie und Pharmakologie, 1875, iii, p. 216.

<sup>3</sup> RINGER and SAINSBURY: British medical journal, 1883, ii, p. 265.

<sup>4</sup> RINGER: Journal of physiology, 1886, vii, p. 306.

<sup>5</sup> BRODIE and DIXON: Journal of physiology, 1904, xxx, p. 486.

as shown by a rapid decrease in outflow and a diminution in size of the pressure curve oscillations. The actual latent period before the reaction occurred was zero. No sign of recovery was present, although one experiment was continued for two hours. The vessels

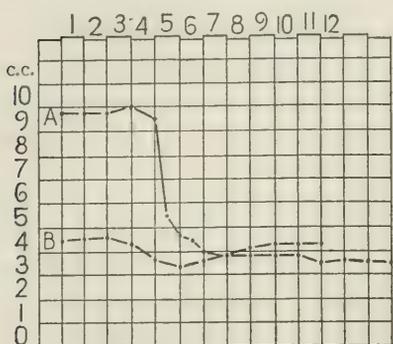


FIGURE 5.— Outflow from the cerebral vessels per thirty seconds after application of barium chloride. (A) The effect of strong solutions  $\text{BaCl}_2$ , 1-10, (B) effect of weaker solutions  $\text{BaCl}_2$ , 1-50. No recovery in two hours.

no longer reacted either to heat or cold, either to the nitrites or an increased perfusion pressure (Fig. 5, A).

In weaker applications the drug caused, without any actual latent period or preliminary dilation, a smaller but easily recognizable constriction, which was followed by recovery in three minutes, as shown by changes in oscillations and outflow (Fig. 5, B). A 1 : 500 solution seemed to be the weakest to cause any distinct reaction.

After recovery from such dilutions other drugs were still able to act. The following synopsis of a typical experiment shows the effect of weak solutions more in detail:

*Experiment 65. March 12, 1907.*— Dog decapitated at 2 P. M. Brain perfused at 3 P. M.

- 3.15. Barium chloride 1 : 1000, 3 c.c. No result.
- 3.19. Barium chloride 1 : 1000, 3 c.c. No result.
- 3.26. Barium chloride 1 : 500, 3 c.c. Very slight, slow constriction.
- 3.30. Barium chloride 1 : 200, 3 c.c. Constriction. Latent period, 0 ; maximal constriction in one hundred seconds ; change in outflow per thirty seconds from 1.8 to 1.3 c.c. ; recovery in six minutes to 1.8 c.c. Diastolic pressure rose.
- 3.38. Barium chloride 1 : 200, 3 c.c. Constriction. Latent period, 0 ; maximal constriction in one hundred and seventy seconds ; change in outflow per thirty seconds from 1.8 to 0.7 c.c.
- 3.52. Chloretone 1 : 125, 3 c.c. Dilation. Change in outflow per thirty seconds from 0.7 to 1 c.c.

The kidney vessels showed essentially the same reactions.

The reactions obtained in these experiments differ from those obtained by Brodie and Dixon in the absence of any latent period

and in the rapid course of onset. Their latent period results can probably be accounted for by inclusion of the period of the apparatus.

3. **The action of chloroform.** *Literature.*— In the Transactions of the Royal Society of Edinburgh<sup>1</sup> Schäfer and Scharlieb review the literature on the peripheral action of chloroform. They themselves studied the action of chloroform on the coronary, limb, and renal vessels by the perfusion method, and found that a constriction occurred with dilutions as great as 1 : 20,000, in the coronary and limb vessels, while the kidney vessels were dilated by those beyond 1 : 1000. The actual number of cubic centimetres of the chloroform solution allowed to go through the organ was not stated, but one infers that it was quite a large quantity.

The action of chloroform on the cerebral vessels has never been made the subject of any very accurate work. Schüller<sup>2</sup> thought he could observe a progressive constriction of the pial vessels with each inhalation of chloroform. Gaertner and Wagner,<sup>3</sup> by measuring the rapidity of flow from the jugular vein, and Hürthle,<sup>4</sup> by measuring the pressure in the peripheral end of the internal carotid, came to the conclusion that an active dilation occurred. Bayliss and Hill<sup>5</sup> showed that these results could be as well explained by an increased general venous pressure, and assumed that no active process in the brain vessels occurred. Roy and Sherrington<sup>6</sup> had previously reported a diminution in the size of the brain during narcosis, and therefore assigned a constricting power to chloroform. Pick<sup>7</sup> found an increased outflow from the external jugular vein at the same time that the flow from the femoral vein was slowed,—a combination of events which he deemed sufficient proof of an active cerebral dilation.

*Solution used.*— In all experiments a saturated chloroform solution (1 : 200) was used for a stock supply from which dilutions were made when necessary. In every case 3 c.c. of the solution to be tested was allowed to flow through the organ.

*Course of reaction.*— In studying reactions of the vessels to chloroform it was soon discovered that even a continuous outflow record did

<sup>1</sup> SCHÄFER and SCHARLIEB: 1904, xli, p. 311.

<sup>2</sup> SCHÜLLER: Berliner klinischer Wochenschrift, 1874, p. 295.

<sup>3</sup> GAERTNER and WAGNER: Wiener medicinische Wochenschrift, 1887, p. 602.

<sup>4</sup> HÜRTHLE: Archiv für die gesammte Physiologie, 1889, xliv, p. 561.

<sup>5</sup> BAYLISS and HILL: Journal of physiology, 1895, xviii, p. 334.

<sup>6</sup> ROY and SHERRINGTON: Journal of physiology, 1890, xi, p. 85.

<sup>7</sup> PICK: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xlii, p. 412.

not present as trustworthy a picture of the behavior of the vessels as a record of side pressure oscillations, and these were largely made use of in interpreting results. The kidneys of dogs, cats, and rabbits were used, while the study of the brain reactions was of necessity limited to dogs. The results of twenty applications show that in both brain and kidneys a 1:200 solution of chloroform constricted the vessels. The differences between the reactions in these organs were similar to those found with adrenalin, and were probably attributable to similar causes. The constriction occurred without an appreciable latent period, and reached its maximum rapidly in both brain and kidney. Recovery was slower in the former. With progressive dilution of a 1:200 solution to a 1:2000 solution, the vessels not only reacted less and less by constriction, but this diminished constriction was followed by a dilation more and more marked. Thus, in Experiment 59, on a cat's kidney, 3 c.c. of 1:200 solution caused constriction from which the vessels recovered in sixty seconds. A 1:400 solution caused a similar constriction of shorter duration. A 1:600 solution caused a slight constriction, as shown by the oscillation curve, but not in the outflow record. This was followed by a dilation distinctly evident in both curves. A 1:1000 solution showed no evidence of constriction, but only a dilation.

The same type of reaction was true of the cerebral vessels. In Experiment 64 a 1:4000 chloroform solution caused a dilation only, with recovery in eighty seconds. A 1:2000 solution as well as a 1:1000 solution caused this dilation only after a preliminary constriction. A 1:500 solution caused a constriction only. Similar results were obtained in other experiments. These are again referred to.

These results warrant the following deductions concerning chloroform:

1. The kidney vessels are not the only vessels, as Schäfer and Scharlieb claim, on which the weaker solutions of chloroform exert a dilating influence. The cerebral vessels at least react like those of the kidney. This makes it questionable whether such a difference between various vessels of the body, as Schäfer and Scharlieb describe, exists.

2. No definite concentration of the chloroform in the perfusion fluid can be placed as the limit which determines the reaction as being one of constriction or dilation. The strength which in 3 c.c. doses produces dilation first may be 1:4000, as in one brain ex-

periment: it may be 1:600, as in one kidney experiment. In fact, the amount injected and the rapidity with which this flows through the organ largely determines the result.

3. Chloroform in solution has a twofold action on blood vessels, constricting and relaxing. In many cases where a dose was adjusted to the outflow so as to fall between the doses producing only constriction and those producing only dilation, it could be determined that a dilation followed the constriction. Any stronger dose diminishes the constriction, while any weaker dose increases the following dilation. The predominant changes regulate the amount of fluid passing through the organ in definite time intervals.

4. **The action of digitalis.** *Previous work.*— It has been shown repeatedly by perfusion experiments that digitalis in different preparations has a peripheral action on blood vessels. Gottlieb and Magnus<sup>1</sup> have given a complete review of the work done. These authors studied its action on the cerebral vessels by the use of three methods, namely, (1) by inspecting the pial vessels, (2) by recording changes in the volume of the brain, and (3) by noting changes in outflow from a cerebral vein consequent to the injection of the drug. As a result, they report that strophanthin as well as digitoxin constricted the cerebral vessels. As these results were obtained, however, by methods which introduced the variable general blood pressure as a factor, they should be accepted with reserve, and should be subjected to reinvestigation.

*Preparations used.*— Two preparations of digitalis were chosen for this work, the pure principle digitalein (Merck), and an infusion prepared to U. S. P. strength by steeping 15 parts of bruised digitalis in 1000 parts of Locke's solution. These preparations were found most suitable, as they were easily dissolved in Locke's solution and contained no substances which modified the pure digitalis action. The latter objection was found against such preparations as the tincture, fluid extract and digitalone, which were also tested.

*Nature of the reaction.*— The active principles of digitalis, as shown by the use of digitalein and the infusion, affect the peripheral blood vessels of the brain as well as the kidney by inducing a constriction. In no instance was a dilation produced. Only small doses of digitalein were required to cause a reaction (0.01 mg. in the kidney and 0.05 in the cerebral vessels). The constriction resulting from such

<sup>1</sup> GOTTLIEB and MAGNUS: *Archiv für experimentelle Pathologie und Pharmakologie*, 1901, xlvii, p. 135; also 1902, xlviii, p. 262.

doses reached its maximum rapidly (7-11 seconds), and complete recovery followed (Curve 1, Fig. 6). Doses larger than 0.1 mg. caused a more marked constriction, from which the vessels either failed to recover at all or did so only slightly. The induction of a tonic state of contraction seemed to be the most characteristic action of the drug (Curve 2, Fig. 6). Each succeeding dose of digitalein caused more and more of a permanent constriction. After the vessels were thus

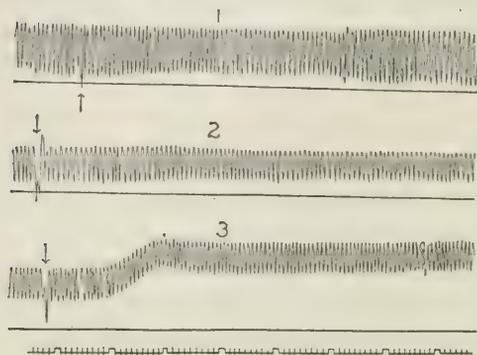


FIGURE 6. — Effect of digitalein on blood vessels as determined by changes in the diastolic pressure. (1) 0.05 mg. on the cerebral vessels. (2) 2 mg. digitalein on the renal vessels. (3) 3 mg. digitalein on renal vessels. Time in seconds.

markedly contracted by these doses, they still retained the power of reacting to such drugs as adrenalin, chloroform, and barium chloride, and responded to an increase in the perfusion pressure with a greater outflow. It may be added that the reaction to the infusion of digitalis was of the same nature.

5. **The action of chloretone.** *Literature.* — As chloretone represents a product closely related to

chloroform, and as no exact investigations of its peripheral action have come to my notice, a few observations in regard to this drug were made on the brain and kidney vessels with the hope that it might prove an important drug for us.

The only reference to its peripheral action that could be found was that of Impens,<sup>1</sup> who observed a dilation of the blood vessels of a frog's foot after its injection. This, however, he attributed to a paralysis of the vaso-constrictor centre, inasmuch as a saturated chloretone solution perfused through a decapitated frog did not increase the outflow, but decreased it. Thus he supposed its purely peripheral action to be one of constriction.

*Solution used.* — One part of chloretone is soluble in 125 parts of Locke's solution. In this strength it was perfused through the brain and kidney vessels.

*Results.* — Chloretone caused only a dilation of the cerebral and

<sup>1</sup> IMPENS: Archives internationales de pharmacodynamie et therapie, viii, p. 77.

renal vessels. The dilation of the brain vessels lasted from one to two minutes, while in the kidney vessels their action was, for mechanical reasons, about half as long. The synopsis of two typical experiments show the general character of the reactions.

*Experiment 52.* December 16, 1906. — Dog decapitated at 2 P. M. Perfusion of kidney at 10.25 P. M.

10.59. Chloretone 1 : 125, 3 c.c. Maximal dilation in twenty-five seconds ; change in outflow per thirty seconds from 10 to 11.3 c.c. ; recovery not waited for.

*Experiment 36.* March 21, 1906. — Dog decapitated at 5 P. M. Perfusion of brain at 7 P. M.

7.08. Chloretone 1 : 125, 3 c.c. Maximal dilation in twenty-three seconds ; change in outflow per thirty seconds from 10.5 to 12.2 c.c. ; recovery in fifty seconds.

Some estimate of the power of chloretone to dilate vessels may be gained by studying its neutralizing action of some known constricting drug. Digitalis supplies a convenient drug for this purpose, and the following experiment illustrates the neutralizing effect. Three cubic centimetres of the infusion of digitalis caused a constriction reaching its maximum in twenty seconds, with partial recovery in fifty seconds. Then 3 c.c. of a mixture of equal parts of the above infusion and chloretone (1 : 125 solution) were perfused. The constriction occurring after digitalis alone was now replaced by a dilation lasting for about two minutes. In one case the dilation was preceded by a temporary constriction lasting five seconds. As small a quantity as three drops of a 1 : 125 solution of chloretone added to 3 c.c. of digitalis infusion was sufficient to diminish the constriction and the permanency of its duration. We may conclude, then, that chloretone has a marked, direct dilating effect on blood vessels, and, added to digitalis preparations, abolishes their action. It does not, however, in any way modify the subsequent action of digitalis ; for if a dose of chloretone was injected and allowed to cause a dilation, and then this was followed, before recovery, by the injection of a dose of digitalis infusion, the ordinary constriction course is run by the latter. As far as other experiments were conducted, they failed to show that chloretone affected, to any extent, the subsequent action of other drugs, as chloroform, barium chloride, or adrenalin.

6. **The action of apocodein.** *Previous work.* — Brodie and Dixon,<sup>1</sup> in their study of peripherally acting drugs, demonstrated some valu-

<sup>1</sup> BRODIE and DIXON: *Journal of physiology*, 1904, pp. 97 and 476.

able physiological reactions by means of apocodein. Injected into an animal in moderate doses, it caused a permanent fall of blood pressure, sometimes preceded by a slight preliminary rise. This fall was uninfluenced by section of the vagi, but was abolished by nicotine and diminished by section of the splanchnics. They assumed the action to be primarily on the ganglion cells, and the nature of the reaction a paralysis. If additional larger doses of apocodein were then injected, they caused no further fall, but a rise in blood pressure, which was attributed to a peripheral action of the drug. The peripheral action was more clearly demonstrated in perfusion experiments. For the characteristics of this peripheral action the above-mentioned authors may be quoted (page 497): "The first effect of an injection of apocodein during the course of a perfusion through the limbs, kidneys, or intestines, is to produce a constriction. This quickly passes off, and subsequent injections produce less and less effect, until after the injection of a few cubic centimetres of a 1 per cent solution no constriction is produced, and in most cases a dilation takes place. It is now found that the nerve endings are paralyzed. Stimulation of the vaso-constrictor fibres is entirely without effect." Adrenalin was also found to be without effect.

*Solution used.*—A  $\frac{1}{2}$  to 1 per cent solution of apocodein was used. In every case the solutions were prepared just before their use.

*Results.*—The results here reported were gathered from fifteen applications of the drug to the brain vessels, and a like number of controls on the kidney. All experiments were made on dogs. Applied to the cerebral and renal vessels in doses ranging from 0.5 to 20 mg., the drug invariably induced a constriction, attaining its maximum rapidly. This constriction never passed off to any extent, whether caused by weak or strong solutions, there being only a difference in the degree of reaction. In the kidney vessels the constriction was sometimes preceded by a slight temporary dilation, as shown by the diastolic pressure change.

The reaction of the cerebral vessels to successive injections varied in different experiments. The experimental data collected show that after 2 c.c. of a 1 per cent solution, further injection was without effect; while in other cases this dose was repeatedly introduced into the cerebral vessels, each time causing a constriction superimposed on that already existing. This reaction was well shown by the pressure oscillations as shown in Fig. 7, which represents segments of

the oscillations after the injections of this drug. After several applications of such doses, the reaction of the vessels was only temporary, with a prompt return to the tonic state of constriction established by the previous doses. Such reactions were usually slight constrictions (Fig. 7), but in two cases slight dilations occurred.

The kidney vessels, like those of the brain, reacted to each successive dose by an additional tonic constriction. In the experiments in which the vessels reacted to the first dose by a temporary dilation

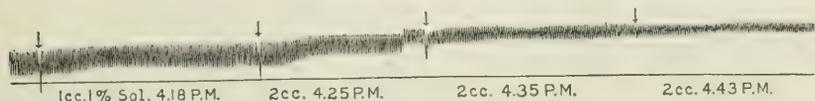


FIGURE 7. — Effect of successive injections of apocodein on the cerebral vessels as shown by sections of pressure oscillations.

followed by constriction, each succeeding dose caused less constriction, but the temporary dilation increased in degree and duration. When this dilation was maximal, the calibre of the vessels never approximated the normal, as shown by a diminished outflow rate, and the height of the diastolic pressure. Even after large doses had been introduced, the vessels still reacted to an increase in the perfusion pressure by a greater outflow. This was tested in two experiments on the cerebral vessels and in three on those of the kidney.

*The action of apocodein after digitalein.* — The great similarity in the tonic constriction produced by these two drugs suggested the question, To what extent does the previous action of digitalein modify the action of apocodein? Only two experiments were made on the cerebral, and six on the renal vessels. The kidney was better suited for this investigation than the brain, because of its greater vascular supply and because a control could be made on the opposite organ. The results in each case show, not only that digitalein possesses the power of reducing the action of apocodein, but also that large doses could convert its action into a temporary constriction, followed even by a slight dilation. In Fig. 8 curve 1 was made as a control on the right kidney of a dog. Curves 2, 3, and 4 were taken on the left kidney of the same dog after previous application of digitalein had been made, under as similar conditions as possible. These latter curves show that the degree of constriction, as well as its duration, was markedly diminished. This could not be assigned entirely to the fact that the vessels were constricted to their greatest degree, for in the

same experiment adrenalin and chloroform still caused their typical reactions.

**7. The action of drugs after apocodein.** *The action of adrenalin.*— Experiments performed on both the brain and kidney vessels showed that the action of adrenalin could be entirely abolished by previous application of apocodein. The dose of apocodein necessary to do this was found to vary. In some experiments 10 mg. were sufficient while in others three times this quantity did not entirely abolish its action. The dose required was smaller if injected in a few larger doses than if introduced in numerous small ones. The time interval between the injection of the drugs, apocodein and adrenalin, varied from one hour to one minute, and seemed to be without influence on the effect.

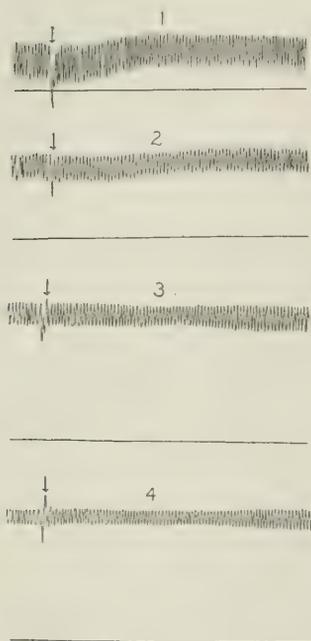


FIGURE 8.— Effect of apocodein after digitalein: (1) One-half c.c. apocodein (1 per cent) on normal right kidney. (2) One-half c.c. apocodein (1 per cent) on left kidney after 3 mg. digitalein. (3) Same after 6 mg. digitalein. (4) Same after 12 mg. digitalein.

If the dose of apocodein was not sufficient to cause complete abolition of the adrenalin action, it reduced it. A comparison of the pressure curves resulting from the introduction of adrenalin after previous action of various-sized doses of the apocodein showed that the latter shortened both the degree and duration of the reaction of the former (Fig. 9).

*The action of barium chloride.*— After the administration of apocodein in such dose that adrenalin failed to act, barium chloride, applied from seven to twenty-eight minutes after, did so. The reactions did not differ in their general character from those obtained on the normal vessels of the brain and kidney, although there was no longer so marked a tendency for the reactions from weaker solutions to pass off. This was probably partly due to some mechanical cause, as discussed under adrenalin. In two experiments the reactions to strong solutions (2 to 5 per cent) differed from those normally obtained by an augmentation of the dilation preceding the constriction (Exp. 33 and 35).

*The action of chloroform.*—As far as the reaction to chloroform was concerned, the vessels were not altered by the previous introduction of apocodein. This may be said both of strong solutions, which still constricted, and weak ones, which still dilated after the vessels failed to react to adrenalin. Moderate doses still caused both dilation and constriction, and, as far as could be determined by the limited number of experiments, the relative degree and duration of each was not affected.

*The action of digitalis.*—It will be recalled that digitalis, by inducing a tonic constriction, had the power in suitable doses of preventing the subsequent action of apocodein. The results cannot so readily be reversed, as the experiments, with one exception, show, for digitalein still acted after large doses of apocodein had been administered from two and one half to sixteen minutes before. In each case the constriction was superimposed on the one previously induced by apocodein, and for this reason was necessarily less marked than on the normal vessels.

*The general relation of apocodein to other drugs.*—Two experiments—one on the brain, the other on the kidney—could be regarded as model ones, for they permitted, before œdema set in, the introduction of a number of drugs, and thus condensed into one the results of other experiments showing the relation of apocodein to other drugs. A brief account of the experiment on the cerebral vessels will be given. That on the kidney was practically identical. Seven milligrams of digitalein were injected in three doses, each causing an additional constriction. After this neither a 1 nor a 2 c.c. dose of apocodein (1 per cent) caused any reaction. Three cubic centimetres of adrenalin (1:1000) caused a slight reaction, lasting for one hundred and thirty seconds. Again 2 c.c. of apocodein (1 per cent) were injected, producing a very slight dilation. Subsequent to this, adrenalin failed to act, 2 mg. of digitalein still caused an additional reaction; 3 c.c. of a 1:200

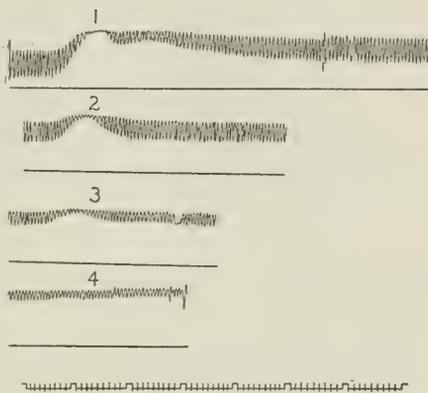


FIGURE 9. — Effect of adrenalin after apocodein. Time in seconds. (1) Adrenalin (1:10,000) on normal vessels of kidney. (2) Same five minutes after 1 c.c. apocodein (1 per cent). (3) Same after 2 c.c. apocodein (1 per cent). (4) Same after 3 c.c. apocodein (1 per cent).

solution of chloroform caused a slight reaction, and in spite of the marked œdema present at this time, 2 c.c. of a 2 per cent barium chloride solution caused a further permanent constriction.

### III. SUMMARY OF RESULTS.

**Adrenalin.**—Weak solutions, 1 : 100,000 to 1 : 5000.

Constrict vessels of brain ; recovery complete in 2 to 3 minutes.

Constrict vessels of kidney ; recovery complete in 0.5 to 1.5 minutes.

Moderate strength solutions, 1 : 4000 to 1 : 1000.

Constrict vessels of brain ; recovery incomplete in 2 to 5 minutes.

Constrict vessels of kidney ; recovery complete in 1 to 1.5 minutes.

Strong solutions, 1 : 750 to 1 : 250.

Constrict vessels of brain ; no recovery.

Constrict vessels of kidney ; recovery incomplete in 2 to 6 minutes.

**Barium chloride.**—Weak solutions, 1 : 500 to 1 : 50.

Constrict cerebral vessels ; recovery complete in 3 to 6 minutes.

Constrict renal vessels ; recovery complete in 1.5 to 6 minutes.

Strong solutions, 1 : 40 to 1 : 10.

Constrict cerebral and renal vessels, preceded sometimes by a slight temporary dilation ; no recovery.

**Chloroform.**—Weak solutions, 1 : 4000 to 1 : 1000.

Dilate the cerebral vessels ; recovery complete in 1 to 1.3 minutes.

Dilate the renal vessels ; recovery complete in 0.5 to 1.5 minutes.

Moderate strength solutions, 1 : 1000 to 1 : 400.

Constrict the cerebral vessels for about 0.5 minute, then dilate ; recovery in 1 to 1.5 minutes.

Constrict the renal vessels for about 0.6 minute, then dilate ; recovery in 1.5 to 2 minutes.

Strong solutions, 1 : 300 to 1 : 200.

Constrict cerebral vessels ; recovery in 2 to 4 minutes.

Constrict renal vessels ; recovery in 1 to 1.5 minutes.

**Digitalis.**—Weak doses, 0.01 to 0.1 mg. digitalein.

Constrict cerebral and renal vessels ; recovery almost complete in 0.5 to 1 minute.

Moderate doses, 0.1 to 1 mg. digitalein.

Constrict cerebral and renal vessels tonically ; no recovery ; subsequent action of apocodein diminished ; subsequent action of adrenalin and chloroform not affected.

Large doses, 1 to 10 mg. digitalein.

Constrict cerebral and renal vessels ; no recovery ; subsequent action of apocodein entirely prevented or changed into a slight dilation ; constricting power of adrenalin only slightly diminished.

**Chloretone.** — Saturated solutions, 1 : 125.

Dilate cerebral vessels ; recovery in 1 to 2 minutes.

Dilate renal vessels ; recovery in 0.5 to 1 minute.

Do not prevent subsequent action of any drug.

**Apocodein.** — Weak solutions, 0.1 to 0.4 c.c. of 0.5 per cent solution.

Constrict cerebral vessels ; no recovery.

Sometimes dilate temporarily, then constrict renal vessels ; no recovery.

Strong solutions, 1 to 2 cc., 1 per cent solution.

Constrict cerebral vessels ; no recovery.

Dilate renal vessels temporarily, then constrict ; no recovery.

Diminish or abolish subsequent action of adrenalin ; somewhat reduce the action of digitalein ; but do not affect the subsequent power of chloroform, chloretone, or barium chloride.

From the above summary the following general conclusions may be drawn :

1. It is evident that the cerebral as well as the renal vessels react to the various drugs, and sometimes to different dilutions of the same drug in very characteristic ways. Thus it is found that chloretone is capable only of dilating vessels separated from the central nervous system, while adrenalin and digitalein only constrict them. With another group of drugs dilation or constriction, or one following the other, may be produced, depending on the strength employed. Some drugs, as chloroform, cause dilation in weak solutions, constriction in stronger ones ; others, as apocodein, cause constriction in weaker solutions, and tend to dilate in stronger ones. If a moderate dose of these drugs is injected, both constriction and dilation may follow. In these cases the dilation may precede the constriction, as with apocodein, or follow it, as with chloroform.

2. The statement made by other workers that apocodein is capable of abolishing or diminishing the action of adrenalin, the strongest vaso-constricting drug known, while other less powerful drugs, as chloroform, chloretone, digitalein, and barium chloride, still react, has been corroborated in this research, and extended to the cerebral vessels.

3. The results also show that digitalis, which does not prevent the action of adrenalin, is capable of diminishing or abolishing the action of apocodein, or of converting it into a dilating drug.

## IV. THE PHYSIOLOGICAL SIGNIFICANCE OF THESE REACTIONS.

These varied reactions given by the cerebral and renal vessels are difficult to explain unless it be assumed that apocodein and adrenalin, and probably other drugs, act elsewhere than on the muscle substance of the vessel walls. Since there remains as a point of action for such drugs only the nerve terminals, or some junctional structure between nerve end and muscle, the results of this research seem to supply proof of a cerebral innervation.

The evidence on which it is assumed that adrenalin stimulates the nerve terminals in the blood vessels depends largely on the paralytic action of apocodein on the same structure. For this reason it becomes necessary to analyze more in detail those reactions of apocodein which might cast doubt on the existence of this paralyzing power.

In the first place, it was found in many experiments, especially on the renal vessels, that repeated injections of apocodein failed to abolish, but merely diminished, the action of adrenalin. This may be explained by supposing that in these cases apocodein had a parietic rather than paralytic effect, lowering rather than destroying the irritability possessed by these particular structures.

In other experiments the action of adrenalin was abolished only when the vessels were already extremely constricted by the apocodein. The question arises, Did apocodein abolish or lessen the action of adrenalin by constricting the vessels so far that the latter could produce little or no additional constriction? The fact that chloroform, barium chloride, digitalein, etc., which normally have a much weaker action than adrenalin, were still able to act after this extreme degree of constriction had been reached, disproves this. By some this argument might not be accepted, as it has been asserted that some of these drugs produce their changes by a rigor process. Experimental evidence refutes this statement, as the vessels still react to other drugs (presence of contractility), and passively respond to an increase in the perfusion pressure (presence of elasticity).

Lastly, doubt is cast on the paralytic action of the drug by the fact that, contrary to the results of Brodie and Dixon, the vessels remained tonically constricted after an application of apocodein, and each succeeding dose caused an additional constriction. In an occasional experiment there occurred, after many injections of apocodein, a very slight and temporary dilation, but even then the vessels were far from

reaching their normal calibre, whereas, were the reaction due to paralysis, they should pass beyond. The action of apocodein after digitalein suggests an explanation for this tonic constriction. The action of either of these drugs was characterized by a persistent tonic constriction. That induced by digitalein could apparently replace that of apocodein in the sense that when the latter drug was injected after the former, its characteristic action was absent. Digitalein, however, differed from apocodein in its action in that it could not abolish the subsequent action of adrenalin. When digitalein was followed by apocodein, however, the latter drug, although giving no evidence of a reaction itself, was capable of doing this. These facts seem to indicate that apocodein also has a muscular action which causes a tonic constriction replaceable by that of digitalein.

In summing up the action of apocodein we find that it apparently paralyzes some nervous mechanism in the vessel walls, as shown by its abolishing the action of adrenalin while other drugs still acted. On the other hand, the tonic state of constriction, shown in its reaction, seems to be due to a stimulating muscular action of the drug. These paradoxical reactions may be explained by assuming that the constriction due to muscular stimulation prevented the paralysis of the nerve terminals from being indicated by a dilation. It is a most plausible assumption that the action of any peripherally acting drug is not limited to any definite structure, histological or imaginary, but that it has a graded affinity for all the structures comprising the peripheral mechanism. It may be assumed that, as the nerve-end substance gradually shades into that of the muscle, the affinity which the terminals have for a drug is passed over to some extent with it, there being no point where the action of the drug abruptly ceases.

By the judicious application of one set of drugs, the action of another set may be profoundly altered. This may happen because the first drug destroys the affinity of the second for a certain structure, or substitutes a similar action for it. The experiments of this research, though far from being conclusive, have been suggestive enough to point to a tentative classification of peripherally acting vaso-motor drugs, not according to any action on one definite structure, but according to a system of graded neuro-muscular action. This was done by the following process of eliminating the point where it seemed improbable for each drug to act:

1. Apocodein abolishes the action of adrenalin, while weaker drugs, as digitalis, barium chloride, and chloroform, still act. Therefore adrenalin is classified as having more affinity for the nervous side of the peripheral mechanism, while these other drugs have more affinity for the muscular side. Apocodein, accordingly, must be supposed

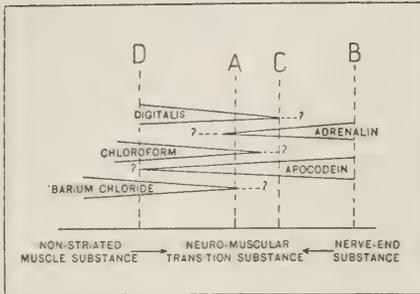


FIGURE 10.—Diagram to express the overlapping action of a number of vaso-motor drugs. No definite division between muscle substance (toward left) and nerve-end substance (toward right) is made. Greatest points of predilection for various drugs are represented by the widest portion of the decrescendo, points least affected by the apex. The uncertainty as to how far action occurs is expressed by question marks. Lines A, B, C, and D are referred to in the text.

as nerve-end action. The second shows that its affinity for the muscular portion is exceeded by that of digitalis (Fig. 10, C to D).

4. The action of either weak or strong doses of chloroform and barium chloride is not appreciably affected by large doses of apocodein or digitalis. Therefore their action is represented as leaning still more toward the muscular side.

Present-day methods are still too crude to reveal much more than the structure for which each drug has the greatest affinity, while the extent to which it acts on neighboring structures cannot be so clearly established. A careful analysis of drug reactions, however, indicates that probably no drug possesses an action absolutely confined to either nerve terminal or muscle substance. This hypothesis that a graded affinity exists for all peripherally acting drugs does not interfere with their use to prove the presence or absence of an innervation of blood vessels as long as the greatest action of these drugs may be assumed

to paralyze the nerve-end portion which adrenalin stimulates (Fig. 10, A to B).

2. Digitalis does not abolish, nor react in the same manner as adrenalin; therefore this drug probably does not act to any great extent, at least, on the same structure as adrenalin (Fig. 10, C to D).

3. Full doses of digitalis completely prevent the subsequent tonic constriction of apocodein, sometimes even converting it into a dilating drug, while full doses of the latter drug merely weaken the subsequent constriction of digitalis. The first of these results indicates that apocodein has a muscular as well

to be on the nervous side. The reactions of the cerebral vessels to adrenalin and apocodein are such as to necessitate the assumption that their greatest action is on the nervous side. Thus physiological evidence of a functioning of the nerve terminals, histologically discovered in the cerebral vessels, is partly supplied by the reactions obtained in this research.

My thanks are due to Professor Lombard for his useful suggestions in this research.

## THE NUTRITIVE VALUE OF GELATIN. — II. SIGNIFICANCE OF GLYCOCOLL AND CARBOHYDRATE IN SPARING THE BODY'S PROTEID.

By JOHN R. MURLIN.

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IN a recent communication by the writer<sup>1</sup> it was shown that nitrogen equilibrium at or near the fasting level may be maintained in man and dogs, for short periods of time at least, after approximately two thirds of the supply of proteid (meat) nitrogen has been replaced by gelatin nitrogen. This is the highest replacement of proteid by gelatin thus far reported, for no one has before obtained a replacement of as much as one half of the proteid at the fasting level. This result, however, was obtained only when the amount of carbohydrate in the food was very high (two thirds of the total supply of energy), and when the total supply of energy was considerably in excess of the requirement. The advantage of high carbohydrate calories was not unexpected in view of the well-known protecting power over the body's proteid of this class of foodstuffs. But it suggested the idea that the high sparing effect of gelatin itself might possibly be due in part to carbohydrate capable of being synthesized from it rather than to the amino-acids which it is capable of yielding.

The line of reasoning was as follows: Landergren<sup>2</sup> had performed some experiments which he interpreted as showing the body's *absolute requirement for carbohydrate*. For example, by exclusive feeding of carbohydrate to the amount of 38 to 45 Cal. per kgm., he was able to reduce the amount of nitrogen output of a man weighing 70 kgm. to between 3 and 4 gm.; by exclusive fat feeding, to the amount of 54 Cal. per kgm., the nitrogen output was 5.7 gm. This great superiority of carbohydrate in sparing approximately twice as much proteid as

<sup>1</sup> MURLIN, J. R.: This journal, 1907, xix, p. 285.

<sup>2</sup> Reviewed by HAMMERSTEN in MALY'S Jahresbericht, 1902, p. 685

fat is met with only in exclusive ingestion of carbohydrate or of fat, for with a certain minimum of carbohydrate added to the diet, fat spares proteid as well as carbohydrate itself. Hence Landergren concludes that the increased proteid combustion which takes place in exclusive fat feeding corresponds to that quantity of proteid which is required for the formation of carbohydrate when no carbohydrate is being fed.

Whether Landergren's interpretation as to the quantitative requirement of the body will stand or not, there is no doubt that under certain circumstances (*c. g.*, *Diabetes mellitus*, phlorhizin glycosuria, etc.) *proteid can yield carbohydrate*. Moreover, according to modern ideas of protein metabolism,<sup>1</sup> when the amino nitrogen is split off normally in the form of ammonia and transformed into urea (because it is of no use to the organism), *there remains a carbonaceous residue* capable of being burned. We do not know that in the case of every amino-acid this residue is convertible into carbohydrate, but it has been demonstrated that alanin, asparagin, and others can yield dextrose.<sup>2</sup>

It has been shown by Reilly, Nolan, and Lusk,<sup>3</sup> that *gelatin, like proteid, can yield carbohydrate*. They observed, after feeding gelatin to a dog which was under the influence of phlorhizin, that the increase in the amount of dextrose eliminated in the urine was as much as 60 per cent of the weight of the gelatin fed. May not this dextrose from gelatin (equal in potential energy, gram for gram, with the gelatin itself) be formed normally also, and be available for the protection of the body's proteid? It has long been generally believed that the nitrogen of gelatin cannot be retained in the body, and that its effect of reducing the body's waste of nitrogen is due to the fact that it furnishes something which can take the place of proteid in combustion. Is that something carbohydrate, *i. e.* the non-nitrogenous residue, or does the gelatin protect the proteid in virtue of the amino bodies which it furnishes?

It appears, from a study of the literature, that nobody has observed the effect on protein metabolism, as measured by nitrogen elimination, of small quantities of carbohydrates fed alone. Landergren's experiments show the effects of carbohydrates in large quantities, and from his results, together with those of Kirchmann<sup>4</sup> on gelatin

<sup>1</sup> FOLIN: This journal, 1905, xiii, p. 117.

<sup>2</sup> STILES and LUSK: This journal, 1903, ix, p. 380.

<sup>3</sup> REILLY, NOLAN, and LUSK: This journal, 1898, i, p. 395.

<sup>4</sup> KIRCHMANN: Zeitschrift für Biologie, 1900, xl, p. 54.

fed alone, it may be calculated that the amount of dextrose derivable from large quantities of gelatin would have a sparing effect equal to that of the gelatin itself. But we know that gelatin in small quantities can exert an effect almost as great as when fed in large quantities,<sup>1</sup> and we do not know that this smaller proportional effect of gelatin in large quantities may not be due to its incomplete denitrogenization.<sup>2</sup> If it were not completely denitrogenized, the non-nitrogenous portion would not all be available for combustion. At all events, there is nothing in these experiments, or in any others on gelatin or carbohydrates with which I am acquainted, to show that gelatin in small quantities may not spare the proteid in virtue of the carbonaceous part of its molecular complex rather than because of the amino-nitrogen which it affords.

#### METHOD.

The question may be put to test (1) by feeding an animal whose fasting metabolism is known, a small quantity of gelatin, and comparing its effect with 60 per cent of that quantity of dextrose. It is scarcely possible that gelatin can yield more than 60 per cent of its weight as dextrose. Hence, if the reduction in nitrogenous waste from the body is as great with this amount of dextrose as with the amount of gelatin from which it could be derived, we may safely conclude that gelatin owes its sparing effect to the non-nitrogenous compounds which it can furnish. (2) The amino-acids contained in gelatin may be fed separately and their effects compared with those of gelatin itself.

#### EXPERIMENTS WITH DEXTROSE.

**Dog A.** — Female fox terrier in good condition and weighing 13.8 kgm., was made to fast for four days and was then fed with 34 gm. gelatin, containing approximately 20 per cent of the required energy. This was immediately followed by a three-day period in which 12 per cent of the required energy was supplied by carbohydrates, and the experiment was terminated by another fasting day.

<sup>1</sup> Cf. KIRCHMANN's curve, *loc. cit.*, p. 81.

<sup>2</sup> Or even incomplete digestion, since disturbances of digestion are always apparent when gelatin is fed in excess. RUBNER, for example, has reported the presence of gelatin itself in the urine. It is possible also that the destruction and elimination of a large quantity of gelatin may itself entail a loss of nitrogen from the body.

TABLE I.

DOG A.

Date. 1906	Weight.	Food.	Cal. of requirement.	N in food.	N in urine.	N in feces.	Total N.	Fasting N.	Body N spared.
July 27	kgm. 13.4	4th fasting day	per cent. ....	....	2.591	0.09	2.681	....	per cent. ....
28	13.2	SiO <sub>2</sub> 34 gm. gelatin	20	5.242	6.932	0.13	7.062	2.6	35.0
29	13.1	34 gm. gelatin	20	5.242	.... <sup>1</sup>	0.13	....	2.5	30.0
30	12.9	34 gm. gelatin	20	5.242	.... <sup>1</sup>	0.13	....	2.4	30.0
31	12.8	SiO <sub>2</sub> 19.5 gm. cane sugar	12	....	2.566	0.10	2.666	2.3	-13.0
Aug. 1	12.6	20.4 gm. dextrose.	12	....	2.232	0.10	2.332	2.2	-4.5
2	12.5	18.8 gm. corn-starch	12	0.028	2.205	0.10	2.305	2.1	-8.0
3	12.3	fasting	....	....	1.966	0.10	2.066	2.0	....

<sup>1</sup> A portion of the gelatin was vomited during the night and was found mixed with the urine, making analysis impossible. Accordingly the sparing effect for the second and third days of gelatin feeding are estimated from Kirchmann's curve (*loc. cit.*).

It is clear that in this experiment the small quantity of carbohydrate which might be available by synthesis from the gelatin fed would have no sparing effect. The fasting nitrogen, it will be seen, has fallen, in the eight days of the experiment, from 2.7 gm. to 2.0 gm. If we suppose this decline to have been a gradual one, and estimate the fasting nitrogen for the carbohydrate days at 2.3, 2.2, and 2.1 gm., respectively, the results indicate an additional waste of nitrogen because of the carbohydrate ingestion. This I have expressed as a minus sparing of 13, 4.5, and 8 per cent, respectively, for the three days.<sup>1</sup> No particular significance should be attached to the different figures obtained with the different carbohydrates fed on the three days. Except between the cane-sugar day and the dextrose day the difference is not greater than might be expected between any two days with the same carbohydrate, and the period is entirely too short to make the one considerable difference of any value.

**Dog B.** — Female mongrel, weighing 12.7 kgm., was made to fast for 5 days and was then given a small quantity of beef heart for three days. For

<sup>1</sup> It was learned subsequently that the dog was in the early stages of pregnancy at the time of the experiment. I am not prepared to say what influence, if any, this condition might have had on the results as tabulated above.

three days immediately following this, 20 gm. of dextrose were added to the meat, and the experiment was closed with another fasting period. The dog's appetite was very poor, so that even the small quantity of meat had to be given by hand. The dextrose was taken readily dissolved in 100 c.c. water (see Table II).

TABLE II.

Date 1906.	Weight.	Food.	N in food.	N in urine.	N in fæces.	Total N excreted.	N dif.	Fasting N.	Body N spared.
Nov.	kgm.								per cent.
18	12.04	5th fasting day	....	2.540	0.06	2.600	....	2.6	..
19	11.98	6th fasting day	....	2.440	0.06	2.500	....	2.5	..
20	11.90	SiO <sub>2</sub> 30 gm. beef heart	? <sup>1</sup>	2.641	0.16	2.801	?	2.4	..
21	11.56	30 gm. beef heart	0.88 <sup>2</sup>	2.474	0.16	2.634	1.754	2.3	24
22	11.44	30 gm. beef heart	0.88	2.407	0.16	2.567	1.687	2.2	23
23	11.36	30 gm. beef heart 20 gm. dextrose	0.88	2.674	0.19	2.864	1.984	2.1	..
24	11.18	30 gm. beef heart 20 gm. dextrose	0.88	2.457	0.19	2.647	1.767	2.0	11
25	11.10	30 gm. beef heart 20 gm. dextrose	0.88	2.356	0.19	2.546	1.666	1.9	12
26	11.02	Fasting	....	2.356	0.06	2.416	....	..	..
27	10.84	Fasting	....	1.538	0.06	1.598	....	1.6	..

<sup>1</sup> Dog vomited part of the meat, probably owing to the silicic acid given with it as a fæcal mark.

<sup>2</sup> Owing perhaps to the poor appetite for the meat, it was imperfectly digested, and consequently a small amount of meat fibre was found in all the fæces of the two feeding periods. This was carefully separated, and its nitrogen deducted from the nitrogen contained in the food. The figure given is the average per day after deduction.

Here the singular effect of a small quantity of carbohydrate to increase the nitrogen loss is again seen. The sparing of the body proteid effected by the meat alone is twice as great as the sparing effected by the meat plus 20 gm. of dextrose.<sup>1</sup>

**Dog C.** — Female bull-terrier which had been very much reduced by suckling a large litter of puppies. The dog was made to fast for eight days and was extremely emaciated at the time feeding was begun. The nitrogen in the urine rose from 2.581 gm. on the second fasting day to 3.104 on the eighth. The dog was fed 21 gm. of dextrose (12 per cent of the energy

<sup>1</sup> It is possible that this dog also may have been in the early stages of pregnancy. The dog was killed for another purpose before the importance of determining this point was appreciated. Dog C is known not to have been pregnant.

requirement) for four days, after which another fasting period of two days was taken. On the second day of this period the nitrogen output rose suddenly to a very high figure (probably the premortal rise), and the experiment was terminated (Table III).

TABLE III.

Date 1907.	Weight.	Food.	N in urine.	N in fæces.	Total N excreted.	Fasting N.	Body N spared.
Feb. 18	kgm. 11.90	7th day of fasting	3.067	0.028	3.095	....	....
19	11.62	8th " " "	3.104	0.028	3.132	3.1	....
20	11.52	21 gm. dextrose	2.829	0.039	2.878	3.2	+0.33
21	11.38	" "	3.033	0.039	3.072	3.4	+0.33
22	11.22	" "	3.135	0.039	3.174	3.5	+0.33
23	11.12	" "	3.237	0.039	3.276	3.6	+0.33
24	10.82	Fasting	3.833	0.028	3.861	3.8	....
25	10.65	"	4.499 <sup>1</sup>	0.028	4.527	....	....

<sup>1</sup> Premortal rise.

In this experiment there is an evident sparing effected by the dextrose. Estimating the fasting metabolism by the amount of nitrogen which would have been eliminated if nothing had been fed on the four dextrose days, we learn that the sparing is exactly 0.33 gm. N, or 2.06 gm. proteid on each day. In other words, 12 per cent of the body's requirement fed alone in the form of dextrose has protected the body proteid (held back the nitrogen) to the extent of 10 per cent of the fasting requirement. This is the only dog in which any sparing has been obtained with 12 per cent of the requirement, — a result probably due to the extreme state of impoverishment to which the dog had been reduced previous to the experiment. Judging by the steady rise in the nitrogen output previous to the feeding, there could not have been a large reserve of fat available for the fasting metabolism; hence the living substance was levied upon to a greater and greater extent. In the other dogs the nitrogen output was on the decline, as is usual in all but the very last stages of fasting. Hence it would appear that under the circumstances which this dog presents, a small quantity of carbohydrate is much more readily sub-

stituted for proteid (if that be the action of the carbohydrate) in the katabolic processes of the body than when, as in the other dogs, sufficient fat (it may be presumed) was available. The sparing is slight, however, even under these circumstances, and is by no means to be compared with the protecting influence of that quantity of gelatin from which, at the best, this quantity of dextrose could be derived. In the next experiment the protecting influence of gelatin is directly compared with that of 60 per cent of its weight of dextrose.

**Dog D.** — A small female mongrel in a fairly good condition as regards body fat, weighing 6.28 kgm. at the beginning of the experiment, was made to fast for thirteen days. The nitrogen output declined from 2.665 gm. on the second day to 1.959 gm. on the eleventh day, then remained stationary at 2.010 gm. on the twelfth and thirteenth days. For four days thereafter 12 gm. of dextrose (equivalent to 12 per cent of the requirement for potential energy) dissolved in 100 c.c. water were given in five equal quantities at ten, twelve, two, half-past three, and five o'clock. A single fasting day followed, and for four days more 20 gm. of gelatin (equivalent to 20 per cent of the energy requirement) were given at a single feeding. The gelatin used in this and subsequent experiments reported in this paper was a powdered commercial article<sup>1</sup> found to be entirely free of proteid (see Table IV).<sup>2</sup>

There is no sparing in the dextrose period, but about the usual sparing (31 per cent) for the amount fed in the gelatin period. This dog was absolutely normal so far as could be determined. It is clear, therefore, that even in the condition of extreme nitrogen hunger in the dog the protection of the body's proteid by gelatin is not due to dextrose, which may be formed from it, but to some nitrogenous constituent.

In the following experiment on a man it will be seen that the same must be true also of the human organism.

#### EXPERIMENT ON MAN (A. I. R.).

Subject was a medical student twenty years of age, slender of build and weighing at the beginning of the experiment 45.4 kgm. He was first placed in nitrogenous equilibrium, or nearly so, on a diet containing two thirds

<sup>1</sup> PETER COOPER'S, made in New York.

<sup>2</sup> A 5 per cent solution gave no turbidity with acetic acid and potassium ferrocyanide, and no colored precipitate with MILLON'S fluid.

of the estimated requirement for nitrogen in beefsteak, the remaining third in oatmeal, soda biscuit, etc. Then the beefsteak was replaced by gelatin containing the same amount of N for six days, at the end of which time the gelatin was dropped and in its place 60 per cent of the weight of gelatin was given in dextrose. The gelatin had the usual effect on the appe-

TABLE IV.

Date 1907.	Weight.	Food.	Cal. requirement.	N in food.	N in urine.	N in fæces.	Total N excreted.	Fasting N.	N dif.
Feb. 10	kgm. 5.00	12th fasting day	..	....	2.010	0.036	2.046	..	....
11	4.86	13th fasting day	..	....	2.010	0.036	2.046	2.0	....
12	4.74	12 gm. dextrose	12	....	<sup>1</sup>	....	....	1.9	....
13	4.70	12 gm. dextrose	12	....	1.768	0.047	1.815	1.8	....
14	4.68	12 gm. dextrose	12	....	1.601	0.047	1.648	1.7	....
15	4.63	12 gm. dextrose	12	....	1.567	0.047	1.614	1.6	....
16	4.60	Fasting	..	....	1.533	0.036	1.569	1.5	....
17	4.58	20 gm. gelatin	20	3.120	.... <sup>1</sup>	....	....	..	....
18	4.56	20 gm. gelatin	20	3.120	4.156	0.057	4.213	..	-1.093
19	4.51	20 gm. gelatin	20	3.120	4.156	0.057	4.213	..	-1.093
20	4.47	20 gm. gelatin	20	3.120	4.021	0.057	4.078	..	-0.958
21	4.34	Fasting	..	....	1.341	0.036	1.377	1.4	....
22	4.24	Fasting	..	....	1.211	0.036	1.247	1.3	....

<sup>1</sup> Not analyzed.

tite, and on the last days of the period was eaten only by the greatest effort. The subject also experienced considerable languor and disinclination to do mental work. A light wine was allowed by way of a tonic for the stomach, and on the last two gelatin days a light dose of *Tinct. Zingiberis* was given just before mealtime. Two meals each day at eight A. M. and five P. M. were eaten. The diets follow:

ALL PROTEID NITROGEN.

*Breakfast.*

40 gm. oatmeal . . . (2.14 per cent N) = 0.86 gm. N and 158 Cal.  
 75 gm. beefsteak . . (3.4 per cent N) = 2.55 gm. " 75<sup>1</sup> "

<sup>1</sup> 1.5 per cent fat estimated.

*Breakfast (continued).*

50 gm. cream . . .	(0.3 per cent N) = 3.15 gm.	and 220 Cal.
50 gm. cane sugar . .	....	195 "
35 gm. soda biscuit .	(1.68 per cent N) = 0.58 gm. N	" 125 "
10 gm. butter . . .	(0.1 per cent N) = 0.01 gm. N	" 76 "
200 gm. coffee . . .	....	0.05 gm. N " 00 "
		4.29 gm. N " 849 "

*Supper.*

125 gm. beefsteak . .	(3.4 per cent N) = 4.25 gm. N	and 126 <sup>1</sup> Cal.
40 gm. rice . . .	.... = 0.52 gm. N	" 137 "
75 gm. cane sugar . .	....	287 "
50 gm. soda biscuit .	(1.68 per cent N) = 0.84 gm. N	" 180 "
50 gm. cream . . .	(0.3 per cent N) = 0.15 gm. N	" 220 "
20 gm. butter . . .	(0.1 per cent N) = 0.02 gm. N	" 152 "
200 gm. coffee . . .	....	0.05 gm. N " 00 "
50 c.c. light wine . .	....	0.02 gm. N " 20 "
		5.85 gm. N " 1122 "
Total for day . . . .	10.05 gm. N	" 1971 <sup>2</sup> "
		= 43 cal. per kgm.

TWO THIRDS (67 PER CENT) GELATIN NITROGEN + ONE THIRD PROTEID NITROGEN.

*Breakfast.*

40 gm. oatmeal . . .	(2.14 per cent N) = 0.86 gm. N	and 158 Cal.
50 gm. cream . . .	(0.3 per cent N) = 0.15 gm. N	" 220 "
50 gm. cane sugar . .	....	195 "
35 gm. soda biscuit .	(1.68 per cent N) = 0.58 gm. N	" 125 "
10 gm. butter . . .	(0.1 per cent N) = 0.01 gm. N	" 76 "
200 c.c. coffee . . .	....	0.05 gm. N " .... "
Total . . . . .	1.65 gm. N	" 774 "

*Half at Each Meal.*

{ 43.5 gm. gelatin . .	(15.4 per cent N) = 6.7 gm. N	and 165 Cal.
{ 50 c.c. light Rhine wine . . .	....	0.02 gm. N " 20 "

*Supper.*

40 gm. rice . . .	(1.3 per cent N) = 0.52 gm. N	and 137 Cal.
50 gm. cream . . .	(0.3 per cent N) = 0.15 gm. N	" 220 "
75 gm. cane sugar . .	....	287 "

<sup>1</sup> 1.5 per cent fat estimated.

<sup>2</sup> 50 per cent of total calories were from carbohydrate.

*Supper (continued).*

50 gm. soda biscuit . . .	(1.68 per cent N) =	0.84 gm. N and 180 Cal.
20 gm. butter . . .	(0.1 per cent N) =	0.02 gm. N " 152 "
200 c.c. coffee . . .	....	0.05 gm. N " .... "
		1.58 gm. N " 996 <sup>1</sup> "
Grand total for day . . .		9.95 gm. N " 1935 "
		= 42 cal. per kgm.

ONE THIRD PROTEID NITROGEN; DEXTROSE INSTEAD OF GELATIN.

*Breakfast.*

Same as in previous diet . . . . . 1.65 gm. N and 774 Cal.

*Supper.*

Same as in previous diet . . . . .	1.58 gm. N and	996 Cal.
plus 24 gm. dextrose . . . . .	....	88 "
Total for day . . . . .	3.23 gm. N "	1858 "
		= 40 cal. per kgm.

TABLE V.

SUMMARY OF EXPERIMENT. FOR DETAILS SEE TABLE X AT END OF PAPER.

Source of N.	Number of days.	Weight kgm.	Cal. per kgm.	N in food.	Total N excreted.	N. dif. per day.
All-proteid N . . . . .	4	46.4	43	10.05	10.447 <sup>1</sup>	-0.39
Two thirds (67%) gelatin N + one third proteid N . . . . .	6	46.4	42	9.95	11.284	-1.44
One third proteid N only . . . . .	3	46.4	40	3.25	6.269	-3.02

<sup>1</sup> Average of last two days only.

The amount of nitrogen supplied in the gelatin period was 0.1 gm. less than in the all-proteid period, but the average loss was fully 1.0 gm. more. In my former paper on the subject of gelatin, I reported an experiment on myself, in which I was able, with the help of extra carbohydrate calories, to maintain almost exactly the same state of nitrogen balance on two thirds (63 per cent) gelatin nitrogen plus one third proteid nitrogen as on all-proteid nitrogen. The present subject (not being fond of sweets) was unable to take the extra carbohydrate in the form of sugar, and an attempt to readjust the diet so

<sup>1</sup> 50 per cent of total calories were from carbohydrate.

as to give two thirds of the total calories in carbohydrate (see Table X at the end of the paper for details) by substituting a cornstarch pudding for rice was not successful. The subject experienced nausea and more or less pain in the stomach. In spite of this, however, on the last gelatin-proteid day the sparing was only a little less favorable than on the best all-proteid day, and on the fourth gelatin-proteid day, when the amount of carbohydrate calories was only 50 per cent of the total, the sparing was a little better than on the best all-proteid day. The experiment is not to be construed as contradicting the former one, for the reason that the conditions were not identical in the two.

The object of the experiment was to observe the effect of substituting for the gelatin 60 per cent of its weight in dextrose, and the result is perfectly positive. The advantage, from the standpoint of nitrogen loss, of the gelatin over dextrose is very evident, for the average daily loss in the latter period is more than twice as great as in the former. The conclusion from this experiment on a man and from the preceding ones on dogs is, obviously, that gelatin does not protect the body's proteid in virtue of dextrose synthesized from it, even if as much as 60 per cent of its weight should pass through a dextrose stage. Its value to the organism must consist in the fact that it contains nitrogen, even though that nitrogen itself may be, as is generally supposed, immediately eliminated. Possibly a detailed study of the nitrogen distribution in the urine in fasting and after feeding gelatin alone will afford some light as to the exact rôle played by the gelatin nitrogen.

#### EXPERIMENTS WITH GLYCOCOLL.

It has been customary,<sup>1</sup> in explaining the behavior of gelatin in metabolism, to refer its inadequacy as a substitute for proteid to the absence of tyrosin and indol groups in its molecular complex. Kauffmann,<sup>2</sup> in fact, proceeding on this idea, attempted to restore gelatin to the full nutritive value of proteid by adding to it appropriate quantities of tyrosin and tryptophan and of these two together with cystin. His conclusion was that the latter mixture "probably had the full physiological value of proteid." Kauffmann's results, however, have recently been called in question by Rona and Müller,<sup>3</sup> who failed to

<sup>1</sup> Cf., for example, Tigerstedt in NAGEL'S *Handbuch*, i, p. 423.

<sup>2</sup> KAUFFMANN: *Archiv für gesammte Physiologie*, 1905, cix, p. 440.

<sup>3</sup> RONA and MÜLLER: *Zeitschrift für physiologische Chemie*, 1907, l, p. 363.

confirm his findings after adding tyrosin and tryptophan. Whether it is possible thus to raise gelatin to the physiological status of proteid by addition of artificially prepared amino-acids must, therefore, be left in abeyance.

It seems to me, however, that this conception that gelatin falls short of proteid merely in that it lacks tyrosin and indol or these and a sulphur-containing group, overlooks the most important (from the quantitative point of view) constituent of gelatin, namely, glycocoll. Let us see what is the nutritive significance of this constituent. It is well known that glycocoll fed alone to an otherwise fasting organism is completely oxidized at once to urea. Thus v. Brügsch and Hirsch<sup>1</sup> fed 20 gm. of glycocoll to a fasting woman on the twelfth day of her fast, and observed that all of its nitrogen appeared in the urine as urea.<sup>2</sup> Samuely<sup>3</sup> had likewise observed this fate (chiefly) of glycocoll nitrogen when glycocoll was added to meat and other foodstuffs in the diet of dogs suffering from artificial anæmia. But Lühje<sup>4</sup> reports a nitrogen retention with asparagin and glycocoll as the only sources of nitrogen, *provided abundant carbohydrate* was fed at the same time. He thinks there may have been here a synthesis of amino-acids with carbohydrate, *i. e.*, a formation of "amino-sugar" which can escape the destructive processes of the body.

Whether the nitrogen retained in Lühje's experiment was the nitrogen of glycocoll or of asparagin or both, there was no way of deciding, just as in my own experiments (where nitrogen was retained after feeding but little more than the fasting requirement, two thirds of it in the form of gelatin and one third in the form of meat, but under the protection of abundant carbohydrate) there was no way of telling whether the nitrogen retained was nitrogen of gelatin or meat or of both. But if it could be shown that glycocoll can be retained, even temporarily, under conditions similar to those under which I obtained nitrogen retention with two thirds gelatin, we should have an additional explanation of my results; while if it should prove that the retention with glycocoll is *only* temporary, even under the most favorable circumstances for retention, we should have strong ground for believing that the inadequacy of gelatin as a source of

<sup>1</sup> BRÜGSCH and HIRSCH: Zeitschrift für experimentelle Pathologie und Therapie, 1906, p. 638.

<sup>2</sup> This was not the case with alanin and leucin.

<sup>3</sup> SAMUELY: Deutsches Archiv für klinische Medizin, 1906, p. 220.

<sup>4</sup> LÜTHJE: Kongress für innere Medizin, 1906, p. 440.

nitrogen might, in large measure, be due to its high content of glycocoll, and this condition would need to be taken into account in any attempt to raise gelatin to the physiological status of proteid.

#### METHOD.

It has appeared in my experiments with gelatin<sup>1</sup> that the tendency to nitrogen retention is stronger the lower the proteid condition of the animal at the time of feeding. For the purpose of testing the power of retaining glycocoll nitrogen, therefore, I prepared the dogs by subjecting them to long periods of fasting or under-nutrition.

**Dog D.** — Female mongrel, weighing in good condition 6.3 kgm., was fasted for thirteen days, during which the weight fell to 4.8 kgm. Then for two weeks more the dog was kept in undernutrition for the purpose of the dextrose and gelatin experiment reported on page 240. At the end of the month the weight had been reduced to 4.2 kgm. The total output of nitrogen on the second day of a fasting period taken at this time was 1.247 gm.<sup>2</sup> Ten days later, on a diet containing 0.491 gm. N in the form of beef heart and carbohydrate enough to make 150 Cal. per kgm. (weight still, 4.2 kgm.), the dog was almost in nitrogen equilibrium (—0.021 gm. N). To this diet were then added 5 gm. of glycocoll, containing 0.951 gm. nitrogen, bringing the total amount of nitrogen ingested up to 1.442 gm. This was continued for five days, after which the glycocoll was dropped for two days and the experiment was terminated with a fasting period. The results are given in Table VI.

On four of the five glycocoll days there was retention of nitrogen, — a total for the period of 0.259 gm. Whether the nitrogen retained was glycocoll nitrogen or nitrogen of the meat, we do not know positively. All we can say with certainty is, that so long as the diet con-

<sup>1</sup> MURLIN: *Loc. cit.*, p. 303.

<sup>2</sup> By feeding for a period of five days 6.4 gm. of proteid-free gelatin containing 0.990 gm. N and supplying over 100 Cal. per kgm. of energy (chiefly in the form of carbohydrates), the output of nitrogen was reduced to 0.406 gm. — less than one third of what it was on the fasting day just preceding. Theoretically it should have been possible now, by adding to the diet this one-third of the fasting requirement for nitrogen in the form of proteid, to establish perfect nitrogen equilibrium. Some difficulty, however, was experienced; for when 4.07 gm. of plasmon, containing 0.495 gm. N, were added, making the total supply 1.485 gm., there was a loss of nitrogen on the second, third, and fourth days amounting to 0.114, 0.249, and 0.148 gm. respectively. Since the total supply of energy was not changed, it would appear that the utilization of the plasmon itself involves an expenditure of nitrogen.

tained but little more than one third the fasting requirement for nitrogen, all of it in the form of proteid, there was considerable loss of nitrogen from the body; but as soon as the glycocoll, containing approximately two thirds the requirement for nitrogen, was added, there was nitrogen retention.

TABLE VI.

Date 1907.	Weight.	Food; source of N.	Total cal.	Total N fed.	N in urine.	N in feces.	Total N excr.	N dif.
April 4	kgm. 4.20	16 gm. beef heart	per kgm. 109	0.491	0.780	0.038	0.818	-0.317
5	4.20	" " "	150 <sup>1</sup>	0.491	0.713	0.038	0.751	-0.260
6	4.26	" " "	150	0.491	0.474	0.038	0.512	-0.021
7	4.34	Fasting <sup>2</sup>	....	....	0.943	0.036	0.979	....
8	4.16	16 gm. beef heart 5 gm. glycocoll	150+	1.442	1.324	0.058	1.382	+0.060
9	4.26	16 gm. beef heart 5 gm. glycocoll	150+	1.442	1.324	0.058	1.382	+0.060
10	4.33	16 gm. beef heart 5 gm. glycocoll	150+	1.442	1.429	0.058	1.487	-0.045
11	4.36	16 gm. beef heart 5 gm. glycocoll	150+	1.442	1.293	0.058	1.351	+0.092
12	4.48	16 gm. beef heart 5 gm. glycocoll	150+	1.442	1.293	0.058	1.351	+0.092
13	4.54	16 gm. beef heart	150	0.491	0.868	0.058	0.926	-0.435
14	4.48	" " "	150	.... <sup>3</sup>	....	....	....	....
15	4.34	Fasting	....	....	1.055	0.036	1.091	....
16	4.24	"	....	....	1.157	0.036	1.193	....

<sup>1</sup> Raised by addition of 50 gm. cane sugar.  
<sup>2</sup> For the purpose of removing glycogen previously stored.  
<sup>3</sup> Dog vomited during night, contaminating urine.

The retention, however, is not permanent; for on the day following when the glycocoll was dropped, and there should have been nitrogen equilibrium, as there was previous to the glycocoll feeding, there was a loss of 0.435 gm. N. It is more than probable that this includes the very nitrogen retained during the previous period, and it is perhaps safe to say that the nitrogen thus retained temporarily represents glycocoll itself.

**Dog E.** — Very much like dog D, weighing in good condition 5.4 kgm. Was fasted for ten days, during which the weight fell to 4.6 kgm. The nitrogen

output in the urine on the last fasting day was 1.697 gm. For five days immediately following this 1.506 gm. N were given, two thirds of it (1.006 gm.) in the form of proteid-free gelatin and one third (0.500 gm.) in the form of beef heart. Then for two days glycocoll was substituted for gelatin; and finally, the glycocoll was dropped and the beef heart continued alone for two days. A total energy supply of 130 cal. per kgm. (chiefly carbohydrates) was maintained throughout. Only the urine was analyzed. The conditions throughout the feeding periods being so nearly the same as regards absorbability, it was not considered necessary to analyze the feces (Table VII.)

TABLE VII.

Date 1907.	Weight.	Food: source of N.	Cal. per kgm.	N fed.	N in urine.	N dif.	Mean loss.	Fasting N.
April 29	Egm. 4.72	10th day fasting	..	....	1.697	....	....	1.7
30	4.62	6.5 gm. gelatin 16.3 gm. beef heart	130	1.506	.... <sup>1</sup>	....	....	1.65
May 1	4.78	6.5 gm. gelatin 16.3 gm. beef heart	130	1.506	.... <sup>1</sup>	....	....	1.6
2	4.88	6.5 gm. gelatin 16.3 gm. beef heart	130	1.506	1.593	-0.087	} 0.053	1.55
3	4.82	6.5 gm. gelatin 16.3 gm. beef heart	130	1.506	1.525	-0.019		1.5
4	4.80	6.5 gm. gelatin 16.3 gm. beef heart	130	1.506	1.559	-0.053		1.45
5	4.80	5.3 gm. glycocoll 16.3 gm. beef heart	130	1.508	1.559	-0.051	} 0.220	1.4
6	4.90	5.3 gm. glycocoll 16.3 gm. beef heart	130	1.508	1.898	-0.390		1.35
7	4.86	16.3 gm. beef heart only	130	0.500	1.050	-0.550	} 0.414	1.3
8	4.80	16.3 gm. beef heart only	130	0.500	0.779	-0.279		1.25
9	4.82	Fasting	..	....	1.017	....	....	1.25
10	4.68	Fasting	..	....	1.152	....	....	1.25
13 <sup>2</sup>	4.32	Fasting	..	....	1.288	....	....	1.25
<sup>1</sup> Not analyzed.			<sup>2</sup> Fifth fasting day.					

Estimating the decline in the fasting proteid metabolism from the time the feeding began, at the rate of 0.5 gm. N per day, we find that on the best gelatin day, when the animal was receiving exactly the requirement of nitrogen (1.5 gm), two thirds of it in the form of gelatin, and the other one-third in the form of proteid, there was a sparing of practically 100 per cent, and the average sparing for the last two gelatin days was 90 per cent.

The amount of nitrogen in the urine on the first glycocoll day is exactly the same as on the last gelatin day. This might be taken on first sight to mean that glycocoll has an equal "sparing power" with gelatin; but it is probably mere coincidence, meaning rather that on the first day some of the glycocoll escaped oxidation. On the second day there is a considerable increase in the amount eliminated, or, calculated as a "sparing," 84 per cent for the two days, as against 90 per cent for the best two gelatin days. When we remember that the amount of nitrogen fed on these two days was considerably greater in proportion to the absolute requirement, as determined by the fasting metabolism, than on the gelatin days, we see that from the standpoint of any sparing effect the glycocoll suffers still further by comparison with the gelatin.

With the meat alone the loss from the body is twice as great as with the meat and glycocoll. Here, again, we must give a guarded interpretation of the results. Do the figures mean that glycocoll serves in some way to reduce the destruction of the body proteid, which immediately rises as soon as the glycocoll is withdrawn, or do they mean that some of the glycocoll has escaped oxidation until the meat period? The difficulty, of course, lies in the fact that we do not know whether the nitrogen held back is the nitrogen of glycocoll or the nitrogen of the meat or the nitrogen of the body itself. The presumption is, I think, that the nitrogen held back is the nitrogen of glycocoll itself. The fact that the loss is twice as great on the day immediately following its removal from the diet as on the second day would favor that interpretation. Besides, judging from the experiment with dog D (April 6, Table VI, page 247), it is likely that on a third day with meat alone the dog would have been in nitrogen equilibrium, and it is quite possible that this would have been the case on May 5th and 6th if glycocoll had not been fed. That is to say, we know that the conditions for nitrogen equilibrium were already present without the glycocoll.

It would simplify matters if glycocoll alone were given as the source of nitrogen. In the following experiment this is the case. A large quantity of carbohydrates and a small quantity of lard (10 gm.), making, all told, a supply of energy equal to 142 Cal. per kgm., were fed with glycocoll for three days and then for two days without the glycocoll. Assuming that all the glycocoll was absorbed, I paid no attention to the faecal nitrogen. The results are given in Table VIII.

The non-nitrogenous diet should have been continued for one or

two days more, since, as Landergren's<sup>1</sup> experiments have shown, it is possible with carbohydrates alone to reduce the output of nitrogen to about one third of what it would be in fasting. At the same rate of decrease a third day in this period would have shown a nitrogen waste of 0.437 gm., which is just one third of the assumed requirement (1.3 gm.) for the beginning of the glycooll period, though probably a little more than one third for the day in question. The average loss

TABLE VIII.

Date 1907.	Weight in kgm.	Food.	Cal. per kgm.	N in food.	N in urine.	N dif.
May 13	4.32	5th day fasting	....	....	1.288	....
14	4.22	7 gm. glycooll	142	1.332	1.457	-0.125
15	4.38	" "	142	1.332	1.612	-0.280
16	4.50	" "	142	1.332	2.006	-0.674
17	4.54	No glycooll	140	....	0.987	....
18	4.54	" "	140	....	0.713	....
....	....	....	....	....	0.437(?)	....

of nitrogen, however, for the three days during which glycooll was fed was only 0.359 gm. This shows either that glycooll has caused some of the body nitrogen to be held back, *i. e.*, it has "spared" some body proteid from destruction, or that some of the glycooll itself escaped oxidation and elimination. In either case it is clear that the effect is not permanent; for as soon as the glycooll is dropped from the diet an increase in the amount of nitrogen eliminated takes place.

Again, the weight of evidence is that we have to do here with a temporary retention of glycooll itself. Two considerations at least favor this idea: First, if glycooll had not been fed on these three days it is practically certain that the loss of nitrogen on the two carbohydrate days (May 17th and 18th) would have been considerably less. Secondly, if glycooll were to reduce the metabolism of proteid in the body itself, the reduction, we should expect, would be greater on the second day than on the first and possibly also greater on the third than on the second. Instead of that we find the "protection" to be less and less. A much more plausible explanation is

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

that on the first day more of the glycocoll escaped elimination than on the second, and more on the second than on the third.

The observation of Parker and Lusk<sup>1</sup> that the amount of glycocoll<sup>2</sup> formed in metabolism and capable of being removed from rabbits in combination as hippuric acid is greater on the first day of the administration of benzoic acid than on subsequent days of a fasting period, may be cited as favoring the possibility of a temporary storage of glycocoll.

In all three of the experiments here reported, therefore, it appears that we have a temporary retention of glycocoll. But glycocoll fed alone to an otherwise fasting organism is at once completely oxidized to urea and removed. This is shown by the experiments of v. Brügsch and Hirsch, already cited (p. 245). This is ordinarily the case also in feeding glycocoll with other foods, as may be seen in the experiments of Samuely. How, then, shall we account for a temporary retention of glycocoll such as we have witnessed in the above experiment?

#### SIGNIFICANCE OF CARBOHYDRATES.

In my previous paper,<sup>3</sup> and in fact as early as 1904,<sup>4</sup> I drew attention to the large part played by carbohydrates in obtaining the comparatively high replacement of two thirds the fasting requirement of proteid by gelatin with maintenance of nitrogen equilibrium. Luthje has recently emphasized the same factor in obtaining nitrogen retention with asparagin and glycocoll, and, as already quoted (page 245), believes that the important condition is a chemical union of the carbohydrate with the amino-acid (amino-sugar). Still further support for this idea of a specific relationship between nitrogen retention and carbohydrates is to be found in the old experiments of E. Voit and Korkunoff,<sup>5</sup> by which it was learned that with carbohydrates much

<sup>1</sup> PARKER and LUSK: This journal, 1900, iii, p. 472.

<sup>2</sup> The statement of these authors that "in a fasting rabbit which had been frequently fed with lithium benzoate the amount of glycocoll eliminated in the urine as hippuric acid compared with the total nitrogen eliminated indicates that 4.0 grams of glycocoll may be derived from the metabolism of every 100 grams of the body's proteid," becomes very striking in the light of the work of ABDERHALDEN, GIGON, and STRAUSS, which shows (*Zeitschrift für physiologische Chemie*, 1907, li, p. 321) that 100 gm. of the proteid matter composing the rabbit's body may yield 3.27 gm. of glycocoll by hydrolysis.

<sup>3</sup> MURLIN: This journal, 1907, xix, p. 307.

<sup>4</sup> Proceedings American Physiological Society, this journal, 1905, xiii, p. xxix.

<sup>5</sup> VOIT, E., and KORKUNOFF: *Zeitschrift für Biologie*, 1895, xxxii, p. 58.

less proteid is necessary for maintenance of nitrogen equilibrium than with fats; in those of Parker and Lusk,<sup>1</sup> where it may seem that the output of glycocoll in combination with benzoic acid is smaller when carbohydrates are fed than in starvation; in those of Landergren,<sup>2</sup> where it is observed that carbohydrates reduce the metabolism of proteid considerably more than do fats; and finally, in those of Heilner,<sup>3</sup> where a reduction of from 12 to 25 per cent in the proteid metabolism (nitrogen output) was obtained by feeding carbohydrate enough to replace the fat of the fasting metabolism. Whether we call this specific relationship, with Landergren, a "specific need" on the part of the body for carbohydrate, in order that it may protect its living substance, or refer it to a specific affinity between the carbohydrate and the amino-acids by which a larger molecule is formed and the nitrogenous bodies escape oxidation for a longer time, in accordance with Lüthje's idea, there is sufficient warrant for the belief that in the case of glycocoll in the experiments above reported the essential condition for the temporary retention is the presence in the circulation of abundant carbohydrate.

#### INFLUENCE OF EXTRA-METABOLIC CARBOHYDRATE ON NITROGEN EXCRETION.

In the first section of this paper it was seen that small quantities of carbohydrate (12 per cent of the energy requirement) exercise little if any effect on the nitrogen output of the body. And yet, from the considerations reviewed in the preceding paragraph, we have every reason to believe that carbohydrates in large quantity play a determining part in the disposition which the body can make of nitrogenous substances. In attempting to reconcile these facts it occurred to me that it might be only the excess carbohydrate — that which is not needed for purposes of metabolism, or what we might call extra-metabolic carbohydrate — that is especially significant in aiding the retention of nitrogen.

It has been the general belief that carbohydrates protect the body proteid, *i. e.*, tend to reduce its destruction, by taking its place as a source of energy for the vital processes. If this be the only way in which carbohydrates can protect the body proteid, then its action, as

<sup>1</sup> PARKER and LUSK: *Loc. cit.*

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

<sup>3</sup> HEILNER: *Zeitschrift für Biologie*, 1906, *xlvi*, p. 144.

judged by nitrogen excretion, ought to be proportional to the amount fed until the total energy supply of the body is covered, beyond which its action would be *nil*. But if, as might be presumed from Landergrén's experiments with carbohydrates fed exclusively, where from 7 to 14 Cal. per kilogram were in excess of the requirement, and from my own with carbohydrates fed with gelatin, where<sup>1</sup> from 10 to 20 Cal. per k<sub>g</sub>m. were in excess, carbohydrates act in some other way, then, as the amount fed exceeds the requirement, its influence on the amount of nitrogen eliminated ought to be more pronounced. With these considerations in mind the following experiment was planned :

**Dog F.** — Same as dog C, page 238. For nearly two months previous to this experiment the dog had been on a rich proteid diet and was in fairly good condition as regards body fat, weighing 13.54 k<sub>g</sub>m. After fasting for four days the animal was given successively increasing quantities of carbohydrates for periods of two days each, alternating with fasting periods of one day. In all of the urines but two creatinin and ammonia as well as total nitrogen were determined. No attention was paid to the fæcal nitrogen. The urine at no time gave any reaction for albumin nor any reduction of Fehling's solution, even after boiling with HCL (Table IX).

Estimating the energy requirement of the dog by Rubner's<sup>2</sup> method employing v. Meeh's formula, we get for a body weight of 13 k<sub>g</sub>m., 646 Cal. Supplying 12.5 per cent of this requirement in the form of dextrose (22 gm.), we get a mean sparing for the two days of 0.279 gm. N, or 8.3 per cent of the fasting requirement (3.345 gm.). Increasing the supply to 25 per cent of the requirement (42 gm. cane sugar) gives us a sparing of 0.460 gm. N, or 13.3 per cent of the fasting requirement. In other words, by doubling the supply of carbohydrate calories we have increased the sparing by only 5 per cent.

Through an accident the urine for June 6th became contaminated, so that the figure obtained for the total N, was plainly too high. The number given for that day is, therefore, estimated so as to make this period yield the same percentage increase as the preceding period. The surprising thing is that by so doing we make the increased sparing resulting from the next 100 per cent increase in the supply exactly the same, namely, 5 per cent. That is, from each doubling of the energy supply, up to 100 per cent of the requirement, we get the same percentage increase in the effect on nitrogen elimination.

<sup>1</sup> *Loc. cit.*, p. 285.

<sup>2</sup> RUBNER : *Zeitschrift für Biologie*, 1885, xxi, p. 370.

TABLE IX.

Date 1907.	Weight kgm.	Food.	Calories.			Creatinin.	NH <sub>3</sub>	Total N.	Mean fasting N	Mean sparing	
			Total.	per kgm.	% of req.					Gm. N.	Fasting N, %.
May 29	13.04	4th day fasting . . .	..	..	..	0.279	3.345		0.279		
" 30	12.94	22 gm. dextrose . . .	81.4	6.3	12.5	0.250	3.148	3.345	0.279	8.3	
" 31	12.84	22 gm. dextrose . . .	81.4	6.3	12.5	0.297	2.984				
June 1	12.66	Fasting . . . . .	..	..	..	0.242	3.345				
" 2	12.46	42 gm. cane sugar . .	163.8	13.2	25.0	0.260	3.017	3.443	0.460	13.3	
" 3	12.30	42 gm. cane sugar . .	163.8	13.2	25.0	0.271	2.952				
" 4	12.16	Fasting . . . . .	..	..	..	0.191	3.542				
" 5	12.00	84 gm. cane sugar . . .	327.6	27.5	50.0	0.210	3.411	3.788	0.690	18.3	
" 6	11.82	84 gm. cane sugar . . .	327.6	27.5	50.0	....	2.785 <sup>1</sup>				
" 7	11.66	Fasting . . . . .	..	..	..	....	4.034				
" 8	11.44	{ 40 gm. cornstarch 20 gm. dextrose } . .	643.6	56.0	100.0	0.253	3.575	4.131	0.952	23.0	
" 9	11.58	{ 104 gm. cane sugar " } . . . . .	643.6	56.0	100.0	0.177	2.783				
" 10	11.58	{ Fasting . . . . . " } . . . . .	..	..	..	0.172	3.429				
" 11	11.30	{ 40 gm. cornstarch 20 gm. dextrose } . .	807.4	71.0	125.0	0.162	(4.228) <sup>2</sup>	2.582	2.119	49.1	
" 12	11.30	{ 146 gm. cane sugar " } . . . . .	807.4	71.0	125.0	0.305	1.787				
" 13	11.30	{ Fasting . . . . . " } . . . . .	..	..	..	0.165	3.111				
" 14	11.00	{ Fasting . . . . . " } . . . . .	..	..	..	0.181	4.402				

<sup>1</sup> This figure is estimated.<sup>2</sup> Estimated for second fasting day.

It will be observed that there is a steady increase in the fasting nitrogen metabolism throughout the experiment. This rather unusual phenomenon was observed in this same dog in the experiment reported on page 239, and is interpreted here, as it was there, to mean that there was not sufficient fat in the body to meet the needs of the fasting metabolism. This is rendered probable by the fact that the dog became extremely emaciated in a very few days. It is evident, in view of this steady increase in the fasting nitrogen, that the figure obtained on June 10th does not represent the real fasting metabolism, or, in other words, that the carbohydrate fed on the two preceding days contained something more than the requirement of potential energy; hence some glycogen was stored, which served to keep back the body nitrogen. If a second day of fasting had been taken, the figure would probably have been close to the mean between that for June 7th and that for June 14th, or about 4.228 gm. This figure is used in estimating the fasting requirement for the two feeding periods adjacent thereto. The fact of the increase in fasting metabolism, instead of the usual decrease, as well as the two defects in the record just pointed out, may well be held to invalidate the experiment to some extent as a basis for arriving at any definite law of the influence of what we may call *intra-metabolic* carbohydrate on nitrogen elimination. At all events, it will be necessary to confirm the facts before enunciating the law as above indicated.

When, however, the amount of carbohydrate is increased beyond the requirement of potential energy (extra-metabolic), we see a marked difference in the effect on nitrogen output. With 100 per cent (or slightly more, as it proves) of the requirement supplied by carbohydrate, the reduction in the amount of nitrogen eliminated was 23 per cent. Increasing the supply to 125 per cent gives us a reduction of nearly 50 per cent. In passing from 25 to 50 per cent of the requirement (June 2d to 5th) an increase of exactly the same absolute amount (42 gm. cane sugar) gave an increased sparing of not over 5 per cent, whereas here the increased sparing is more than 100 per cent. The contrast may be more sharply expressed in this way: so long as the total amount of carbohydrate is below the full requirement of potential energy, doubling the supply gives a five per cent increase in the effect; beyond the full requirement a 25 per cent increase in the supply doubles the effect.

These facts certainly speak for some influence of the carbohydrate on protein metabolism (nitrogen elimination) other than that of sub-

stitution in combustion. Whether that influence is to be explained by a chemical combination of the unburned sugar with amino-acids, as Lüthje supposes, or by some combination necessary to the storage of glycogen, or by a reduction of the metabolic processes through osmotic effects, or in some other way, must be left for future researches.

#### CREATININ AND AMMONIA.

With the hope of discovering what fraction of the total nitrogen is especially affected by the carbohydrate, a record was kept of the creatinin (Folin's method) and of the ammonia (Schaffer's method) for every day but two. This record, based on the single experiment, is too brief for more than passing mention.

Up to the point where the full energy requirement is supplied by the carbohydrate there is a noticeable tendency for the creatinin output to be higher on the carbohydrate days than on the intervening fasting days. Within the same limit the tendency with respect to the ammonia is just the reverse — higher on the fasting days than on the food days. From the 100 per cent limit on, the creatinin exhibits no fixed relationship, but the ammonia is markedly higher on the food days than on the fast days.

#### SUMMARY AND CONCLUSIONS.

1. The well-known action of gelatin to protect the body's proteid is not due to the influence of any dextrose which may be synthesized from it in the course of metabolism. Its value as a proteid-sparing agent must consist in the fact that it contains nitrogenous bodies.

2. Glycocoll, which is the chief amino-acid contained in gelatin, when fed with abundant carbohydrate, either as the only source of nitrogen or together with proteid (beef heart), can be retained temporarily in the body. This fact may serve in part to explain the unusually high replacement of proteid by gelatin, with maintenance of nitrogen equilibrium, which I reported in a previous paper. The fact that glycocoll cannot be retained permanently, even with large quantities of carbohydrate available, may serve in part to explain the inadequacy of gelatin as a source of nitrogen, and must be taken into account in any attempt to "restore" gelatin to full proteid value.

3. A specific relationship has been shown to exist between carbohydrates ingested and the elimination of nitrogen (or protein me-

tabolism, as measured by nitrogen output). Carbohydrate, not needed for combustion (extra-metabolic) is far more efficacious in reducing the nitrogen output (therefore favoring the retention of proteid) than carbohydrate coming within the requirement for potential energy. This fact indicates the importance of abundant carbohydrates for convalescence and growth, and may explain the almost universal craving for sweets, especially in the young.

TABLE X.  
EXPERIMENT ON MEDICAL STUDENT (A. I. R.).

Date 1906.	Weight in kgm.	Daily diet in grams.										Calories.		N in food.	N Output.			N Dif.
		Beef- steak.	Oat- meal.	Soda biscuit.	Rice.	Cane sugar.	Cream.	But- ter.	Cof- fee.	Wine c.c.	Dex- trose.	Total.	per kgm.		Urine.	Feces.	Total.	
Sept. 26	45.4	200	40	100	60	117	100	30	400	50	..	2067	45	9.822	1.190	11.012	-0.452	
27	45.6	200	40	110	60	100	75	30	400	50	54	2006	44	No anal.	1.190	...	...	
28	45.4	200	40	75	40	125	100	30	400	50	..	1936	43	9.355	1.190	10.545	-0.665	
29	46.5	200	40	85	40	125	100	30	400	50	..	1971	43	9.160	1.190	10.350	-0.300	
30	46.6	43.5 <sup>1</sup>	40	85	40	125	100	30	400	50	..	1935	41	11.391	0.830	12.221	-2.271	
Oct. 1	46.4	43.5 <sup>1</sup>	40	85	40	125	100	30	400	50	..	1935	42	10.564	0.830	11.394	-1.444	
2	46.3	43.5 <sup>1</sup>	40	85	40	125	100	30	400	50	..	1935	42	10.564	0.830	11.394	-1.444	
3	46.4	43.5 <sup>1</sup>	40	85	40	125	100	30	400	50	..	1935	42	9.361	0.830	10.191	-0.241	
4	46.3	43.5 <sup>1</sup>	40	120	70 <sup>2</sup>	125	50	20	400	50	50	2044	44	11.553	0.830	12.383	-2.763	
5	46.4	43.5 <sup>1</sup>	40	121	50 <sup>2</sup>	125	50	20	400	50	70	2036	44	9.294	0.830	10.124	-0.504	
6	46.4	....	40	85	40 <sup>3</sup>	125	100	30	400	50	24	1858	40	5.817	0.608	6.425	-3.195	
7	46.4	....	40	85	40 <sup>3</sup>	125	100	30	400	50	24	1858	40	6.151	0.608	6.759	-3.529	
8	46.4	....	40	85	40 <sup>3</sup>	125	100	30	400	50	24	1858	40	5.015	0.608	5.623	-2.393	

<sup>1</sup> Gelatin.

<sup>2</sup> Cornstarch.

<sup>3</sup> Rice.

## PERISTALTIC RUSH.

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### INTRODUCTION.

SOME writers on the motor phenomena of the intestinal tract distinguish at present three types of movement in the small gut. (1) Pendular movements: rhythmical, swaying motions, which apparently contribute but little to the forward expedition of the intestinal contents. According to Bayliss and Starling, and to Cannon, the pendular movements are chiefly concerned in the thorough mixing of the food with digestive fluids; they are the essential factors in the "rhythmic segmentation" of the intestinal contents observed by Cannon. (2) Peristaltic movements, which consist in a contraction of the gut above a food mass and a relaxation below it; they are chiefly concerned in carrying the food through the intestines in the aboral direction. The progress is very slow, 1 cm. in two to ten minutes in a fasting state and in thirty to forty seconds after a meal (Cash). The peristaltic waves never travel far, the same wave never running through the entire length of the intestines. (3) "Rollbewegungen," a fast, running movement extending over the whole or a large section of the small intestines. It is this motor phenomenon we intend to deal with in the present paper.

### HISTORICAL.

This phenomenon was first observed and described by van Braam Houkgeest<sup>1</sup> with Sanders-Ezn. The observations were made on rabbits whose abdomen was opened and the intestines observed while these parts of the animal were submersed in a warm saline bath.

<sup>1</sup> HOUKGEEST, VAN BRAAM: PFLÜGER'S Archiv für die gesammte Physiologie, 1872, vi, p. 266.

When the animal was killed by suffocation or by inhalation of CO<sub>2</sub>, a few seconds after the convulsions attending asphyxia ceased, a strong peristaltic contraction set in, either at the pylorus or a little distance away, which energetically and rapidly drove the contents before it. This rapid peristaltic wave ran without interruption to the cæcum, especially when the duodenum was strongly filled with contents. Sometimes another wave followed which originated in the ileum, and which also ran down in the same manner to the cæcum. Mostly, however, the duodenal wave was the only one which was observed. When the duodenum was less filled, or when the animal was already for some time in the saline bath, the peristaltic wave did not reach the cæcum, but stopped at the lower end of the jejunum or at the upper end of the ileum; in the latter case another similar wave arose later at some part of the ileum which carried the contents rapidly to the cæcum. None of the waves ever continued their course in the cæcum.

The rapid run of the peristaltic wave through the small intestine gave the coils the appearance of turning wheels; the movements were therefore designated by the investigator as "Rollbewegungen." At first in the protocols, but later for lack of a better expression, van Braam Houkgeest accepted this term in the final presentation of his results. Most of the subsequent writers have adopted this term for the description of this special form of intestinal peristalsis. Van Braam Houkgeest termed them in his protocols sometimes also "post mortem movements" on account of their occurrence only after death of the animal. Only in one case did he succeed in reviving the animal by prolonged artificial respiration after the Rollbewegungen had made their appearance. Later on van Braam Houkgeest was led to believe that under certain conditions he was able to produce the Rollbewegung also while the animal was alive. The discussion of this, however, will be deferred until later.

Observations of a similar character were also made by Engelmann<sup>1</sup> (with van Brakel) about one year previous<sup>1</sup> to the publication of van Braam Houkgeest. In a cat, which was killed by chloroform, and whose intestines were examined about half an hour after death, a mechanical stimulus applied to some part of the intestines caused a strong local constriction which ran down as a peristaltic wave to the cæcum with a rapidity of about 4 cm. in a second; upward the contraction ran as an antiperistaltic wave, stopping at

<sup>1</sup> ENGELMANN: *Archiv für die gesammte Physiologie*, 1871, iv, p. 33.

the stomach. In animals which were killed by exsanguination (rabbits, dogs, and cats) spontaneous movements which originated in the duodenum were seen to run down at times to the ileo-cæcal valve; mostly, however, the rapid wave stopped at the ileum.

In the experiments of van Braam Houkgeest, as well as in those of Engelmann, these movements were seen to occur after death; they were characterized by the rapid propagation of the peristaltic wave; sometimes the wave ran through the entire small intestine without stopping.

Nothnagel<sup>1</sup> later described Rollbewegung, which he studied in the living rabbit. He describes it as a violent peristalsis running rapidly over some 20 cm. of the small intestine and coming suddenly to a standstill, as he suggests, by some inhibitory influence. He has never seen the wave extending over the entire length of the small intestine. Nothnagel believes that the distention of the intestines by liquid and gas is the cause of these Rollbewegungen, but asserts at the same time that observation of these movements had to be confined to their accidental occurrence, and that it was impossible to produce the Rollbewegungen at will.

Bokai,<sup>2</sup> on the other hand, stated that the presence of  $\text{CO}_2$ ,  $\text{CH}_4$ , or  $\text{H}_2\text{S}$  in the intestines will cause "rollende Bewegungen." These movements, when once started, are not propagated to a distant section of the intestines where these gases are absent. The gases produce violent but only local contractions. The presence of oxygen in the lumen of the intestines stops these movements; nitrogen and hydrogen exert no effect.

From the writings of Mall,<sup>3</sup> Cannon,<sup>4</sup> and others it appears that the "rollende Bewegungen" of Bokai are considered as identical with the Rollbewegungen of van Braam Houkgeest.

Cannon describes the Rollbewegungen "as a rapid movement sweeping the food without pause through several turns of the gut," and "is frequently seen when the food is carried on from the duodenum"; and states "that it may readily be produced in other parts of the small intestine by giving an enema of soapsuds."

<sup>1</sup> NOTHNAGEL: Beiträge zur Physiologie und Pathologie des Darms, Berlin, 1884. See also NOTHNAGEL'S Handbuch der speziellen Pathologie und Therapie. Die Darmbewegungen, 1898, xvii, p. 2.

<sup>2</sup> BOKAI: Archiv für experimentelle Pathologie und Therapie, 1887, xxiii, p. 209.

<sup>3</sup> MALL: Johns Hopkins Hospital reports, 1896, i, p. 51.

<sup>4</sup> CANNON: This journal, 1902, vi, p. 251.

We shall return now to a few statements of van Braam Houkgeest. In the first place, he states that the Rollbewegungen did not occur post mortem if both vagi were previously cut; only irregular Rollbewegungen and constrictions appeared in various sections of the small intestines. If however at the onset of these irregular contractions the peripheral ends of the cut vagi were stimulated, regular Rollbewegungen then ran through the entire small intestine. Furthermore, if in a living rabbit both splanchnics were cut, then stimulation of the peripheral end of either vagus would cause first a strong constriction of the median part of the stomach which was followed soon by a "true Rollbewegung," which in most cases ran through the entire small intestine. In strong animals such results could be obtained repeatedly by repeated stimulation of the peripheral end of either vagus. Van Braam Houkgeest believes that the Rollbewegungen produced by the stimulation of the vagi, after cutting the splanchnics, are identical with the post mortem Rollbewegungen.

In summing up this review we find that Engelmann as well as van Braam Houkgeest have observed in dead animals Rollbewegungen of spontaneous origin running through the entire intestine; that Engelmann produced in dead animals "Rollbewegungen" by mechanical stimulation. Van Braam Houkgeest produced true Rollbewegungen in living animals by stimulating the peripheral end of one vagus after both splanchnics were cut. Nothnagel has seen in the living animal Rollbewegungen, but they never traversed the entire small intestine, and they could not be produced at will. Bokai produced by the introduction of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{H}_2\text{S}$  into the lumen of the intestine of living animals violent peristaltic movements which he termed "rollende Bewegungen."

We should add here that the statement of van Braam Houkgeest concerning the production of true Rollbewegung in the living animal by stimulation of the peripheral end of the vagus was, as far as we know, never tested by any one; in fact, we did not come across any reference to that statement, nor to the statement that the post mortem Rollbewegungen depend upon the vagi being intact.

The true Rollbewegungen were not seen by many students of intestinal movements. Those who had seen them would not have failed to dwell upon them, as they present a striking phenomenon.

In the extensive studies of Bayliss and Starling<sup>1</sup> this form of intestinal movement was apparently not observed by them. Had they observed this mode of peristalsis, they would not have failed to comment upon it, since it illustrated, as we shall show later, their law of intestinal contraction in a striking way. Starling<sup>2</sup> says that "the post mortem vermicular contraction described by Engelmann in the rabbit is probably merely an exaggerated wave just described," meaning pendular movements. He states there further that Mall places this form of contraction in a class by itself which he terms "vermicular." Mall, however, applies the term vermicular to the normal peristalsis as well as to the "irregular rapid wave." The latter is not designated by Mall by any special name. The term Rollbewegung does not occur in Mall's paper in which the description of Engelmann of the effect of mechanical stimulation of the intestines serves as a basis for the analysis of this mode of intestinal movement. Mall considers the rapid irregular wave as a pathological phenomenon "to rid the intestine rapidly of irritating products of decomposition," having in mind the above recorded statements of Bokai.

We came across the phenomenon of Rollbewegungen in our studies of the effects of saline purgatives,<sup>3</sup> ergot,<sup>4</sup> and of magnesium salts.<sup>5</sup> We have seen it occasionally occurring in the living animal in the very same striking manner as was described by van Braam Houkgeest in animals dying from asphyxia. In a series of experiments especially devoted to that subject we have traced at first the conditions under which Rollbewegungen accidentally occurred, and then attempted to bring out the phenomenon at will. It is the object of this paper to give a brief account of the results we have thus obtained. However, before entering upon a detailed description of our experiments we shall dwell upon the general appearance of the phenomenon of Rollbewegung and its essential features, by means of which it may be distinguished from other forms of intestinal movements.

**Rollbewegung, or peristaltic rush.** — In moderately distended and

<sup>1</sup> BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 99, and 1900-1901, xxvi, p. 125.

<sup>2</sup> STARLING: SCHAEFER'S *Textbook of physiology*, ii, p. 329.

<sup>3</sup> AUER: *This journal*, 1906-1907, xvii, p. 15.

<sup>4</sup> MELTZER and AUER: *Ibid.*, p. 143.

<sup>5</sup> MELTZER and AUER: *Ibid.*, p. 313.

moderatively active small intestines, suddenly, frequently without warning, a rushing wave appears, which sweeps with great rapidity over the entire small intestine to stop only at the cæcum. Each coil, as the rushing wave passes through it, gives the appearance of a rapidly turning wheel. On account of twisted and intricate relations of the intestinal convolutions which do not permit the simultaneous observation of the entire gut, the peristaltic rush presents a confusing spectacle of whirling coils appearing, disappearing, and reappearing, until the entire rush comes to a standstill. The entire rush is at times accomplished in less than fifteen seconds. In the wave, as it hurries through each coil, two parts may be distinguished which present completely different aspects. In the aboral part of the wave the moving intestine appears in the shape of a turning wheel, is greatly distended, perfectly smooth, and offers apparently not the slightest resistance to the onward movement of its contents which is rapidly driven through it, and which consists of a dark brown or yellowish fluid intermingled with some gas bubbles. This part is apparently completely relaxed, all tonic or rhythmic contractions are inhibited. Closely at the foot of this section, at the oral end of it, the other part of the wave follows in which the intestine is contracted to a cord. The lumen of that part of the intestine is completely obliterated, but the constriction fails to produce such complete anemia as often attends strong contractions of the intestines caused by artificial stimulations, by the administration of barium, etc. Even at the height of the contraction the color is still pinkish. The contraction lasts only a few seconds, after which that part of the intestine looks patulous and empty; it retains, however, the rounded shape for some time. On account of the very rapid propagation of the wave of constriction the contracted piece of intestine appears sometimes to have a length of 5 to 10 cm. The preceding wave of inhibition extends apparently over a considerable section of the intestine; but its aboral end is usually lost in a hidden loop and cannot be ascertained.

A complete peristaltic rush begins somewhere in the duodenum and terminates at the cæcum. The exact starting-point of the wave is difficult to establish on account of the deep location of the duodenum.

Such complete Rollbewegungen are sometimes followed immediately by an incomplete wave which begins at some place in the

jejunum or ileum, and runs either a full course terminating at the cæcum or dies out, terminating at some distance from it.

As a rule, after such rushing waves, the small intestine remains completely quiescent, and for some time neither normal peristalsis nor pendular movements make their appearance.

Accordingly, the true and complete Rollbewegungen, as we have seen them, are characterized (1) by the great rapidity of progress of the circular constriction; (2) by the extensive and complete inhibition preceding the contraction, and (3) by the complete course of the wave, which traverses without interruption the entire small intestine.

In many instances, however, Rollbewegungen appear which are incomplete in one or the other of the characteristics mentioned. In the first place many rushing waves appear which run only short distances, beginning at the duodenum and terminating in some part of the jejunum or ileum, or beginning in a still lower section and dying out before reaching the cæcum. It is this kind of Rollbewegungen which were seen by Nothnagel in the living animal. In the second place, the progress of the wave may sometimes be of only moderate rapidity. Such slower waves may exceptionally traverse the entire intestine; as a rule, such slow waves die out in the middle of the circuit. However, even these slower waves move incomparably more rapidly than the waves of normal peristalsis. Finally, in some cases of Rollbewegungen the constriction as well as the relaxation may not be so extreme as described above; but here again both features of the rushing wave are even in such incomplete cases much more pronounced than in normal peristalsis.

Incomplete forms of Rollbewegungen are easily distinguishable from normal peristalsis by the size of the section which is involved, by the rapidity of the progress of the wave, and by the intensity of the processes, especially by the striking relaxation of the part of the intestine in front of the progressing constriction.

For those who have seen the phenomenon of Rollbewegungen it is hardly necessary to point out the particulars which distinguish them from pendular movements. The two types of movement can hardly be confounded, as they have practically very little in common.

It is, however, necessary to state expressly that the Rollbewegungen should not be confused with simple, violent constrictions of the small intestines, such as are frequently seen after intravenous

administration of barium chloride, of eserine, or even of ergot, and in many other conditions. These constrictions may even be stronger than those seen in Rollbewegungen, and may extend over 6 or 8 cm.; they might even show some travelling. But it is not difficult to distinguish them from the Rollbewegung. The constrictions in violent intestinal movements last a good deal longer than those of the Rollbewegungen, occur simultaneously at many sections of the small intestine; they do not travel progressively in an aboral direction, but move irregularly to and fro in a slow fashion. The striking differential point, however, is the absence in these violent constrictions of any inhibition in front of a constricted section. The contents of the intestine which is driven out by such violent constrictions have to pass through a more or less tonically contracted piece of intestine at the other end of which the way is frequently blocked by another strongly contracted part of the intestine.

We may say here that the above-mentioned "rollende Bewegungen," observed by Bokai after the introduction of  $\text{CO}_2$  and other gases into the lumen of the intestines, were simply violent movements of the type just mentioned, and do not belong to the true Rollbewegungen. Bokai himself states positively that the inhibitory factor of the intestines was not involved in the action of these gases upon the intestines. The gases introduced into the lumen of the intestines stimulate the gut by contact to violent constriction, extending over a few centimetres, which constriction may extend farther down if the gases travel downward.

A few words more with reference to the nomenclature of this phenomenon. As stated above, the term Rollbewegungen was used by van Braam Houckeest in his protocols. For lack of a better term he retained it also in the final publication of his studies. However, Rollbewegung indicates only the incidental feature of the phenomenon, the rapidity of the wave through the coils calling forth the illusion of a turning wheel. In the English literature there is no special name for this phenomenon. Cannon and other writers use the German term Rollbewegungen. As a fitting English designation for this phenomenon we adopted the term Peristaltic Rush, indicating in the first place its main feature, namely, the rushing character of the forward movement. Furthermore, "peristaltic" conveys the important fact that these movements possess the essen-

tial characteristic of peristalsis, namely, a contraction above and inhibition below (Law of Intestine of Bayliss and Starling). When the rushing wave runs through the entire small gut, we designate it as *complete*; when the wave runs through only a part of the course, or is deficient in other ways, we designate it as *incomplete*.

#### EXPERIMENTAL OBSERVATIONS.

**Method.** — The observations were made exclusively on rabbits, some of which received subcutaneous injections of morphin. The intestines were invariably observed in a warm saline bath. The saline consisted of a solution of 0.92 per cent sodium chloride, which is considered isotonic with the serum of the rabbit. (The solutions employed by van Braam Houkgeest consisted of 0.6 per cent sodium chloride.) In the present series of experiments the “receptacle for the bath” was prepared by flaps from the abdominal skin in the manner described in our paper on the action of ergot.<sup>1</sup> After an incision in the middle line of the abdomen the skin was extensively dissected on both sides from the underlying musculature, and by an appropriate suspension a deep receptacle was formed. This was filled up with a warm saline solution, and under cover of this the abdomen was freely opened in the linea alba. By slightly retracting the muscular walls by light weights the escaping intestines were bathed on all sides with the warm saline. The solutions were kept warm and at the proper level by frequent addition of fresh warm saline.

**Movements in normal rabbits.** — At the outset we may state that in normal living animals we have never seen movements of the small intestines which we could designate as rushing peristalsis according to our definition. In the present series the behavior of the intestines was watched for some time before the effect of any substance was tested. It must be admitted that the time given to such preliminary observations was necessarily not very long. But we have had sufficient opportunities in various series of experiments, carried out for other purposes, to watch the behavior of the uninfluenced intestines. Never have we noticed intestinal movements belonging to the rushing type.

**After intestinal stimulants.** — Neither have we seen rushing peri-

<sup>1</sup> MELTZER and AUER: This journal, 1906-1907, xvii, p. 143.

stalsis in experiments in which the intestines were stimulated to greater activity by subcutaneous or intravenous injections of some purgatives. In the experiments of one of us (A.) with subcutaneous and intravenous injections of sodium sulphate, sodium phosphate, and sodium citrate in which it was found, in agreement with the statement of J. B. MacCallum,<sup>1</sup> that the movements of the small intestine are increased, no peristaltic rush ever occurred, although the intestines were watched for hours. In experiments with intravenous injections of barium chloride and of eserine, in which the intestines were stimulated to violent and extensive contractions, no peristaltic movement was ever observed which bore the criteria of peristaltic rush as set forth above. The same we may state with regard to the effects of ergot, when employed alone. The intestines were stimulated to rhythmic, travelling, and tonic contractions, but none of them possessed the characteristics of rushing peristalsis; especially was the marked inhibitory wave absent from all the motor phenomena produced or aggravated by the injections of ergot or any of the other intestinal stimulants.

**Stimulating and inhibitory factors.** — The phenomenon of peristaltic rush we have seen to occur in such experiments in which apparently two opposing elements were in operation, — factors which increase intestinal activity and factors which as a rule inhibit this activity. Of the elements of the first class there were employed sodium phosphate, sodium sulphate, sodium citrate (“saline purgatives”), ergot, barium chloride, eserine, and destruction of some part of the dorsal cord. As inhibitory agents we have employed: calcium chloride, magnesium chloride, and magnesium sulphate. The greatest number of experiments were made with ergot and calcium, which gave, as we shall see later, very reliable results. We have obtained, however, satisfactory results also in other combinations. We shall illustrate our results only by a few greatly abbreviated protocols.

**“Saline purgatives” and calcium. —**

*Experiment 1.* — Gray female rabbit, 1750 gm. . . .

3:27 P. M. Abdomen opened in saline bath. . . . No movements of small intestines.

3:37 P. M. Injected subcutaneously 15 c.c. sodium phosphate (4 per cent). . . .

<sup>1</sup> J. B. MACCALLUM: This journal, 1904, x, p. 107.

3.47 P. M. Slight contraction of duodenum, balance of small gut empty and quiet. . . .

3.52 P. M. Duodenum full of light yellow fluid, quite active; jejunum and ileum, still empty, show swaying movements. . . .

During next eighty minutes only slight changes.

5.10 P. M. Upper part of jejunum full, but not distended, shows constantly swaying movements. Duodenum shows only moderate swaying. . . .

5.17 P. M. Injected through the external jugular vein 2 c.c. sodium sulphate  $m/8$  solution, followed by an injection of 1 c.c. saline. Small intestines show soon after a moderate increase of swaying motions.

5.23 P. M. Movements definitely less again.

5.30 P. M. Injected 2 c.c. sodium citrate  $m/8$  solution, followed by 1 c.c. saline. Soon after the injection the movement of small intestines definitely increased, swaying and shortening movements, no constriction waves seen.

5.35 P. M. Intravenous injection of 2 c.c.  $\text{CaCl}_2$   $m/8$  solution, followed by 1 c.c. saline.

Before injection was finished jejunum and ileum became quiet, but a part of the duodenum showed very active pendular movements and strong circular constrictions such as were not seen before.

5.40 P. M. Small intestines show again good swaying movements. . . .

5.50 P. M. Intravenous injection of 3 c.c.  $\text{CaCl}_2$   $m/8$ , followed by 1 c.c. saline. . . . No marked change in either direction.

6.00 P. M. Intravenous injection of 1 c.c.  $\text{CaCl}_2$   $m/1$  solution, followed by 1 c.c. saline. Shortly after, a powerful contraction of the duodenum set in, shooting the fluid contents swiftly through the coils of the small intestines and stopping at the cæcum — a *complete peristaltic rush*.

Repeated twice with 1 c.c.  $\text{CaCl}_2$   $m/1$ , each time with the same result.

The above experiment, the protocol of which was here greatly abbreviated, is instructive in many directions. For two hours the intestines were watched while they were under the influence of subcutaneous and intravenous injection of the "purgative salts." As a result of these injections the small intestines showed a moderate increase of their motility which was confined to the swaying motions. At no time was there an indication of a rushing peristalsis. After the injection of 2 c.c.  $\text{CaCl}_2$   $m/8$ , which was equal to the preceding dose of sodium citrate, the activity of the jejunum and ileum became inhibited, which is in harmony with the statement of J. B. MacCallum<sup>1</sup> that calcium counteracts the stimulating effect of the purgative salts.

<sup>1</sup> MACCALLUM, J. B.: This journal, 1904. x, p. 107.

The duodenum, however, became more active. After a few minutes the activity of the small intestines returned, and a second injection of a similar dose of  $\text{CaCl}_2$  had no decided effect. Finally, when a dose of 1 c.c. of a *molecular* solution of calcium chloride was administered, which had to be injected very slowly, a powerful wave of rushing peristalsis swept over the entire small intestine. Repeating the injections with similar doses at proper intervals brought out similar results.

While we can confirm in general the discovery of MacCallum regarding the inhibitory effect of the calcium salts upon intestinal movements, we found at the same time that it is just the addition of the calcium salts which brings out the rushing peristalsis.

Similar results were obtained in some other experiments in which injections of calcium salts followed those of purgative salts.

However, we have not made many experiments with the purgative salts. Our main experiments were made, as stated before, with ergot and calcium, and we shall quote two abbreviated protocols to illustrate the various results obtained with this combination.

#### Calcium and ergot. —

*Experiment 2.* — Black female rabbit, 2030 gm. . . . Abdomen opened. . . . Recovering from ether.

11.15 A. M. No movements of gut. Intravenous injection of 1 c.c.  $\text{CaCl}_2$  *m/1*, followed by 2 c.c. saline.

11.23 A. M. Intestines relaxed, flat, no movements anywhere. . . .

11.35 A. M. Intravenous injection of 1 c.c.  $\text{CaCl}_2$  *m/1*, followed by 2 c.c. saline.

11.42 A. M. Lower small gut shows slight motions, coils flat. . . .

11.54 A. M. Occasional good swaying of some loops of the small intestine.

11.55 A. M. Intravenous injection of 1 c.c. of fluid extract of ergot (Squibb), followed by 2 c.c. saline. Shortly after, movements of small gut definitely increased. . . .

11.58 A. M. Strong contraction, a few centimetres long, drives contents swiftly into cæcum — *complete peristaltic rush*.

12.11 P. M. Intravenous injection of 1 c.c. ergot, followed by 2 c.c. saline.

12.13 P. M. Slight increase of swaying of small gut.

12.24 P. M. Swaying becomes once in a while more marked, but no sign of Rollbewegung.

12.26 P. M. Intravenous injection of 1 c.c.  $\text{CaCl}_2$  *m/1*, followed by 2 c.c. saline.

12.27 P. M. Strong "Rollbewegung," driving contents into cæcum (stronger than before).

12.30 P. M. Small gut relaxed, but not empty.

12.40 P. M. Intravenous injection of 1 c.c. ergot, followed by 2 c.c. saline.

12.50 P. M. Pendular movements increasing, gut gradually filling up, no Rollbewegung.

12.52 P. M. 1 c.c.  $\text{CaCl}_2$   $m/1$ , followed by 2 c.c. saline. Swaying movements subsided for a while and then started again.

12.55 P. M. A good *wave of rushing peristalsis*. . . .

Animal killed by asphyxia. After convulsions subsided "good travelling peristalsis of small gut."

In this experiment the first injections of  $\text{CaCl}_2$  in molecular solution produced no effect, there were previously no movements to be inhibited; the additional injection of 1 c.c. of ergot brought on within three minutes a wave of rushing peristalsis. A further injection of ergot brought out only swaying movements, but now an injection of  $\text{CaCl}_2$  brought out within two minutes strong Rollbewegungen. Further injections of 1 c.c. ergot and 1 c.c. calcium brought on another peristaltic rush.

Calcium chloride alone had no effect at all (when there were no previous movements), and ergot alone brought only an aggravation of the usual intestinal movements; but when ergot followed calcium and when calcium followed ergot, the result was usually a complete peristaltic rush.

*Experiment 3.* — Female rabbit, 1530 gm. . . .

11.35 A. M. Abdomen open, all operations finished. No movements of intestines visible.

11.50 A. M. Occasional slight movements of small intestine.

11.54 A. M. Intravenous injection of 1 c.c.  $\text{CaCl}_2$   $m/1$ , followed by 2 c.c. saline.

11.57 A. M. Moderate, but distinct, pendular movements of all visible coils of small gut.

12.01 P. M. Movements improved; some coils filled up, are round; duodenum invisible.

12.11 P. M. Intravenous injection of 1 c.c.  $\text{CaCl}_2$   $m/1$ , followed by 2 c.c. saline. Before injection of the calcium was finished all movements disappeared.

12 15 P. M. Slight movements visible again.

12.20 P. M. Movements improved, present in nearly all visible coils.

12.28 P. M. Intravenous injection of 1 c.c. of ergot, followed by 2 c.c. saline. Before the injection of saline is finished a *moderate incomplete peristaltic rush* appeared.

12.32 P. M. Pendular movements in all coils.

12.34 P. M. Nearly all coils are perfectly quiet.

12.44 P. M. Intravenous injection of 1 c.c. of ergot, followed by 2 c.c. saline.

12.46 P. M. Pendular movements in all coils, gradually increasing.

12.58 P. M. No Rollbewegung occurred. Intravenous injection of  $\text{CaCl}_2$  *m/1* was given again, followed by 2 c.c. saline. Before injection of calcium was finished, an *incomplete but strong peristaltic rush* set in.

1.02 P. M. All coils very active.

1.12 P. M. *Complete peristaltic rush.*

1.27 P. M. Slight pendular movements in some coils.

1.28 P. M. Intravenous injection of 1 c.c. of ergot, followed by 2 c.c. saline.

1.30 P. M. Pendular movements increased everywhere.

1.38 and 1.44 P. M. *Incomplete but good waves of rushing peristalsis* reach the cæcum. Loops remain full, round, and show good swaying movements.

1.45 P. M. Intravenous injection of 1 c.c. of  $\text{CaCl}_2$  *m/1*, followed by 2 c.c. saline.

1.47 P. M. Entire gut quiet.

A few more alternating injections of calcium and ergot brought out only increased normal activities and their inhibition, but no waves of rushing peristalsis.

In this experiment the various alternating injections of calcium and ergot brought out only one complete peristaltic rush and a few incomplete rushes.

In many of the experiments upon normal animals, in which ergot and calcium were used, more than one complete peristaltic rush occurred, besides a few incomplete waves. Frequently, however, after one or two rushes occurred, further injections of these substances became less effective and an incomplete Rollbewegung was the most that could be obtained. We had no experiment in which the injection of calcium and ergot failed completely to cause rushing peristalsis, and we had only one experiment in which only incomplete Rollbewegung occurred. In this experiment morphine alone was used as an anesthetic, the respiration became very slow, and ergot failed otherwise to cause the customary stimulating effect upon the intestinal movements.

**Destruction of cord and administration of calcium.** — In the following instructive experiment the administration of calcium alone brought out a complete peristaltic rush.

*Experiment 4.* — White female rabbit, 1560 gm. Morphine subcutaneous 0.015. . . . Spinal cord destroyed below fifth dorsal vertebra. . . . Abdomen opened.

11.45 A. M. Good swaying motions and constrictions all over the small gut.

12.00 M. Intravenous injection of 1 c.c. of  $\text{CaCl}_2$  *m/l*. Before injection was finished, a strong wave swept over entire small intestine (duodenum not visible) and drove contents into cæcum. *Complete peristaltic rush.* After this wave passed, gut became quiet.

12.06 P. M. Another wave swept down, stronger than before. Small gut, after wave, empty and tape-like, moderately relaxed and shows some swaying.

Three more injections of  $\text{CaCl}$  did not bring out any rushing peristalsis.

Here the first injection of calcium brought out two waves of rushing peristalsis. The destruction of the cord, which usually greatly increases the activity of the intestines, supplied the stimulating factor; the intestines were very active while the calcium injection was given.

**Magnesium salts and ergot.** — Rushing peristalsis was rarely brought about when in addition to ergot (and destruction of the cord) magnesium sulphate or chloride was injected instead of calcium. Out of five experiments only in one two incomplete Rollbewegungen occurred. As we have shown elsewhere,<sup>1</sup> magnesium salts inhibit completely the movements of the intestines produced by the injection of ergot. In the present line of experiments the effect of ergot is apparently nearly completely lost as a stimulating factor in the presence of the strongly inhibitory effect of magnesium.

**Calcium chloride and barium.** — On the other hand, the inhibitory effect of calcium is apparently a less reliable factor for the production of peristaltic rush in the presence of such strongly stimulating substances as  $\text{BaCl}$ . In five experiments in which calcium chloride and barium chloride were injected intravenously, in three no peri-

<sup>1</sup> MELTZER and AUER: This journal, 1906-1907, xvii, p. 318.

stalsis of a rushing type made its appearance. In the two other experiments one complete peristaltic rush occurred in each one, besides one or two incomplete waves.

We are here reminded of the statement of MacCallum<sup>1</sup> that "the peristaltic movements produced by barium chloride are usually not stopped by the administration of calcium."

**Magnesium and barium.** — Of four experiments in which barium and magnesium chloride were alternately injected, in three there were a few complete peristaltic rushes as well as incomplete waves, and only in one all signs of Rollbewegungen were missed. In our previous experiments<sup>2</sup> we reported that magnesium salts are capable of inhibiting the violent intestinal constrictions produced by barium. While the inhibiting effect of magnesium is apparently much greater than that of calcium, and is strong enough to overpower temporarily the violent constrictions produced by barium, the stimulating effect of the latter is, however, too strong to be completely annihilated, even by magnesium. The result of the alternating injection of the two strong antagonistic factors is therefore often a compromise in the shape of a peristaltic rush.

In the following experiment calcium as well as magnesium was employed:

*Experiment 5.* — Gray female rabbit, 1320 gm. Morphine 0.01. . . .

Abdomen opened at 11.50.

11.55 A. M. No sign of motion anywhere. Intravenous injection of 8 c.c.  $\text{CaCl}_2$  *m/8* followed by 2 c.c. saline.

12.00 M. No effect.

12.05 P. M. Injection of 0.5 c.c.  $\text{BaCl}_2$  *m/8*. Strong contractions of small gut, no "running."

12.15 P. M. 8 c.c.  $\text{CaCl}_2$  *m/8*, later 0.5 c.c.  $\text{BaCl}_2$  *m/8*.

12.20 P. M. Again 0.3 c.c.  $\text{BaCl}_2$  *m/8*. "No runs or anything approaching them."

12.30 P. M. 4 c.c.  $\text{CaCl}_2$  *m/8* and 2 c.c. saline. Small gut quieter at first, then attempt at running, but no definite wave of contraction swept along.

12.50 P. M. 4 c.c.  $\text{CaCl}_2$  *m/8* and 2 c.c. saline. Soon after 0.1 c.c.  $\text{BaCl}_2$  *m/8*. Same as before: tonic contractions of some parts of small gut.

1.40 P. M. 0.9 c.c.  $\text{MgCl}_2$  *m/1* and 0.1  $\text{BaCl}_2$  *m/1*. Shortly after a definite run occurred over entire small gut. Later two short runs occurred.

<sup>1</sup> MACCALLUM: This journal, 1904, x, p. 107.

<sup>2</sup> MELTZER and AUER: *Ibid.*, 1906-1907, xvii, p. 318.

The several alternating injections of calcium and barium brought no success, while the first injection of  $MgCl_2$  brought on a true Rollbewegung.

**Eserin with calcium or magnesium.** — We also made a few experiments with eserin. In the experiments in which calcium alone was used with eserin, there was practically no success. In one experiment, however, in which at first eserin was alternated with  $MgSO_4$ , no running waves occurred. Later, however, when calcium was substituted for magnesium, the injections of calcium at first quieted the contractions, but a few minutes after each injection a definite complete peristaltic rush set in.

**Destruction of cord.** — The cord was destroyed in seven experiments (below the 5th, 3d, or 2d dorsal vertebra). In four experiments the abdomen was opened soon after the destruction and watched only for fifteen to twenty minutes before an injection of any kind was given. The intestinal activity was increased in all four experiments, but no rushing peristalsis was seen. In three experiments the abdomen was opened about two hours after the destruction of the cord. In two of these experiments there were a few spontaneous peristaltic rushes, complete and incomplete. In the third experiment there was only one incomplete peristaltic rush, but the intestines in this experiment have also otherwise shown very little activity.

From these few experiments we learn at least in a general way that the phenomenon of peristaltic rush may occur in rabbits whose cord was so destroyed as to eliminate the influence of the splanchnics and who otherwise did not receive any substance capable of inhibiting intestinal movements.

**Section of vagi.** — According to van Braam Houkgeest, as will be remembered, the post mortem Rollbewegungen did not occur when both vagi were previously cut. We have made four experiments in which  $CaCl_2$  and ergot were given alternately after both vagi were cut. In none of these cases did a complete Rollbewegung occur. In three experiments there were some incomplete Rollbewegungen, in two of which the course of these waves was short and sluggish and marked by a very slow relaxation of the contracted part. In one of these experiments the vagi were cut after the animal had already received a few doses of ergot and calcium, but only one good but incomplete Rollbewegung was produced. After cutting the vagi and continuation of the injections, one other incomplete

Rollbewegung occurred which was as good as the one before cutting the vagi. In this case after killing the animal by asphyxia an incomplete Rollbewegung occurred exactly like those observed while the animal was alive.

These few observations seem indeed to justify the assumption that the occurrence of a true complete peristaltic rush is to a great measure dependent upon the integrity of the vagi.

We may call to mind here that cutting the vagi interferes greatly, for some time at least, with the normal movements of the stomach and also, as we have shown recently,<sup>1</sup> with the normal movements of the rabbit's cæcum.

**Stimulation of the vagi.**—Van Braam Houkgeest has also stated that stimulation of the peripheral end of one vagus will also cause in the living rabbit Rollbewegungen, provided both splanchnics are previously cut. We have tested this claim in two rabbits whose splanchnics were cut, and in three others in which the cord was destroyed, which was equivalent to cutting the splanchnics. In no case could we find that stimulation of the vagi brings out complete or incomplete peristaltic rushes. Even in an experiment in which, after destruction of the cord, rushing peristalsis occurred spontaneously and with readiness, stimulations of the peripheral end of the vagi, although visibly aggravating the other motor activities of the intestines, did not contribute to the production of a peristaltic rush.

Our experiments have shown that the phenomenon of peristaltic rush occurs when the animal receives injections of two groups of substances. The first group comprises sodium sulphate, sodium phosphate, sodium citrate, ergot, barium chloride, and eserine. The second group comprises calcium chloride, magnesium chloride, or magnesium sulphate. The substances of the first group are intestinal stimulants; that is, by their injection the intestines are stimulated to greater activity. The second group we consider as inhibitory substances for the intestinal movements; that is, we assume that by their injection intestinal movements, when present, are reduced or completely inhibited for some time. The stimulating character of the first group of substances is well understood and requires little discussion. Among this group the sodium salts are the weakest stimulants, ergot acts much more strongly, and barium

<sup>1</sup> Proceedings of the Society for Experimental Biology and Medicine, 1907, iv, p. 37.

and eserine have the strongest effect. With regard to the inhibitory group a few explanatory remarks would not be out of place. The inhibitory effect of calcium upon the intestinal movements was observed by J. B. MacCallum. These movements, which were started or aggravated by the injection of "purgative salts" were inhibited by the injection of calcium chloride. This discovery was made on the basis of J. Loeb's well-known view of the general inhibitory effect of calcium salts. The strong effect of barium chloride could not be inhibited by the injection of calcium. From the observations in the present series of experiments as well as on many other occasions, we can confirm the statement that the injection of calcium chloride causes as a rule an inhibition of intestinal movements when normally present or brought on by the injection of stimulating agents.

Regarding the inhibitory effect of magnesium salts upon intestinal peristalsis, we have dealt recently in a special article on that subject.<sup>1</sup> We have found that these salts are capable of inhibiting intestinal movements of whatever source. We may state again expressly that the violent movements of the intestines caused by barium or eserine can also be inhibited by the injection of magnesium salts.

We may mention here that MacCallum also observed that intravenous injection of magnesium chloride inhibits the movements of the intestines produced by sodium citrate, sulphate, etc., although he surprisingly stated that subcutaneous injection of magnesium sulphate has a stimulating effect upon the intestines. In our experience we found no difference between magnesium sulphate and magnesium chloride; both inhibit the intestinal movements.

We may also state expressly that according to our experience the inhibition exerted by magnesium salts is distinctly stronger than that produced by calcium; the contractions are more completely inhibited, the effect lasts longer, and we met with no kind of movements which could not be reduced or abolished by magnesium, while calcium, according to MacCallum, cannot overcome the effect of barium chloride.

As to the meaning of inhibition we may refer to our first paper on the magnesium salts.<sup>2</sup> We started from the hypothesis that magnesium favors such an action as that of the vagus nerve. For

<sup>1</sup> MELTZER and AUER: This journal, 1906-1907, xvii, p. 313.

<sup>2</sup> This journal, *loc. cit.*

the intestines we may say that the effect of magnesium is similar to the well-known inhibitory action of the splanchnics.<sup>1</sup>

Are our present results in harmony with this hypothesis? Did we not find that by the injection of calcium or even magnesium a most remarkable intestinal movement takes place? We must admit that any investigator who would come across the phenomenon of peristaltic rush occurring after an injection of calcium or magnesium chloride without having much experience with these salts, might be inclined indeed to insist that calcium and magnesium are stimulating agents for intestinal movements.<sup>2</sup>

We shall, however, call attention to the following facts. If injections of magnesium or of calcium are given in large or small doses to an animal which previously received no stimulating salts and whose cord was not destroyed or whose splanchnics were not cut, no movement of the intestines ever follows those injections. For magnesium we may state that this is the absolute rule, to which apparently there is no exception. For calcium there is once in a while an exception; we have occasionally seen after an injection of calcium chloride a constriction appearing in some part of the gut, but this was a rare occurrence, and the effect was circumscribed and very brief. The general rule is that calcium produces no intestinal contractions. Furthermore, if there have been slight spontaneous movements of the intestines, or slight movements brought on by the injection of some intestinal stimulants, an injection of magnesium or calcium will invariably stop these movements for a shorter or longer period. Finally, even when the intestines show strong activity, "spontaneous" as well as those brought on by artificial means, in the great majority of the cases an injection of

<sup>1</sup> See also A. G. MAYER: Rhythmical pulsation in Scyphomedusa, Carnegie Institution publications, 1906.

<sup>2</sup> The situation with which Loeb was confronted in his studies upon the hydromedusa *Polyorchis* (The stimulating and inhibitory effects of magnesium, etc., Journal of biological chemistry, 1905-1906. i, p. 427) is of a similar misleading character. When to a solution of NaCl in which the medusa does not show any movements, magnesium chloride is added, the characteristic swimming movements soon appear. This conveys the impression that magnesium acts as a stimulating agent. The probable interpretation, however, is that the sodium chloride solution keeps the muscle in a state of contraction, a systolic state, and that the addition of magnesium causes a relaxation of the tonus, thereby favoring the reappearance of rhythmic diastoles. In favor of that view LOEB mentions the fact that the mouth and tentacles are permanently contracted in any solutions without magnesium.

magnesium or calcium will cause at least a preliminary inhibition of the intestinal movements.

We therefore assume that the injection of magnesium or calcium introduces an inhibitory factor, and that the appearance of the phenomenon of peristaltic rush occurs only as a compromise between two opposing factors, the stimulating and inhibitory elements. When we stated above that we were enabled to produce at will the occurrence of peristaltic rush in living rabbits, we did not mean to claim that certain injections will invariably bring out the phenomenon. We claim only that we are now in a position to create a situation in which the phenomenon in all probability is likely to occur. The peristaltic rush by no means promptly follows each injection. On the contrary, in a prolonged experiment in which many alternating injections were given, it frequently happened that only one or two complete waves of rushing peristalsis made their appearance.

The phenomenon of peristaltic rush consists, as we have analyzed above, of two parts, — of a stimulating part, in which the circular constriction is strong and its propagation rapid, and of an inhibiting part, in which a long section of the intestine in front of the rushing wave of constriction is completely relaxed, so as to offer no obstacle to the swiftly driven contents. The peristaltic rush is in its composition very similar to the normal peristalsis of the œsophagus in which an inhibitory wave runs rapidly ahead of the contraction to clear the path of all obstructing constrictions. By introducing at the same time into the body stimulating and inhibiting agents, conditions are created which permit the occurrence of such specific combinations of stimulation and inhibition as to start off the wave of peristaltic rush.

The antagonistic factors, to be favorably combined, must be mated in proper proportions. The stimulating effect of ergot, which is not very strong, is best combined with calcium, the inhibitory effect of which is also not too strong. The inhibitory effect of magnesium is too strong and is apt to completely overpower the stimulation of ergot. On the other hand, the strong stimulating effect of barium is better paired with magnesium than with calcium in order to bring out peristaltic rush.

The increased activity of the intestines after release from the inhibitory grip of the splanchnics (*i. e.*, after their section) is apparently just of the right proportion to enter into a satisfactory com-

bination with the inhibitory effect of calcium. Hence the occurrence of peristaltic rush after an injection of calcium when the splanchnics were previously cut.

The occurrence of peristaltic rush after destruction of the dorsal cord would seem to require some explanation. The character of the compromise is here not very evident, since the destruction of the cord rather removes an inhibitory factor. More facts will have to be collected before we could discuss this point satisfactorily. But we may say that in our experiments on the rabbit's cæcum<sup>1</sup> we have established the fact that simple opening of the abdomen even in a warm saline bath is an inhibitory stimulus of a local character for the cæcum. It is probably an inhibitory stimulus also for the small intestine.

Finally, we have to recall here our observation according to which the vagi are apparently controlling factors in the management of peristaltic rush. In the absence of their influence no complete peristaltic rush takes place. Incomplete, short, sluggish runs do occur even after the vagi are cut, and they may be of peripheral origin, either myogenic or neurogenic. But a complete true peristaltic rush seems to require the assistance of the central nervous system conveyed through the vagi.

Peristaltic rush is probably not an infrequent occurrence in various pathological conditions, and is probably also an essential factor in purgation. As to normal conditions, we have stated above that we have never seen Rollbewegungen in the opened abdomen of a normal animal. But an opened abdomen is not a normal state. We have shown that the clearly visible movements of the stomach<sup>2</sup> and of the cæcum<sup>3</sup> completely disappear after opening of the abdomen. We will, however, not enter here into a discussion of that subject.

#### RÉSUMÉ.

Peristaltic rush (Rollbewegungen) consists of a rapidly progressing wave of contraction preceded by a completely relaxed long section of the intestine through which fluid contents mixed with gas bubbles is rapidly driven. A complete peristaltic rush is one

<sup>1</sup> MELTZER and AUER: *Zentralblatt für Physiologie*, 1907, xxi, p. 71.

<sup>2</sup> AUER: *This journal*, 1906-1907, xvii, p. 15.

<sup>3</sup> MELTZER and AUER: *Proceedings of the Society of Experimental Biology and Medicine*, 1907, iv, p. 37.

which sweeps down from the duodenum to the cæcum without stopping.

Peristaltic rush was seen to occur in living animals with opened abdomen when intravenous injections of stimulating and inhibitory substances were given. As stimulating substances were used some purgative salts, ergot, barium chloride, and eserine; and as inhibitory substances, calcium chloride, magnesium chloride, and magnesium sulphate were used. The best success was obtained by ergot and calcium chloride.

Cutting the vagi prevents the occurrence of complete peristaltic rush.

As a result of a general character we may consider the fact that the simultaneous administration of stimulating and inhibitory substances did not lead to a mutual neutralization, but rather to a condition in which both effects are manifest and are combined in such a co-ordination as to favor effective motion, namely, an increase of the motor factors and an inhibition of the antagonists, — a condition which was designated by one of us (M.) as *contrary innervation*.



# THE ACID CONTROL OF THE PYLORUS.

By W. B. CANNON.

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

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**T**HREE years ago I called attention to the fact that when the different food-stuffs, uniform in amount and consistency, are fed, they are discharged through the pylorus at different rates. At that time I offered in explanation of this differential discharge a

theory<sup>1</sup> which accorded with the facts then established. Later I reported further observations on the discharge of the three food-stuffs, singly and in combinations, and stated that a discussion of these observations and an account of explanatory experiments would be deferred to a later paper.<sup>2</sup> They are presented in the following pages.<sup>3</sup>

Opposing views have been set forth as to the manner in which the stomach empties. The statements of Richet<sup>4</sup> and of Rossbach,<sup>5</sup> that gastric contents are first thoroughly mixed with gastric juice and after three or four hours or more are passed rapidly into the duodenum, have given way before contrary results obtained under more natural conditions. Clinical studies with the stomach tube,<sup>3</sup> observations on the undisturbed subject by means of the X-rays,<sup>7</sup> and investigations through duodenal fistulae,<sup>8</sup> combine to prove that the stomach is emptied *progressively* during the course of gastric digestion. The observations by means of X-rays and the investigations through duodenal openings have further demonstrated that the chyme does not pass through the pylorus in a continuous small stream, nor at the approach of every peristaltic wave, but occurs *occasionally*, at irregular intervals.

#### DISCUSSION OF PREVIOUS VIEWS AS TO THE CONTROL OF GASTRIC EVACUATION.

Both mechanical and chemical agencies have been invoked to explain the emptying of the stomach. These agencies have been supposed by some investigators to act in the stomach, by others to act in the intestine. It is necessary, first, to consider these views and the evidence adduced in their favor.

<sup>1</sup> CANNON: This journal, 1904, x, p. xviii.

<sup>2</sup> CANNON: *Ibid.*, xii, p. 418.

<sup>3</sup> Some of this evidence has been reported in previous communications (see CANNON: Journal of the American Medical Association, 1905, xlv, p. 15; This journal, 1906, xv, p. xxv).

<sup>4</sup> RICHEL: Comptes rendus, Académie des Sciences (Paris), 1877, lxxxiv, p. 451.

<sup>5</sup> ROSSBACH: Deutsches Archiv für klinische Medizin, 1890, xlvi, pp. 296, 317.

<sup>6</sup> EWALD and BOAS: Archiv für pathologische Anatomie und Physiologie und für klinische Medizin, 1885, ci, p. 365.

<sup>7</sup> CANNON: This journal, 1898, i, pp. 368, 369, 377.

<sup>8</sup> SCHIFF, Physiologie de la digestion, Florence and Turin, 1867, ii, p. 326. KÜHNE: Lehrbuch der physiologische Chemie, Leipzig, 1868, p. 53. Also v. MERING: Verhandlungen des Congresses für innere Medizin, 1897, xv, p. 433.

**Mechanical agencies in the stomach.** — The claim has been made by those who believe that chyme is discharged only after several hours of gastric digestion, that the pyloric sphincter, although able to withstand the recurrent peristaltic pressure in the earlier stages of chymification, is overcome by the more intense constrictions in the later stages.<sup>1</sup> As already stated, the proof is conclusive that a delay of several hours in the discharge from the stomach is abnormal. The moving constriction-rings do indeed press deeper into the gastric contents as digestion proceeds, but this late augmentation of contraction does not explain the normal gradual exit during earlier stages of chymification. In these earlier stages I have many times looked carefully for any relation between the moment of discharge and any momentarily greater intensity of peristalsis. Wave after wave passes with almost no perceptible variation of depth. Yet, as the waves are passing with such notable uniformity, the pylorus may open before the pressure of an approaching constriction, and the mass in the antrum, then released, will be driven forth into the duodenum. The next wave, and perhaps many thereafter, of approximately the same depth, may fail to press the food onward.<sup>2</sup> The occasional discharge of chyme from the stomach cannot therefore be attributed to an occasional increase of intensity of the peristaltic constrictions.

**Mechanical agencies in the intestine.** — In 1897 v. Mering<sup>3</sup> reported that the introduction of a large amount of milk into a duodenal fistula checked the exit of water from the stomach. The next year Marbaix<sup>4</sup> published a paper on evacuation of the stomach as affected by a state of repletion of various parts of the intestine. He found that in the upper half of the small intestine a state of repletion, induced by injections through fistulae, inhibited the discharge from the stomach.<sup>5</sup> In order to cause the reflex, however, even in the first fourth of the intestine, the injected liquid had to

<sup>1</sup> See LESSHAFT: *Archiv für pathologische Anatomie und Physiologie und für klinische Medicin*, 1882, lxxxvii, p. 80.

<sup>2</sup> CANNON: *This journal*, 1898, i, p. 369.

<sup>3</sup> v. MERING: *Loc. cit.*, p. 434.

<sup>4</sup> MARBAIX: *La cellule*, 1898, xiv, p. 251.

<sup>5</sup> An investigation of the motor functions of the stomach after pyloroplasty (see CANNON and BLAKE: *Annals of surgery*, 1905, xli, p. 707) has proved that although the upper part of the small intestine may become filled with food, there is no cessation of peristalsis. The effect noted by v. MERING and MARBAIX would therefore probably be due to closure of the pyloric sphincter.

occupy a considerable extent of gut. For example, filling the gut from 10 cm. to 25 cm. beyond the pylorus caused no inhibition of the discharge. But much less than 15 cm. of continuous content is normally present in the upper intestinal tract. A radiograph and tracings of X-ray shadows already published<sup>1</sup> show that the intestinal contents are normally disposed in separate short masses. Under natural conditions, therefore, the extensive uninterrupted surface of contact required by v. Mering's and Marbaix's explanation, in order to prevent a continuous outpouring from the stomach, does not exist. As the continuous outpouring nevertheless does not occur, their results do not explain the normal control of gastric discharge.

Von Mering's and Marbaix's results are confirmed by Tobler's observation<sup>2</sup> that the rapid inflation of a balloon in the duodenum checks the passage of food from the stomach. This experiment, like v. Mering's and Marbaix's, does not explain normal conditions, because, as already shown,<sup>3</sup> chyme normally gathers in the duodenum gradually, by repeated small additions, and even when accumulated lies as a slender strand which does not distend the gut. Each strand thus formed is soon hurried forward some distance along the tube, thus clearing the duodenum for new accumulations.

Though the passage of food from the stomach may be checked by artificially filling a long piece of the upper intestine or by sudden distention of the gut at one point, such conditions cannot account for any natural control of gastric discharge from the intestinal side, because such conditions are not normally found. The evidence, therefore, is opposed to the conception that mechanical agencies, acting either in the stomach or in the intestine, play an important part in controlling the normal gastric evacuation.

**Chemical agencies in the stomach.** — More than twenty years ago Ewald and Boas found,<sup>4</sup> by use of the stomach tube on man, that there was a considerable development of free hydrochloric acid before the gastric contents began to be notably diminished in amount. Where the acid may have had its effect — whether on peristalsis or on the pyloric sphincter — was not determined.<sup>5</sup>

<sup>1</sup> CANNON: This journal, 1902, vi, p. 255; and 1904, xii, p. 389.

<sup>2</sup> TOBLER: Zeitschrift für physiologische Chemie, 1905, xlv, p. 195.

<sup>3</sup> CANNON: This journal, 1902, vi, p. 262.

<sup>4</sup> EWALD and BOAS: *Loc. cit.*, p. 364.

<sup>5</sup> HAMMARSTEN'S statement that HCl seems to act as a stimulus to open the pylorus (*Lehrbuch der physiologischen Chemie*, 3d ed., Wiesbaden, 1895, p. 246)

Penzoldt<sup>1</sup> has studied extensively the periods during which various common foods remain in the stomach, and has noted that foods delaying the appearance of free hydrochloric acid remain longest. Verhaegen,<sup>2</sup> on the other hand, has declared that it matters little for the passage through the pylorus whether the food is acid or neutral.

As a basis for explaining the factors in control of gastric evacuation, Penzoldt's results are inconclusive. In general the foods used were not fairly representative food-stuffs, but were often complicated mixtures. The amounts given at different times were not equal, nor was the consistency uniform. For his judgments Penzoldt was dependent on securing remnants of the gastric contents by means of the stomach tube, — a procedure not distinguishing the differences in the chemical reaction of the food in the two ends of the stomach, and furnishing no data as to the progressive rate at which the stomach is emptied. Ignorance of the effects of varying composition of foods, varying amounts and varying consistencies, and ignorance of the rapidity of gastric discharge as digestion proceeds, renders it difficult to arrive at exact conclusions from Penzoldt's results.

In the presence of strong opposing evidence Verhaegen's contention that neither acidity nor neutrality of the chyme has any effect on the emptying of the stomach, may reasonably be doubted, for his observations were made with the stomach tube on only four individuals, two of whom were pathologic.

**Chemical agencies in the duodenum.** — In 1893 Hirsch observed that solutions of inorganic acids left the stomach slowly, and he concluded that the slow exit was due to the stimulating effect of the acid on the mucosa of the duodenum.<sup>3</sup> Later Serdjukow, one of Pawlow's students, inhibited gastric evacuation by introducing acid into the duodenum through a fistula,<sup>4</sup> thus confirming the conclusion of Hirsch. Tobler's results<sup>5</sup> also substantiate it.

was an inference from the observations of EWALD and BOAS (Personal communication). The statement does not occur in later editions.

<sup>1</sup> PENZOLDT: *Deutsches Archiv für klinische Medicin*, 1893, li, p. 535; 1894, liii, p. 230.

<sup>2</sup> VERHAEGEN: *La cellule*, 1897, xii, p. 69.

<sup>3</sup> HIRSCH: *Centralblatt für klinische Medicin*, 1893, xiv, p. 383.

<sup>4</sup> SERDJUKOW: Reviewed in HERMANN'S *Jahresbericht über die Fortschritte der Physiologie*, 1899, viii, p. 214.

<sup>5</sup> TOBLER: *Loc. cit.*, p. 198.

The main defect of all the above methods as means for determining the nature of the chemical control of gastric discharge is their failure to distinguish between the two factors concerned in the passage of food through the pylorus. Before pointing out how essential the distinction is, it will be well to consider the part played by each of the two factors.

#### THE TWO FACTORS CONCERNED IN GASTRIC EVACUATION.

One of the two factors necessary for the emptying of the stomach is the pressure to which the food at the pylorus is subjected by recurring peristaltic waves; the other is the action of the pyloric sphincter. Not until the X-ray method was used to study the mechanical processes in digestion was it possible to watch, under normal conditions, both the movement of gastric peristalsis and the exit of food through the pylorus. Until the application of the X-ray method, therefore, a clear distinction between the normal effects of these two factors could not be made.

That the natural passage of food through the pylorus is occasional might be due to occasional peristaltic constrictions, or occasional specially strong peristaltic constrictions, pressing the gastric contents against an easily opened pylorus; or, on the other hand, the occasional passage might be due to an occasional relaxation of the pylorus in the presence of a fairly constant pressure.

It is true that some of the investigators whose work has already been mentioned have ascribed the control of gastric discharge solely to the action of the pyloric sphincter. Marbaix, for example, writes of the influence of the repletion of the intestine on the closure of the pylorus.<sup>1</sup> His evidence for this limitation is not clear. Von Mering, on the other hand, recognized that intestinal repletion might check gastric discharge by stopping gastric peristalsis, and he resected the pylorus in order to differentiate, if possible, between the peristaltic and the pyloric factors. The failure to make this differentiation is the essential flaw, from the point of view of this paper, in the methods of Ewald and Boas, Penzoldt, Hirsch, Serdjukow, and Tobler. Their results, therefore, while significant, cannot serve for a conclusive determination of the control of gastric evacuation;

The possible confusion of the two factors is illustrated in Paw-

<sup>1</sup> MARBAIX: *Loc. cit.*, p. 273.

low's report of Serdjukow's experiments. He states, without giving evidence, that acid chyme entering the duodenum reflexly occludes the pyloric orifice "and at the same time reflexly inhibits the propulsive movements of the organ (stomach)."<sup>1</sup> Clearly the occlusion of the pyloric orifice alone would account for Serdjukow's results. What is the evidence that peristalsis also is affected?

In my first paper on the stomach I stated<sup>2</sup> that gastric peristaltic waves in normal conditions are continuously running, so long as food remains. Hundreds of observations made since that time on various animals — mainly on cats, but also on dogs, guinea pigs, and white rats — and likewise records of stomach sounds in man,<sup>3</sup> have confirmed the view that peristalsis continues uninterruptedly until the stomach is swept clear of its contents. In my experience, neither ejaculation of acid chyme, nor stretching of the duodenum with food pressed through the cut pylorus (see footnote, p. 285), has any tendency to interrupt the sequence of waves.

The continuously passing peristaltic waves of the stomach, as remarked in discussion of mechanical agencies in the stomach, do not show from moment to moment notable variation of intensity. One of the two factors concerned in gastric discharge—the pressure in the antrum — is therefore recurrently constant. The control of the discharge, consequently, must reside with the other factor, *i. e.*, with the action of the pyloric sphincter. If the sphincter holds tight, the recurring waves churn the food in the antrum; if the sphincter relaxes, these waves press the food out into the duodenum. The pylorus is the "keeper of the gate."

#### THE FACTS TO BE EXPLAINED.

The discharge from the stomach, as already demonstrated, is occasional. The foregoing analysis proves that this occasional discharge must be due to occasional relaxations of the pyloric sphincter. To explain the action of the pylorus, therefore, it is necessary to consider agencies which maintain an intermittent closure,— which usually keep the passage shut, yet open it at intervals to allow portions of the chyme to depart. None of the researches on

<sup>1</sup> PAWLOW: The work of the digestive glands, English translation, London, 1902, p. 165.

<sup>2</sup> CANNON: This journal, 1898, i, p. 367.

<sup>3</sup> CANNON: *Ibid.*, 1903, viii, p. xxii; and 1905, xiv, p. 344.

the control of gastric evacuation, discussed in the preceding pages, were definitely concerned with this intermittent closure. Further investigation was desirable to explain the repeated opening and shutting of the pyloric orifice.

To explain also the differences in the rate of discharge of different food-stuffs from the stomach further investigation was necessary. In the report of the research mentioned at the beginning of this paper I called attention to the fact that when representative carbohydrate, proteid, and fat foods, of uniform amount and consistency, are separately fed, the carbohydrates begin to leave the stomach soon after ingestion (within ten minutes) and are passed out rapidly; proteids commonly do not leave the stomach at all during the first half hour and sometimes not for an hour,<sup>1</sup> then they are expelled only slowly; and fats, because of a continuous slow exit, remain in the stomach for a long period. Since the major portion of a diet is more likely to be composed of carbohydrate or proteid or of the two combined, than of fat, it becomes especially important to understand the difference in the mechanical treatment of these two main food-stuffs. What is the pyloric mechanism whereby carbohydrates, not digested by the gastric juice, are permitted to pass quickly into the small intestine to be digested, whereas proteids, digested in the stomach, are there retained to undergo digestion?

#### A THEORY OF THE CONTROL OF THE PYLORUS.

The investigators whose views have been presented have regarded factors in the stomach, or factors in the intestine, as controlling gastric evacuation. An interaction of agencies in the two situations has not been considered. The theory referred to at the beginning of this paper, propounded to explain the differential discharge of the different food-stuffs, is based on evidence of opposed effects from a single stimulus acting first in the stomach and later in the duodenum.

<sup>1</sup> MARBAIX'S declaration that when any food is introduced into the stomach a portion passes directly into the empty intestine, and immediately causes *v. MERING'S* reflex (MARBAIX: *Loc. cit.*, p. 296), I have never been able to verify. Animals that had fasted several days were given lean beef mixed with bismuth subnitrate, which they eagerly devoured. Radiographs taken a half-hour later showed that in spite of continuous peristalsis there was no sign of food in the intestine.

The first statement in the theory is that acid coming to the pylorus causes a relaxation of the sphincter. Thus would be explained why the initial discharge is longer delayed when proteids are fed than when carbohydrates are fed. Both carbohydrate and proteid stimulate gastric secretion in abundance, as researches on dogs by Pawlow and his co-workers,<sup>1</sup> and as clinical studies on men have shown. Inasmuch as carbohydrates do not unite chemically with the acid, free acid is at once present in the stomach; and carbohydrates would therefore begin almost immediately to pass through the pylorus. Proteids, on the other hand, join with the acid and thus retard for some time the development of an acid reaction;<sup>2</sup> the proteid discharge would therefore be retarded.

But acid on the stomach side of the pylorus is not the only determinant of pyloric action. This is proved by feeding carbohydrate food moistened with 0.4 per cent hydrochloric acid. The rate of discharge is not increased. If acid in the stomach is the stimulus relaxing the pylorus, why in this case is the rate of discharge not increased? The observations of Hirsch and Serdjukow now have their bearing. Since it has been shown that acid in the duodenum does not stop gastric peristalsis, the acid reflex from the duodenum must affect the pyloric sphincter. The second statement in the theory naturally follows, — acid in the duodenum closes the pylorus.

It is probable that the pyloric sphincter has normally a greater or less degree of tonic contraction, with occasional relaxations.<sup>3</sup> Certainly it has a tonic contraction persistently strong for some time after food enters the stomach; when proteid, for example, is fed, peristaltic constrictions may press the food against the pylorus repeatedly for an hour (approximately 300 waves) without forcing food through the orifice.

The whole theory of the acid control of the pylorus may now be stated. The pylorus is tonically closed when food is ingested, and remains closed against recurring pressure. The appearance of acid at the pylorus causes the sphincter to relax. The pressing peristaltic waves now force some of the acid chyme into the duodenum. The acid in the duodenum at once tightens the sphincter against further exit. The same acid also stimulates the flow of

<sup>1</sup> PAWLOW: *Loc. cit.*, pp. 36, 100.

<sup>2</sup> DANILEWSKY: *Zeitschrift für physiologische Chemie*, 1881, v, p. 160.

<sup>3</sup> See BASTIANELLI: MOLESCHOTT'S *Untersuchungen*, 1892, xiv, p. 93; and OSER: *Zeitschrift für klinische Medicin*, 1892, xx, p. 291.

alkaline pancreatic juice.<sup>1</sup> Since no inorganic acid is normally present beyond the first few centimetres of the small intestine,<sup>2</sup> and since the acid reaction of the contents in this uppermost region is replaced throughout the rest of the small intestine by practically a neutral reaction,<sup>3</sup> the acid chyme must be neutralized soon after its emergence from the stomach. As neutralization proceeds, the stimulus closing the pylorus is weakened; now the acid in the stomach is able again to relax the sphincter. Again the acid food goes forth, and immediately closes the passage behind it until the duodenal processes have undergone their slower change. And thus, repeatedly, until the stomach is empty.<sup>4</sup> What is the evidence for this theory?

#### EXPERIMENTAL EVIDENCE FOR THE ACID CONTROL OF THE PYLORUS.

As the acid of the gastric juice, according to the theory, may have two opposing effects on the pylorus, it will be well to present first the evidence that acid in the antrum causes the pylorus to open, and second the evidence that acid in the duodenum causes the pylorus to be kept closed.

**A. That acid in the stomach opens the pylorus.** — The evidence for this first half of the theory will be presented under several headings, as follows:

1. *Delaying the appearance of hydrochloric acid delays the initial discharge.* — Observations on the gastric discharge of different food-stuffs, of the same amount and consistency, proved that carbohydrates begin to leave the stomach early and are passed out rapidly. In terms of the above theory this quick exit is due to the early appearance of acid in the stomach. The appearance of acid can be delayed if the carbohydrates are first moistened with sodium bicarbonate. The acid would first be neutralized by the alkaline food near the secreting surface and in the churning antrum; and only after some time would free acid appear in considerable amount. If

<sup>1</sup> BAYLISS and STARLING: *Centralblatt für Physiologie*, 1901, xv, p. 682.

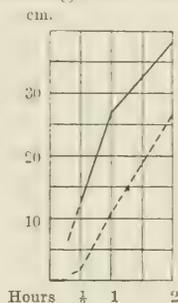
<sup>2</sup> MOORE and BERGIN: *This journal*, 1900, iii, p. 325.

<sup>3</sup> MUNK: *Centralblatt für Physiologie*, 1902, xvi, p. 33.

<sup>4</sup> COHNHEIM, in his summary of the factors controlling the discharge of food from the stomach (NAGEL'S *Handbuch der Physiologie des Menschen*, Braunschweig, 1907, ii, p. 564), mentions the theory here propounded, but states that my evidence for it is not convincing. It is fair to note that this present paper gives for the first time the evidence in a complete and detailed form.

the theory is correct, this postponement of the appearance of acid should delay beyond the normal time the initial discharge of the food.

FIGURE 1. — Curves showing the average aggregate length of the food-masses in the small intestine at the times indicated, after feeding potato, rice, and crackers (4 cases each) moistened with water (continuous line), and the same moistened with 1 per cent NaHCO<sub>3</sub> (interrupted line).



Crackers, rice, and mashed potatoes were chosen as representative carbohydrate foods. The rice was steamed and dried, and the mashed potato was also dried before being used. In all cases one per cent sodium bicarbonate was added to the dried food until a mush was made, of the same consistency as in the standard cases. The carbohydrates thus prepared were mixed with subnitrate of bismuth and fed, as in the standard cases, in 25 c.c. amounts.

In the following figures are presented the average aggregate length of the food-masses in the small intestine, as seen by the Röntgen rays, after feeding potato, rice, and crackers moistened with water (four cases each), and moistened with sodium bicarbonate (four cases each). The figures for the first two hours of observation are given, since they are most significant in judging the rate of discharge.

Hours . . . . .	POTATO.			RICE.			CRACKERS.		
	½	1	2	½	1	2	½	1	2
With water <sup>1</sup> . . . .	9.5	31	43.0	16.5	29	36.0	11	22	35.5
With 1% NaHCO <sub>3</sub>	1.0	11	26.5	1.5	14	29.5	1	8	23.0

The average figures for twelve cases in which the three carbohydrates wet with water were fed, and the twelve cases in which they were fed wet with sodium bicarbonate, are represented graphically in Fig. 1.

<sup>1</sup> See CANNON: This journal, 1904, xii, p. 397. For a description of the method used, see p. 388.

Comparison of the results of feeding carbohydrate food in the two conditions shows that at the end of a half-hour there had emerged only about one tenth as much of the food wet with the alkaline solution as of the same food wet with water (in six of the twelve cases no alkaline food had left the stomach); at the end of an hour, from a third to a half as much; and in two hours, from about a half to five sixths as much. In other words, there has been a marked retardation in the discharge of carbohydrates wet with the alkaline solution. This result is in harmony with the observation by Jaworski on man, that alkalinity of the contents delays the emptying of the stomach.<sup>1</sup>

Sodium bicarbonate delays the appearance of acid in two ways: it checks the secretion of the gastric juice,<sup>2</sup> and for a time it unites with the acid of the gastric juice as rapidly as it is poured out. The evidence here presented shows that experimental conditions delaying the appearance of hydrochloric acid delay the discharge from the stomach.

<sup>1</sup> JAWORSKI: *Zeitschrift für Biologie*, 1883, xix, p. 444.

<sup>2</sup> PAWLOW: *Loc. cit.*, p. 95. Evidence will be presented later in this paper that conditions not favoring gastric secretion are accompanied by low pyloric tonus, and that under these circumstances gastric evacuation may be very rapid. That carbohydrate foods mixed with  $\text{NaHCO}_3$  may be close to the line between a retention at the pylorus till an acid reaction develops, and a swift discharge because the pyloric tonus is low, is indicated by observations on "flaked rice" moistened with  $\text{NaHCO}_3$ . Three cases gave the following figures:

Hours . . . . .	$\frac{1}{2}$	1	2
Centimetres of food masses	0.0	11.0	19.5
	0.0	5.5	14.5
	0.0	7.0	22.0

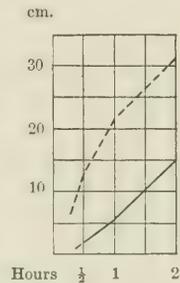
Four other cases, fed in the same manner the same amount with the same consistency, yielded the following figures:

Hours . . . . .	$\frac{1}{2}$	1	2
Centimetres of food masses	21.0	27.0	40.5
	21.5	34.0	47.0
	20.0	36.5	43.5
	14.5	41.5	52.0

These latter figures were extraordinary, and can only be compared with the results of feeding raw egg-white, which likewise does not readily excite gastric secretion (see page 313).

2. *Hastening the appearance of hydrochloric acid hastens the initial discharge.* — Proteids normally begin to leave the stomach only after an interval of about a half-hour after feeding, and then continue going out at a much slower rate than do carbohydrates. According to the theory, as already stated, the slow passage of proteids from the stomach is due to their union with the acid of the gastric juice, which prevents the rapid development of a marked acid state.

FIGURE 2. — Curves showing the average aggregate length of the food-masses in the small intestine, at the times indicated, after feeding fibrin, fowl and lean beef (4 cases each) as natural proteid (continuous line), and the same as acid proteid (interrupted line).



Evidence as to this supposition may be secured by feeding proteid food that has previously been changed to acid proteid. Fibrin, lean beef, and fowl, freed from fat, were chosen as representative proteid foods. They were mixed with ten per cent hydrochloric acid and allowed to stand until changed to acid proteid. The free acid was dialyzed away until test showed none present. As the change to acid proteid was accompanied by swelling of the original substance, approximately the same proteid content was preserved by feeding the acid proteid in twice the standard amount. Doubling the amount of the natural proteid notably retards the outgo from the stomach;<sup>1</sup> if changing the natural to acid proteid has no effect on the outgo from the stomach, doubling the amount should likewise retard the outgo, certainly should not accelerate it.

Fibrin, fowl, and lean beef were fed as acid proteids in 50 c.c. amounts and with the same consistency as in the standard cases. A comparison of the rate of discharge of the natural proteid foods, and the rate of discharge of the same foods given as acid proteids is exhibited in the following table. The figures represent the total length of the food-masses in the small intestine at the indicated intervals after feeding. In each instance the figures are averages of four cases.

<sup>1</sup> See CANNON: This journal, 1904, xii, p. 409.

Hours . . .	FIBRIN.			FOWL.			LEAN BEEF.		
	$\frac{1}{2}$	1	2	$\frac{1}{2}$	1	2	$\frac{1}{2}$	1	2
Natural proteid	2.5	6.5	15.5	2	6.5	13.0	1.5	2.5	16.0
Acid proteid .	11.0	25.0	33.5	10	18.0	28.5	15.5	22.5	31.5

In Fig. 2 are presented the curves for the average figures of the twelve cases in which the natural proteids were fed and the twelve cases in which these same foods were given as acid proteids.

The figures of the foregoing table show that at the end of a half-hour the stomach had discharged from five to ten times as much acid proteid as natural proteid; three to ten times as much at the end of an hour; and in two hours about twice as much acid proteid as natural proteid. Evidently the change to acid proteid and the feeding in increased amount resulted not in slowing, but in remarkably accelerating the exit from the stomach. The proteid in these cases, already united with hydrochloric acid, does not unite with the hydrochloric acid of the gastric juice. The hydrochloric acid of the gastric juice secreted on the acid proteid is at once free acid. Free acid appears earlier, therefore, than when the natural proteid is fed. The evidence given above shows that when experimental conditions hasten the appearance of free acid, the discharge from the stomach is correspondingly hastened.

3. *The appearance of acid in the antrum closely precedes the initial discharge.* Although in the experimental conditions already described the emergence of food from the stomach has occurred as if acid were present to open the pylorus, this judgment is only an inference,—there has been no demonstration that acid was present when the first food passed into the duodenum. It is desirable to determine more exactly the relation between the first development of acid and the first exit of the food. This can be done by establishing in the antrum, close to the pylorus, a fistula.

An antrum fistula holding a simple flanged cannula with a removable plug was established in several cats.<sup>1</sup> The cats recovered readily from the operation and were usually in very good health.

<sup>1</sup> Whenever an operation is mentioned or suggested in this paper, it is understood, of course, that the operation was performed under complete general anæsthesia.

In order that the food could be seen with the X-rays when it first entered the duodenum, it was always mixed with bismuth subnitrate. Various methods were used to determine the first appearance of acid in the antrum. The method causing least disturbance was that used when potato was fed. Mixed with the mashed potato (25 c.c.) were 20 drops of dimethylamidoazobenzol — an amount staining the white potato orange and showing a clearly marked change to pink when hydrochloric acid developed. As soon as the potato was given (usually by stomach tube), the plug was removed from the cylinder of the cannula and replaced by a glass syringe. The syringe consisted of a piece of glass tubing about 25 cm. long into which was passed a close-fitting glass rod 5 cm. longer. Half of a short length of rubber tubing was stretched over the upper end of the glass tube; the other half firmly encircled the projecting rod. Thus the joint was made tight. The glass tube, which slipped snugly into the cannula, was held in place by a ring of rubber tubing stretched around both the cannula and the syringe. By pulling up the rod the thin mushy contents of the antrum were drawn into the glass tube. Then any change of color could be noted. If the original orange color still persisted, the rod was pushed down again, and thus the food was restored normally to the stomach. Usually such observations were made every four minutes; during the intervals X-ray observations showed whether food had yet been passed into the duodenum. When lean beef was fed, the color change could not be clearly seen, and it was necessary to remove through the cannula a sample of the antrum contents in a small pipette. The contents were tested for acid with Congo-red, dimethylamidoazobenzol, and tropäolin oo. The difficulty of this method was the liability of loss of food whenever the plug of the cannula was removed.

In some of the following cases the conditions varied from the normal. These cases are reported, however, because of their double value: they not only present direct evidence that an acid reaction in the antrum precedes the initial gastric discharge, but they also bring to the theory of the acid control of the pylorus the support arising from the concomitant variation of these two processes.

A cat suffering from a severe inflammation of the nose and eyes was given 25 c.c. mashed potato mixed with bismuth subnitrate and dimethylamidoazobenzol, as above described. The animal was examined alternately during fifty-five minutes after feeding, for the presence of acid and for food discharged

from the stomach. There was no sign of acid, and although usually carbohydrate food begins to leave the stomach in about ten minutes, there was no discharge.<sup>1</sup>

Five days later the same cat, in much better health though not yet well, was again examined in the same manner. Gastric peristalsis was seen five minutes after the feeding. Nineteen minutes later no food had left the stomach, and the previous examination for acid had revealed no sign of change. At twenty-one minutes the potato drawn into the syringe was pink. As soon as the X-rays could be applied, the fluoroscope showed that there was food in the duodenum.

A few days later the digestion of some meat was interrupted in the same cat, now in good health, by pulling out through the fistula the larger pieces of the gastric contents, flushing out the remnants, and then washing the stomach from a stomach tube through the fistula until there was no acid reaction in the wash water. The cat was at once given potato as before. As soon as she was looked at with the X-rays gastric peristaltic waves were seen. Five minutes after the feeding the potato in the syringe was pink, and a minute later the X-rays showed that some potato had passed the pylorus.

Other observations after feeding potato have confirmed these results, — an acid reaction of the antrum contents was always noted before the food emerged from the stomach.

Observations after feeding lean beef gave similar results. The following cases are significant:

A cat finished eating voluntarily 25 c.c. lean beef mixed with 5 gm. sub-nitrate of bismuth at 2.16 P. M. At 2.49 gastric peristalsis was prominent; but no food had left the stomach, and the test for acid in the antrum contents was negative. At 3.00 o'clock the condition was unchanged. Then a small amount of 0.4 per cent HCl was introduced into the antrum through the fistula.<sup>2</sup> Within a minute thereafter there were two discharges of food into the duodenum. As there was no further emergence for some time, a little food was removed and tested. It gave no clear acid reaction. A small amount of 0.4 per cent HCl was again introduced into the antrum towards the pylorus, and again food emerged. Nothing more left the stomach for ten minutes. Then the food was once more tested, with no clear sign of acid. The introduction of more acid caused another discharge through the pylorus. It was now 3.40. Nothing

<sup>1</sup> When animals are thus afflicted with "distemper," food has been observed to stagnate all day in the stomach (see CANNON and MURPHY, *Annals of surgery*, 1906, xliii, p. 534).

<sup>2</sup> In none of these injections was the amount introduced sufficient to flood the antrum or even to dilute the gastric contents so as markedly to alter their consistency.

left the stomach during the next five minutes, although deep strong peristaltic waves had been passing continuously and pressing the gastric contents into the cannula whenever the plug was removed. An hour and a half had elapsed and only a very small amount of food had left the stomach, and that had left only when acid had been experimentally introduced. This was an unusual delay. The plug was now removed, and the peristaltic pressure permitted to drive out the gastric contents through the cannula. There was no sign of an acid reaction. It seemed as if the stomach had not been actively secreting. Certainly the acid introduced gave only a temporary and local acidity.

Twenty-five cubic centimetres of the same meat which the cat ate were now tested for alkalinity. More than 10 c.c. of 0.4 per cent HCl were added, and the reaction was still alkaline to Congo-paper blue with 0.4 per cent HCl. Strong HCl was now added; 20 drops were required before a neutral point was reached. Evidently the meat for some unknown reason was strongly alkaline.

It was later found that by mistake the meat had been boiled in a receptacle in which some instruments had previously been boiled with sodium carbonate, and which had not been cleaned.

Another cat with a fistula in the antrum finished eating, with evident relish, 25 c.c. boiled and shredded lean beef plus 5 gm. bismuth subnitrate at 12.05 P. M. At 12.14-15 she was fastened to the holder and examined. The reaction of the antrum contents was not acid, and nothing had left the stomach. At 12.22-23 nothing had left, but the Congo test had changed from a light to a dark red. At 12.30-31 the Congo test showed a still stronger acid change, and at 12.36, when examined by the X-rays, the intestine contained food some distance from the stomach.

The same cat several days later was offered the same kind of food, which she refused to eat. The food was then given to her by spoon, but with much difficulty, for she pushed out the food with her tongue and only received it all finally after repeated refusals. Ten minutes after the last mouthful was swallowed she was fastened to the board and examined as in the previous experiment. She was restless, she mewed and frequently tossed about. For an hour after the feeding the contents did not become acid, and although peristaltic waves were at times clearly seen, no food passed the pylorus. That emotional states inhibit the flow of gastric juice has been pointed out by Bickel.<sup>1</sup> In this experiment it is probable that there was no psychic secretion at the time of eating, and that subsequent secretion was inhibited while the animal was fastened down. The absence of an acid reaction was attended by a failure of discharge from the stomach.

<sup>1</sup> BICKEL: *Deutsche medicinische Wochenschrift*, 1905, xxxi, p. 1829.

The above cases prove that a delay in the appearance of acid in the antrum contents, as tested through a gastric fistula close to the pylorus, is associated with a similar delay in the passage of food from the stomach; that this may occur in spite of vigorous gastric peristalsis; that in these circumstances the introduction of a small amount of acid near the pylorus causes immediately the exit of food through the pylorus; and that whether potato or beef is fed, and whether in the same animal the discharge begins at the usual time or is much retarded, the first delivery of food into the duodenum is normally preceded by the development of an acid reaction in the antrum.

These conclusions from observations on the gastric contents through a fistula in the antrum are completely confirmed by recent studies of the reaction of the discharged chyme. Tobler, London and Sulima, and London and Polowzowa have tested the chyme collected from a duodenal fistula close to the pylorus.<sup>1</sup> Tobler fed lean beef to his dogs. The repeatedly discharged gastric contents are acid from the beginning, and continue during digestion to be "stark sauer."<sup>2</sup> London and Sulima<sup>3</sup> record that when cooked egg-albumin is fed, the discharge from the pylorus is initiated by the pouring forth of an acid fluid. The same condition is recorded by London and Polowzowa<sup>4</sup> after feeding white bread.

4. *Hydrochloric acid opens the pylorus of the excised stomach.*—Magnus has shown<sup>5</sup> that pieces of the small intestine, removed from the body and placed in continuously oxygenated Ringer's solution, will remain alive and, so long as Auerbach's plexus is intact, will manifest the typical local reflex. In a recently published investigation I have given evidence that the mechanism in control of the differential discharge through the pylorus is independent of the central nervous system.<sup>6</sup> It seemed probable, because of the rapidity of closure of the pylorus after food emerged, that the controlling

<sup>1</sup> All these observers report that a few drops of alkaline mucus flow from the cannula soon after observation begins. This flow does not seem to be associated with the repeated gastric discharge.

<sup>2</sup> TOBLER: *Loc. cit.*, p. 197.

<sup>3</sup> LONDON and SULIMA: *Zeitschrift für physiologische Chemie*, 1905, xlvii, p. 215.

<sup>4</sup> LONDON and POLOWZOWA: *Zeitschrift für physiologische Chemie*, 1906, xlix, p. 340.

<sup>5</sup> MAGNUS: *Archiv für die gesammte Physiologie*, 1904, cii, p. 362.

<sup>6</sup> CANNON: *This journal*, 1906, xvii, p. 429.

mechanism resides in the local nerve plexus, and is similar to the typical reaction of the intestinal wall. On this supposition the following experiment has been repeatedly performed.

A cat, which had fasted for twenty-four or thirty-six hours, was killed by etherization. A cut above the cardia and another just below the pylorus separated the stomach from the rest of the alimentary canal. The stomach, which was empty,<sup>1</sup> was cleared of its attachments and placed in warm Ringer's solution (38° C.), through which oxygen continuously bubbled.

A glass tube, with a short rubber tube and a water manometer attached, was tied into the cardiac orifice. A small amount of 0.4 per cent HCl, made blue by the changed Congo-red, was introduced through the tube into the cardiac end of the stomach, which was held lower than the pyloric end. The stomach was now inflated with air until the air bubbled through the pylorus. The rubber tube was next tightly clamped. When the air had ceased escaping from the stomach, *i. e.*, when the pyloric tonus withstood the intragastric pressure, the cardiac end of the stomach was gently and slowly turned until the acid came to the pylorus. In a moment the blue fluid poured forth into the Ringer's solution. The pylorus had opened.

It might be supposed that the acid coming into the antrum caused an increased tonus of the gastric musculature and that thus the pyloric orifice was forced open. The manometer, however, does not show any increase of intragastric pressure. Furthermore the stomach can be tipped so that the acid fluid enters the antrum, but does not come to the pylorus. This does not lead to the driving out of more air, — the acid does not notably stimulate contraction of the gastric wall. The opening of the pylorus, therefore, is due to the presence of the acid.

A one per cent sodium bicarbonate solution colored red, similarly brought to the pylorus, does not begin to emerge for a considerably longer time, and then usually drifts out into the Ringer's solution as if slowly diffusing.

It is justifiable to conclude that in the living excised stomach free acid coming to the pylorus causes the pylorus to open.

**B. That acid in the duodenum keeps the pylorus closed.** — The support for this, the second half of the theory, has already been suggested in part in discussing the experiments on the inhibition of

<sup>1</sup> It is important that the stomach be taken while not digesting. In my experience, if digestion has been going on, the excised stomach exhibits peristalsis as soon as inflated.

gastric discharge by acid in the duodenum. As other observations to the same effect are to be described under the above heading, a brief restatement of the previous experiments and their results will not be out of place, and will serve to bring all the evidence together.

1. *Acid in the duodenum inhibits gastric discharge.*—In 1893, Hirsch,<sup>1</sup> as already noted, found that inorganic acids left the stomach slowly. When he isolated the stomach, however, the acids departed as rapidly as any other fluid. He explained this difference by assuming that the stomach is controlled by acid reflexes from the duodenum. Serdjukow modified Hirsch's experiment by introducing through a duodenal fistula small quantities of acid solutions or pure gastric juice. By repeated injections it was possible to prevent discharge from the stomach for an unlimited time.<sup>2</sup> Tobler's observations were closer to the normal conditions.<sup>3</sup> He allowed a dog with duodenal fistula to eat 100 gm. lean beef. The chyme as it emerged was caused to leave the duodenum through the artificial opening. The stomach was thus emptied in about two hours and fifteen to thirty minutes. The next day the dog was given the same amount of the same kind of food, but whenever a portion of the chyme came through the fistula from the stomach, a similar portion of the chyme of the day before was injected through the fistula towards the intestines. The result was that the chyme left the stomach at considerably longer intervals and was more thoroughly digested. The time of digestion thus became lengthened to three hours and three hours and a half. Tobler's observations have been completely confirmed by Lang.<sup>4</sup>

The evidence of Hirsch, Serdjukow, Tobler, and Lang proves definitely that acid chyme in the duodenum checks the outgo from the stomach. Since gastric peristalsis, as previously shown in this paper, is not stopped by the discharge of acid chyme, the effect must be due to the action of the pyloric sphincter. Acid in the duodenum causes pyloric contraction.

2. *Absence of the normal alkaline secretions from the duodenum retards gastric discharge.*—Pawlow records that the passage of acid solutions out of the stomach is remarkably slower in dogs with

<sup>1</sup> HIRSCH: *Loc. cit.*, pp. 378, 383.

<sup>2</sup> SERDJUKOW: *Loc. cit.* Also PAWLOW: *Loc. cit.*, p. 164.

<sup>3</sup> TOBLER: *Loc. cit.*, p. 197.

<sup>4</sup> LANG: *Biochemische Zeitschrift*, 1906, ii, p. 225.

a pancreatic fistula than in those without one.<sup>1</sup> In order to test whether the discharge of normal gastric contents was likewise retarded by a similar condition in the duodenum, the following experiment was performed. The larger pancreatic duct and also the bile duct were tied so as to prevent the flow of the secretions into the intestine. After several days the animals were given the standard amount of mashed potato and bismuth subnitrate, with the usual consistency. The outgo from the stomach was determined as before by measuring the length of the food-masses in the small intestine. The figures of the following table give the total length of these masses at the times indicated, in normal conditions (four cases) and also after tying the larger pancreatic and the bile ducts (four cases).

POTATO.			
Hours . . . . .	$\frac{1}{2}$	1	2
Normal conditions . . . . .	9.5	31.0	43.0
Pancreatic and bile ducts tied	0.0	7.5	14.5

The observations recorded in this table are represented graphically in Fig. 3. These observations were made six and twelve days

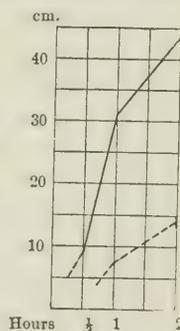


FIGURE 3. — Curves showing the average aggregate length of the food-masses in the small intestine, at the times indicated, after feeding potato (4 cases) in normal conditions (continuous line), and the same after tying pancreatic and bile ducts (interrupted line).

after the operation. It is obvious that there has been a very marked checking of the normally rapid outgo of the potato from the stomach; nothing out in a half-hour, a fourth the normal amount in an hour, and a third the normal at the end of two hours.

Why there should be no exit of the food during the first half-hour

<sup>1</sup> PAWLOW: *Loc. cit.*, p. 164.

is not clear, but the very slow increase of the intestinal contents thereafter — from 7.5 to 14.5 cm. in the second hour of digestion, compared with the increase from 10 to 31.5 cm. in the second *half*-hour in the normal state — is in harmony with the observation that acid in the duodenum closes the pylorus.

Under normal conditions acid in the duodenum stimulates the secretion of pancreatic juice and bile. These alkaline fluids must neutralize the acid chyme, for an acid reaction is not found beyond the first few centimetres of the small intestine.<sup>1</sup> The neutralizing of the acid removes the stimulus keeping the pylorus closed. If the alkaline fluids are prevented from entering the intestine, the acid is necessarily neutralized more slowly, the pylorus is kept closed during longer periods, and the emptying of the stomach therefore occurs at a slower rate.

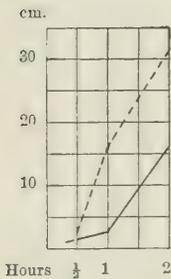


FIGURE 4. — Curves showing the average aggregate length of the food-masses in the small intestine, at the times indicated, after feeding lean beef (4 cases) in normal conditions (continuous line), and the same after setting aside the duodenum (interrupted line).

3. *Destroying continuity between stomach and duodenum hastens gastric discharge.* — Additional evidence as to the relations between the duodenum and the pylorus in the control of gastric evacuation may be secured by setting aside the duodenum and causing the stomach to empty into a lower part of the gut. The intestine was cut through about 1.5 cm. beyond the pyloric furrow, and again about 30 cm. beyond. The upper end of this separated portion was turned in and closed with stitches; the lower end was joined to the gut near the ileocolic opening by an end-to-side junction. The upper end of the main part of the intestine was now united to the small remnant of duodenum contiguous to the pylorus by the simple and effective F. G. Connell suture.<sup>2</sup> Thus the stomach emptied not into the duodenum, but into a piece of the intestine formerly 30 cm. beyond.

<sup>1</sup> See page 292.

<sup>2</sup> See F. G. CONNELL: *Journal of the American Medical Association*, 1901, xxxvii, p. 952. My thanks are due to Dr. F. T. MURPHY for indispensable aid in this operation.

The following figures give the average aggregate length of the food-masses in the small intestine, at the times indicated, after feeding shredded lean beef of standard amount and consistency, in four normal cases and in four cases with duodenum set aside. From two to nineteen days had elapsed since the operation.

Hours . . . . .	$\frac{1}{2}$	1	2
Normal conditions .	1.5	2.5	16.0
Duodenum set aside	1.5	16.0	31.5

These results are represented graphically in Fig. 4. Reference to the table and to Fig. 4 shows at once the difference between the factor which acts inside the stomach and the factor which acts in the duodenum to control the pylorus. Both conditions reported in the above table display the typical retardation of the initial discharge characteristic of proteids. Setting aside the duodenum evidently did not change that. *That* retardation, according to the conclusions already reported, is an affair of the stomach alone. And the figures in the above table serve to confirm those conclusions.

When the food begins to emerge, the results are suddenly quite different. Instead of 3 cm. at the end of an hour, 16 cm.; and twice the normal amount at the end of two hours — such is the effect of destroying the continuity between stomach and duodenum. After the first delay (in one case no food left the stomach for an hour) proteid is poured forth at a remarkably rapid rate.

The above results were secured before the completion of the research on the passage of different food-stuffs from the stomach deprived of its extrinsic nerves, to which reference has been made.<sup>1</sup> That research proved that the differential discharge from the stomach is under local control. As the investigations of Magnus had shown that the local control of intestinal reflexes resides in Auerbach's plexus, it seemed probable that merely cutting a ring around the intestine as close as possible to the pylorus, and deep enough to sever both muscular coats, would yield information as to the path of influence from duodenum to stomach. A ring was cut as above described, and the separated edges of the muscular coats were then

<sup>1</sup> See CANNON: This journal, 1906, xvii, p. 429.

held together by only the mucosa and the submucous connective tissue. When proteid was fed, there was again the initial delay — nothing out at the end of a half-hour — and this was followed by an exit almost as rapid as when the duodenum was set aside. The conclusion may be drawn that the influence from duodenum to pylorus runs through a local reflex, mediated by the myenteric plexus. As Bayliss and Starling have shown that reflex augmentation of intestinal contraction may occur from 1 to 6 cm. above a stimulated point,<sup>1</sup> it is clear that acid chyme may be effective through a considerable extent of the duodenum in causing reflex pyloric contraction.

#### EVIDENCE FOR THE ACID CONTROL DERIVED FROM PREVIOUS OBSERVATIONS ON GASTRIC DISCHARGE.

As already explained (p. 287), the data as to the discharge from the stomach, secured by use of the stomach tube, are of little service for the present investigation, because they do not indicate the rate of gastric evacuation from time to time during digestion. The method used in my earlier research on the discharge of the different food-stuffs from the stomach<sup>2</sup> gave characteristic curves of the rates at which proteids, carbohydrates, and fats pass the pylorus. The present investigation was undertaken to explain these characteristic rates of discharge. To what extent do the results of the earlier research agree with the other evidence that acid in the stomach signals the opening of the pylorus?

**Proteids.** — In the earlier research above referred to, X-ray observations showed that proteids frequently did not begin to leave the stomach during the first half-hour, and that after they began to leave they departed slowly (see curve of natural proteids, Fig. 2). Although in comparing results of different investigations the factor of food consistency cannot be closely estimated, yet observations through duodenal fistulæ on the passage of proteid chyme from the stomach support in general the X-ray observations. Thus, for example, Moritz<sup>3</sup> noted that the exit of the gastric contents began about three quarters of an hour after his dog finished eating 200 gm. raw meat. And Lang reports that the first slight discharges of the

<sup>1</sup> BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 112.

<sup>2</sup> CANNON: *This journal*, 1904, xii, p. 387.

<sup>3</sup> MORITZ: *Zeitschrift für Biologie*, 1901, xlii, p. 574.

gastric contents did not occur for at least fifteen minutes after feeding his dogs 200 gm. fibrin.<sup>1</sup> Peristalsis starts almost immediately after the ingestion of food. The dog's stomach has about four waves per minute.<sup>2</sup> It is clear that in these cases of duodenal fistulæ the food has been churned by numerous peristaltic waves, and these waves have repeatedly pressed food upon the pylorus, before the sphincter has relaxed and permitted an exit into the duodenum. The evidence that the exit does not occur until the contents of the antrum are acid has already been given. The first acid secreted unites with the proteid. The relatively long delay of the initial discharge of proteid from the stomach is thus accounted for by a relatively slow development of a marked acid reaction in the food which is pressed up to the pylorus.

Doubling the amount of proteid food strikingly delays the initial discharge of the proteid from the stomach<sup>3</sup> — a result explicable on the ground that the increased amount of proteid to become acidified in the antrum necessarily delays the proper acid reaction for opening the pylorus.

The continued comparatively slow outgo of proteid into the intestine can also be explained. As proved in my first paper on the stomach, mixing currents do not run throughout the cavity. The mixing occurs only in the pyloric end; the centre of the mass in the cardiac end long remains unchanged in reaction.<sup>4</sup> Since the antrum does not secrete acid, all the acidity of its contents is due to acid pressed in from the cardiac end. But unchanged proteid, stored in the cardiac end, is also continuously being pressed into the antrum. There is thus continuous utilization of the imported acid. Since it is altogether probable that a certain degree of acidity is necessary for opening the pylorus, the fresh proteid masses, by uniting with the acid and thus reducing the acid reaction, would naturally diminish the rate of exit from the stomach. That this factor is important in checking the rapid outgo of proteid food is indicated by the fact that acid proteids, not demanding large amounts of acid, pass the pylorus with almost carbohydrate rapidity (*cf.* Figs. 1 and 2).

Doubtless also the proteid discharge continues to be slow because

<sup>1</sup> LANG: *Loc. cit.*, p. 229.

<sup>2</sup> ROUX and BALTHAZARD: *Archives de physiologie*, 1898, xxx, p. 89.

<sup>3</sup> CANNON: *This journal*, 1904, xii, p. 410.

<sup>4</sup> CANNON: *Ibid.*, 1898, i, p. 378. See also GRÜTZNER: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 463.

proteid chyme presents a greater amount of acid for neutralization than does carbohydrate chyme. Tobler and Lang have shown that acid proteid in the duodenum will check gastric evacuation.<sup>1</sup> Khigine's results prove that when 200 gm. flesh are fed to a dog, 50 per cent more gastric juice is secreted during the first four hours of digestion than is secreted in the same time when the same amount of bread is fed.<sup>2</sup> The neutralizing of the larger amount of acid in the duodenum would necessarily require a longer time, and would result in a slower rate of discharge than would be expected when bread is fed.<sup>3</sup>

Penzoldt's observation that, with due regard to the effects of variation in quantity, those flesh foods with which there is an earlier appearance of acid remain a shorter time in the stomach than those with which the acid appears later; and likewise his observation that of the vegetables legumes, which are richest in proteid and can unite with much acid, are longest delayed in the stomach, — these observations<sup>4</sup> made on man are in agreement with the supposition that the pylorus remains closed until acid appears. The statements of Roux and Balthazard,<sup>5</sup> and of Moritz,<sup>6</sup> based on animal experimentation, that raw meat remains in the stomach a long time and leaves slowly, confirm Penzoldt's results. On the other hand, Cahn's<sup>7</sup> contention that emptying of the stomach begins with peptonization, and Roux's<sup>8</sup> declaration that concentrated peptone seems to accelerate evacuation, do not oppose the idea of the acid control of the pylorus in normal conditions, for when proteid is fed, acid would be present by the time peptonization had occurred in any considerable amount. To suppose that peptone is required for pyloric relaxation is manifestly unwarranted, — the pylorus opens

<sup>1</sup> TOBLER: *Loc. cit.*, pp. 197, 198; LANG: *Loc. cit.*, p. 240.

<sup>2</sup> KHIGINE: Archives des sciences biologiques, St. Petersburg, 1895, iii, p. 461; see also PAWLOW: *Loc. cit.*, p. 35.

<sup>3</sup> KHIGINE'S figures show slight gastric secretion continuing for ten hours after the ingestion of 200 gm. bread, but continuing only eight hours after the ingestion of 200 gm. flesh. I cannot believe that the bread was longer in the stomach than the meat; all observations on the evacuation of the stomach indicate that carbohydrate departs much earlier than the same amount of proteid.

<sup>4</sup> PENZOLDT: Deutsches Archiv für klinische Medicin, 1894, liii, p. 217.

<sup>5</sup> ROUX and BALTHAZARD: *Loc. cit.*, p. 91.

<sup>6</sup> MORITZ: *Loc. cit.*, p. 569.

<sup>7</sup> CAHN: Zeitschrift für klinische Medicin, 1887, xii, p. 41.

<sup>8</sup> ROUX: Comptes rendus, Société de Biologie, 1901, liii, p. 846.

early when carbohydrates are fed, yet under the circumstances no peptone can have been formed.

**Carbohydrates.** — With reference to the departure of carbohydrates from the stomach it is of interest to recall Marbaix's suggestion. He noted that potatoes leave the human stomach rapidly, and that gastric juice cannot attack them to any extent; he pointed out that an important question lay here.<sup>1</sup> The testimony that the delay in the discharge of carbohydrates from the stomach is usually not great,<sup>2</sup> that in conditions of diminished acidity they are more easily borne than meat,<sup>3</sup> that test meals mainly carbohydrate leave the stomach earlier than those containing meat, all points to the possibility that acid on the gastric side of the pylorus signals the relaxation of the sphincter.

A portion of the curve showing the rate of discharge of typical carbohydrate foods (the curve for the first two hours of digestion) is reproduced in Fig. 1. The original curve, published in 1904,<sup>4</sup> corresponds remarkably to that recently published by London and Polowzowa,<sup>5</sup> representing the hourly percentage volume of gastric evacuation collected from a duodenal fistula after feeding white bread. The two curves almost exactly coincide during the first two hours of digestion; thereafter my curve, although similar in shape, is naturally somewhat higher, since it represents not merely the discharge, but the accumulation of the discharge in the small intestine, minus the amount absorbed or passed into the large intestine. The method of study used by London and Polowzowa completely corroborates the X-ray method which I used. Both methods show that carbohydrate foods begin to leave the stomach soon after ingestion. I noted them in the duodenum ten minutes after ingestion; eight to twelve minutes is the time given by London and Polowzowa before the bread chyme emerges, acid in reaction. The carbohydrate foods, once started, pass out rapidly; indeed, they remain in the stomach only about half as long as the same amount of proteid food having the same consistency.

As previously intimated, this early and rapid exit is in accord with the other evidence that acid in the antrum relaxes the pyloric

<sup>1</sup> MARBAIX: *Loc. cit.*, p. 299.

<sup>2</sup> See PENZOLDT: *Archiv für klinische Medicin*, 1893, li, pp. 549, 559.

<sup>3</sup> SCHÜLE: *Therapeutisches Monatschrift*, 1899, xiii, p. 601.

<sup>4</sup> CANNON: *This journal*, 1904, xii, p. 398.

<sup>5</sup> LONDON and POLOWZOWA: *Loc. cit.*, p. 364.

sphincter. It is well known that carbohydrate food stimulates the flow of gastric juice.<sup>1</sup> But carbohydrates do not unite with the acid. Hydrochloric acid is consequently at once present to open the pylorus. And the acid is secreted as rapidly as the duodenum can receive the chyme, for the giving of carbohydrate already mixed with acid, does not increase the rate of the passage into the intestine.

**Combinations of the food-stuffs.** — In the research on the passage of different food-stuffs from the stomach it was found that when carbohydrate was fed first and proteid second, the proteid, filling the cardiac end of the stomach, did not materially check the departure of carbohydrate food, lying in the antrum; but proteid in the antrum, when proteid food is fed first and carbohydrate second, results in the characteristic slow discharge. The proteid holds back the carbohydrate occupying chiefly the cardiac end. In the former case the carbohydrate content of the antrum did not retard the development there of an acid reaction; in the latter case the proteid did retard that development. This observation indicates that the acid, which opens the pylorus, acts close to the pylorus, — a conclusion which is sustained by the effects of acid in the excised stomach (see p. 301).

When carbohydrates and proteids were mixed in equal parts, the mixed food did not leave the stomach so slowly as the proteids, nor so rapidly as the carbohydrates; the discharge was intermediate in rapidity. This result was to be expected, for a large proportion of proteid was present to unite with the acid secreted, and this would tend to retard the discharge in the manner already discussed (see p. 307).

In a mixture of fats and proteids in equal parts the presence of the fat caused the mixture to leave the stomach even more slowly than the proteid alone. This result also is in accord with the supposition that acid opens the pylorus, for fat alone inhibits, and fat mixed with proteid notably retards and diminishes, the flow of gastric juice.<sup>2</sup> Moreover the development of an acid reaction is checked by the union of the acid with the proteid. It is quite natural that this combination of food-stuffs should be slowest of all to pass from the stomach.

Fat mixed with carbohydrate in equal amounts caused the carbo-

<sup>1</sup> PAWLOW: *Loc. cit.*, pp. 36, 100.

<sup>2</sup> PAWLOW: *Loc. cit.*, pp. 97, 103. Also FERMI: *Archiv für Physiologie*, Supplement-Band, 1901, p. 76.

hydrates to pass the pylorus at a rate slower than their normal. In this case the fats again undoubtedly retarded and diminished the gastric secretion, but the carbohydrates, unlike the proteids, did not further hinder the appearance of an acid reaction. The checking of the outgo can therefore be explained solely by the effect of the fats in diminishing the secretion of gastric juice.

A review of the evidence presented in this section shows that observations on the rate of discharge of proteids, carbohydrates, fats, and combinations of these food-stuffs, can be readily explained on the assumption that acid in the stomach opens the pylorus and acid in the duodenum closes it. This fitness of the theory to explain the peculiar differences in the gastric discharge of the different food-stuffs, makes it still more probable as a statement of the normal mechanism of the pyloric passage.

#### OBSERVATIONS NOT ACCORDANT WITH THE ACID CONTROL.

The argument has been suggested against an acid control of the pylorus, that other sphincters of the alimentary canal are not thus controlled, and therefore it is unnecessary to assume such control for the pyloric sphincter. The pyloric sphincter, however, is in several respects unlike any other in the alimentary canal: (1) peristaltic waves are rhythmically pushing food against it (five or six times per minute in the cat), sometimes for half or three quarters of an hour, without causing relaxation; (2) the pylorus has on either side a secretion of opposite reaction — acid above, alkaline below — a condition unlike any other alimentary sphincter except the cardia.<sup>1</sup> Because of these peculiarities it is unjustifiable to argue from the control of other sphincters as to the control of the pylorus, especially since the local changes in chemical reaction can explain the normal pyloric functioning.

It has also been urged that much of the gastric contents must escape before it is conceivable that any great proportion of acid is present. This objection to the acid control fails to take into account the important difference between the two ends of the stomach. What may be true of the bulk of the food lying in the cardiac end may not be at all true of food in the antrum. A small amount of food in the antrum may be thoroughly mixed with acid

<sup>1</sup> A research yet unpublished shows that the cardia may be kept tonically closed during gastric digestion by the acid gastric contents.

when merely the surface of the mass in the cardiac end has been slightly acidified.<sup>1</sup> As has been previously shown in this paper (see pp. 301, 310), it is the acid reaction of the food *at the pylorus* that is significant in causing the sphincter to relax.

**The discharge of water.** — Another objection to the idea that acid on the stomach side opens the pylorus is the fact that water is very rapidly discharged. For example, Moritz, in studying by means of a duodenal fistula the emptying of the stomach, noted that water begins to enter the intestine almost as soon as it enters the stomach; it may pass out in single gushes or continuously. In thirty minutes 500 c.c. of water may go from the stomach into the intestine.<sup>2</sup> Similar results have also been reported by other observers who have studied the exit of water from the stomach.<sup>3</sup> Not only water, but likewise physiological salt solution, may go out rapidly.<sup>4</sup>

It should be noted, in the first place, that water and salt solution are very different in consistency from the foods ordinarily taken into the stomach. Furthermore, water and salt solution produce only a very slight, if any, secretion of gastric juice. When only 100 or 150 c.c. of water are injected, very often not the least trace of secretion occurs. "It is only a prolonged and widely spread contact of the water with the gastric mucous membrane, which gives a constant and positive result (secretion)."<sup>5</sup> The rapid exit of water from the stomach would preclude the conditions which make it even a feeble stimulant of gastric secretion. The failure of water to excite any noteworthy amount of gastric juice favors a rapid exit, so far as the duodenal reflex is concerned, for the acid stimulus closing the pylorus is thereby absent. Within the stomach water certainly has an effect on the pyloric sphincter very different from foods which evoke an abundant flow of gastric juice. When such foods are given, scores of peristaltic waves may sweep up to the pylorus before the sphincter relaxes; but when water is given, it begins to leave the stomach at once.

It seems probable that a state of increased pyloric tonus accompanies the conditions favoring secretion of gastric juice. Pawlow has

<sup>1</sup> CANNON: This journal, 1898, i, pp. 378, 379.

<sup>2</sup> MORITZ: *Zeitschrift für Biologie*, 1901, xlii, p. 584.

<sup>3</sup> See GLEY and RONDEAU: *Comptes rendus, Société de Biologie*, 1893, xlv, p. 517. ROUX and BALTHAZARD: *Archives de physiologie*, 1898, xxx, p. 90.

<sup>4</sup> MORITZ: *Loc. cit.*, p. 589; also CARNOT and CHASSEVANT: *Comptes rendus, Société de Biologie*, 1906, lx, p. 866.

<sup>5</sup> PAWLOW: *Loc. cit.*, p. 94.

shown that the psychic secretion of gastric juice is due to impulses coming to the stomach by way of the vagi.<sup>1</sup> Vagus stimulation also produces an augmentation of the contraction of the pyloric sphincter.<sup>2</sup> Vagus impulses, therefore, cause the initial flow of gastric juice—the psychic secretion—and they also cause increased pyloric tonus. Water does not present the conditions for psychic secretion: it is not chewed with a relish; it is swallowed rapidly; it does not satisfy appetite; and once in the stomach, it begins to pass immediately through the pylorus, as if the sphincter were in a relaxed state. The fact that water may pour through the pylorus not rhythmically but fairly continuously (see p. 312) points definitely to a diminished pyloric tonus. This fact and the failure to stimulate gastric secretion seem related to each other. In these facts may be found a probable explanation of the rapid discharge of water from the stomach.<sup>3</sup>

**The discharge of egg-albumin.**—In the same class with water is raw egg-white. In my observations on the rate of discharge of different food-stuffs from the stomach, I pointed out that egg-albumin formed an exception to the general rule that proteid passes out from the stomach slowly.<sup>4</sup> Since then this observation has been confirmed by London and Sulima in a study on dogs with duodenal fistulæ. They found that raw egg-albumin begins to pass the pylorus immediately after ingestion; it emerges in large gushes at intervals of four or five seconds. These gushes are therefore too frequent to correspond to the occurrence of peristaltic waves. For about twenty minutes the egg-white issues from the stomach with an alkaline reaction; then the reaction becomes acid, and the discharge naturally is more seldom (one to three minute intervals) and

<sup>1</sup> PAWLOW: *Loc. cit.*, p. 51.

<sup>2</sup> OPENCHOWSKI: *Centralblatt für Physiologie*, 1899, iii, p. 4. OSER (*loc. cit.* p. 288) states that vagus stimulation completely closes the open pylorus. See also MAY, *Journal of physiology*, 1904, xxxi, p. 270.

<sup>3</sup> That water does not go rapidly from the stomach merely because of its fluid consistency is shown by observations of MORITZ (*loc. cit.*, pp. 589, 590). Weak HCl, he states, passes out more slowly than water, and beer passes out with even greater retardation. The slow exit of weak HCl may be explained solely by its effect in closing the pylorus from the duodenal side. Beer stimulates gastric secretion not only by its alcohol content, but because it is bitter (see PAWLOW: *Loc. cit.*, pp. 138, 139, and CHITTENDEN, MENDEL, and JACKSON: *This journal*, 1898, i, p. 207). Beer, therefore, must go out slowly, because of the acid control of the pylorus.

<sup>4</sup> CANNON: *This journal*, 1904, xii, p. 399.

less abundant.<sup>1</sup> In this connection it is of interest that Pawlow found fluid egg-white no more effective in exciting gastric secretion than an equal volume of water.<sup>2</sup> Like water, fluid egg-white does not offer the conditions for arousing psychic secretion; and attending that condition there is a state of diminished pyloric tonus, as evidenced by discharges through the pylorus much more frequent than the peristaltic waves in the dog's stomach. The rapid passage of fluid egg-white from the stomach would therefore be explained in the same manner that the rapid outgo of water is explained.<sup>3</sup>

According to the results of my earlier investigations, however, egg-white coagulated by heat also left the stomach at a rapid rate. This observation likewise has been confirmed by London and Sulima. They found, however, that, unlike fluid egg-white, the coagulated form did not begin to leave the stomach immediately, but several minutes after ingestion. When the gastric discharge began, its reaction was acid. First the discharge had only fine particles of the egg-albumin, but later these were much larger.<sup>4</sup> These unchanged particles are significant, for they indicate that the acid has been secreted more rapidly than it could unite with the compact coagulum of the egg-albumin.<sup>5</sup> This failure of the acid to unite with albumin as soon as secreted brings about the same condition that prevails when carbohydrates are fed, — there is an early appearance of free acid in the stomach. London and Sulima report large amounts of free hydrochloric acid in the chyme of coagulated egg-white.<sup>6</sup> Moritz, Tobler, and Lang, on the other hand, declare that although the chyme of beef and fibrin is acid in reaction, it does not contain free hydrochloric acid.<sup>7</sup> This difference in the rapidity of union with the acid as it is secreted would account for the difference in the rate of discharge of these proteids. The slow union of acid with coagulated egg-white and the resultant early appearance of free acid in the stomach explains the rapid departure of this food.

<sup>1</sup> LONDON and SULIMA: *Zeitschrift für physiologische Chemie*, 1905, xlvii, p. 233.

<sup>2</sup> PAWLOW: *Loc. cit.*, p. 96.

<sup>3</sup> The very rapid exit of a rice preparation moistened with  $\text{NaHCO}_3$  (which hinders gastric secretion) may be similarly explained (see p. 294).

<sup>4</sup> LONDON and SULIMA: *Loc. cit.*, pp. 215, 220.

<sup>5</sup> See FERMI: *Loc. cit.*, p. 59.

<sup>6</sup> LONDON and SULIMA: *Loc. cit.*, p. 212.

<sup>7</sup> MORITZ: *Loc. cit.*, p. 569; TOBLER: *Loc. cit.*, p. 197; LANG: *Loc. cit.*, p. 240.

**The discharge of fats.** — In the research on the passage of the different food-stuffs from the stomach, X-ray observations showed that fats remain long in the stomach. The discharge begins slowly and continues at about the rate at which the fat leaves the small intestine by absorption and by passage into the large intestine. These results of X-ray examination are in entire accord with those of Zawilski,<sup>1</sup> Frank,<sup>2</sup> Matthes and Marquadsen,<sup>3</sup> Boldireff,<sup>4</sup> Carnot and Chassevant,<sup>5</sup> and Levites,<sup>6</sup> who used various other methods.

In attempting to understand the discharge of fats, it is necessary, first, to consider their effects both in the stomach and in the duodenum. Observations by Lobassow<sup>7</sup> and Fermi<sup>8</sup> have shown that fat in the stomach does not stimulate the flow of gastric juice. On the other hand, according to Lintwarew,<sup>9</sup> fat in the duodenum, like acid, may cause a prolonged checking of the gastric discharge.

As already shown, there is reason to believe that the taking of food not causing a flow of gastric juice is accompanied by a state of low pyloric tonus. This condition seems to be as true of fats as of water and fluid egg-white, for Boldireff has reported that when fats are fed in considerable amount, a mixture of pancreatic juice, bile, and intestinal secretion flows back into the stomach.<sup>10</sup> This result could not occur unless at times the pyloric sphincter were in a relaxed state, and unless at times the pressure in the stomach were less than that in the duodenum. In this connection it is of interest to recall that of the three food-stuffs fats produce the slowest rate of gastric peristalsis<sup>11</sup> and commonly the weakest (*i. e.*, the shallowest) waves.

As noted earlier in this paper, fats differ from carbohydrates and proteids in very seldom constituting the chief elements of a diet.

<sup>1</sup> ZAWILSKI: Arbeiten aus dem physiologischen Anstalt zur Leipzig, 1876, p. 156.

<sup>2</sup> FRANK: Archiv für Physiologie, 1892, p. 501.

<sup>3</sup> MATTHES and MARQUADSEN: Verhandlungen des Congresses für innere Medicin, 1898, xvi, p. 364.

<sup>4</sup> BOLDIREFF: Centralblatt für Physiologie, 1904, xviii, p. 457.

<sup>5</sup> CARNOT and CHASSEVANT: *Loc. cit.*, p. 866.

<sup>6</sup> LEVITES: Zeitschrift für physiologische Chemie, 1906, xlix, p. 276.

<sup>7</sup> See PAWLOW: *Loc. cit.*, p. 97.

<sup>8</sup> FERMI: *Loc. cit.*, p. 76.

<sup>9</sup> LINTWAREW: Biochemisches Centralblatt, 1903, i, p. 96.

<sup>10</sup> BOLDIREFF: *Loc. cit.*, p. 458.

<sup>11</sup> CANNON: This journal, 1904, xii, p. 392.

They differ also in not arousing gastric secretion. They are further peculiar in acting by themselves in the duodenum, not only to inhibit gastric evacuation, but also to stimulate the flow of pancreatic juice.<sup>1</sup> Clearly fats do not require the secretion of gastric juice for changes in the stomach, or for the control of their exit into the intestine, or for the stimulation of a pancreatic secretion specially favorable to their digestion.

It may be that fats have also a special relation to the pyloric mechanism. But the alternative possibility of an acid control, even when fats alone are fed, should not be overlooked. Fatty acid may be set free in considerable amount in the stomach by gastric lipase, if the fat is fed as an emulsion.<sup>2</sup> A separation of fatty acid also occurs when in the early stages of fat digestion pancreatic juice enters the stomach.<sup>3</sup> If the failure of fats to excite gastric secretion would place them at first with fluid egg-white as substances readily passed through an easily opened pylorus, the later development of acid in the fats contained in the stomach might cause them to control their own discharge like other foods developing an acid reaction of the gastric contents.

In the duodenum it is noteworthy that fats are changed with an effect quite unlike that of the other food-stuffs. Fats cause the pancreatic juice to flow, but the pancreatic juice, instead of diminishing the acidity of the duodenal contents, increases the acidity by separating a still greater amount of fatty acid.<sup>4</sup> Even when dissolved in bile the fatty acids give the solution an acid reaction.<sup>5</sup> To this increasing acidity of the contents of the upper intestine, and also to the weak and sluggish gastric peristalsis which fats evoke, may reasonably be attributed the fact that fats pass from the stomach only as fast as they are absorbed or carried into the large intestine.

**Pathological cases.** — It may be urged that certain cases of pathological secretion of gastric juice — cases of hypo- and hyperchlorhydria, and of achylia gastrica, for example — do not yield results accordant with the acid control of the pylorus. Thus in achylia

<sup>1</sup> DOLINSKY: Archives des sciences biologiques, St. Petersburg, 1895, iii, p. 424.

<sup>2</sup> VOLHARD: Zeitschrift für klinische Medicin, 1901, xlii, p. 429.

<sup>3</sup> See LEVITES: *Loc. cit.*, p. 276.

<sup>4</sup> See LEVITES: *Loc. cit.*, p. 279.

<sup>5</sup> MOORE and ROCKWOOD: Journal of physiology, 1897, xxi, p. 64.

gastrica the absence of acid does not lead to a retention of food in the stomach. But gastric evacuation in the absence of an acid reaction is only one problem to be settled in achylia gastrica, — pancreatic secretion without the natural acid stimulus in the duodenum must also be investigated and explained. As shown by these examples, the discovery of natural relations at once reveals the character of disturbed relations. If in spite of disturbed relations the processes concerned continue to be serviceable to the organism as a whole, an adaptation to the new conditions must have occurred. The ability of organs to adapt their functioning gradually to pathological states is well known in many instances. This adaptation, however, must be studied by itself as a special subject. Thus, after the normal physiology of the pylorus is made clear, it becomes of interest to know to what extent and in what manner disturbances in the stomach and duodenum are attended by changes in pyloric reflexes which are compensatory. Undoubtedly other factors may modify the usual mechanism. Already it has been shown that the pylorus may remain tightly closed against persistent peristalsis for five or six hours, if serious injury is done to the duodenum.<sup>1</sup> But this is a pathological state. It is with the normal physiology of the pylorus that the present research is primarily concerned.

In the foregoing consideration of evidence not in accord with the acid control of the pylorus, it has been necessary to assume that the ingestion of material not stimulating a flow of gastric juice is attended by a weaker tonus of the pyloric sphincter than that prevailing when food is eaten with relish. It has also been necessary to assume that if acid is secreted on proteid more rapidly than the proteid can change to acid proteid, the free acid will then cause a rapid emergence of the food from the stomach. For both these assumptions evidence is presented. If these assumptions are granted, the conclusion remains valid that acid in the stomach opens, and in the duodenum closes, the pyloric sphincter.

#### INFERENCEAL SUPPORT FOR THE ACID CONTROL FROM OTHER PROCESSES IN THE PYLORIC REGION.

The evidence that the appearance of acid at the pylorus is the signal for the relaxation of the pyloric sphincter receives strong

<sup>1</sup> CANNON and MURPHY: *Annals of surgery*, 1906, xliii, p. 515.

support from the relation of this process to other processes in the automatic mechanisms of the stomach and duodenum.

As is well known, the acid of the gastric juice is secreted only in the cardiac end of the stomach. Edkins has reported experiments showing that the condition for the continuance of gastric secretion after the initial psychic secretion, and thus the condition for the continuance of gastric digestion, lies in a chemical stimulation of the glands through the blood stream.<sup>1</sup> The chemical stimulant is produced, not by the mucous membrane of the cardiac end of the stomach, but by that of the pyloric end. It is not produced by the mucosa alone, but by the action upon the mucosa of acid, peptone, or sugar solutions. Evidently on this basis, if the pylorus opened as soon as food entered the stomach, the food would pass from the antrum without presenting to the mucosa of the antrum the acid requisite for maintaining the secretion of gastric juice. That the processes in the stomach may advance in an orderly manner, therefore, it is necessary that the food be retained until the portion in the antrum is acid.

The processes in the duodenum likewise require that food shall be checked at the pylorus until acid in reaction. If the food were allowed to depart before becoming acid,<sup>2</sup> it could not stimulate chemically the duodenal reflex. The pylorus consequently would not be held closed, and the upper small intestine would be crowded full of food through an uncontrolled pyloric sphincter. Furthermore, the chyme, unless held back until acid, would not, on entering the duodenum, excite the flow of pancreatic juice and bile. Thus, if the pylorus relaxed at the approach of the first peristaltic wave (after meat had been fed, for example), the food would not only go out from the antrum wholly undigested by gastric juice, but would bear no provision for being digested by the pancreatic juice. In order that the pancreatic juice may be caused to flow and may have time to become mixed thoroughly with the chyme without being overwhelmed by fresh discharges from the stomach, food must be retained in the antrum until acid in reaction.

If it is granted that the antrum contents must be acid before being permitted to pass the pylorus, it is of interest to note how favorably the stomach is arranged for the utilization of its secretions. Evidence has previously been presented that in order to open the

<sup>1</sup> EDKINS: *Journal of physiology*, 1906, xxxiv, p. 133.

<sup>2</sup> In this discussion the somewhat variant case of the fats is not regarded.

sphincter the acid must be at the pylorus. Clearly, if the antrum secreted acid, the acid would at once open the pylorus and let out the food (meat, for example) before the gastric juice had had opportunity to digest it. But the antrum, in which the acid stimulus acts, does not itself secrete acid. The acid and the food with an acid reaction must be brought from the cardiac end of the stomach and thoroughly mixed with the contents of the antrum before the pylorus relaxes. The necessity of importing the acid into the antrum insures a thorough mixing of the food with the gastric juice before the food departs, and provides time for gastric digestion.

The acid control of the pylorus is therefore an arrangement whereby the food is held in the stomach until provision is made for the continuance of gastric secretion, until the gastric juice has had time to act, and until the food can bear with it the acid needed for processes in the duodenum. In the duodenum the acid chyme stimulates the flow of pancreatic juice and bile, and holds the pylorus closed until this chyme has been thoroughly mixed with these digestive fluids. This thorough mixing stops gastric digestion, injurious to the action of the pancreatic ferments, by neutralizing the acid. As the acid is neutralized, the stimulus holding the pylorus closed is weakened, and then the acid in the stomach is again effective in causing the pylorus to open.

The acid control of the pylorus here described receives further inferential support from the fact that the acid affects the pyloric sphincter just as a stimulus in the intestine affects the intestinal muscle. Bayliss and Starling<sup>1</sup> have shown that in the intestinal wall is a local reflex, such that a stimulus causes a contraction above the stimulated point and a relaxation below. The action of acid on the two sides of the pylorus is in exact agreement with this so-called "law of the intestine"; the acid when above causes a relaxation of the sphincter which is below, and the acid when below causes a contraction of the sphincter which is above. As already noted (see footnote, p. 311), the cardia also obeys this law. It is not impossible that throughout the portion of the alimentary canal consisting of smooth muscle, this reflex is the mechanism for orderly action.

#### SUMMARY.

The stomach is emptied *progressively* during the course of gastric digestion; by occasional discharges through the pylorus.

<sup>1</sup> BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 142.

Mechanical agencies, either in the stomach or in the intestine, play an unimportant part in controlling gastric evacuation; for (1) the occasional discharges through the pylorus are not the result of momentarily deepened peristalsis, and (2) the upper intestine in normal conditions is not sufficiently filled or distended to check the outgo from the stomach.

Observations on chemical conditions in the stomach have hitherto been defective for judging the mechanism of the pylorus, because the food given at different times has not been identical in amount nor uniform in consistency, and the difference in the chemical reaction of the two ends of the stomach has not been distinguished. Furthermore, these studies, like the observations of Hirsch, Serdjukow, and Tobler, that acid in the duodenum checks gastric discharge, have failed to distinguish between two factors always concerned in the passage of food through the pylorus.

The two factors are (1) the pressure at the pylorus due to recurrent peristalsis, and (2) the action of the pyloric sphincter. The X-ray method shows that during gastric digestion peristaltic waves are passing, not occasionally, but continuously. Since the discharge from the stomach is not continuous, but occasional, the control must rest with the pyloric sphincter.

It is necessary to explain the intermittent closure of the pylorus; the usual closure, and the occasional opening. It is also necessary to explain why, for example, carbohydrates begin to leave the stomach early and depart rapidly, whereas proteids of the same amount and consistency begin to leave the stomach only after some time, and then depart slowly.

These facts can be explained on the theory that acid in the antrum opens the pylorus, acid in the duodenum closes it. Because the acid in the duodenum is soon neutralized, the closure of the pylorus is intermittent.

That acid in the antrum signals the opening of the pylorus is indicated by the following evidence: (1) moistening carbohydrates with  $\text{NaHCO}_3$  retards their normally rapid exit from the stomach; (2) feeding proteids as acid proteids remarkably hastens their normally slow exit; (3) observations through a fistula in the antrum show that an acid reaction closely precedes the initial passage of food through the pylorus, that the introduction of acid causes pyloric opening, and that delaying the acid reaction causes retention of the food in the stomach in spite of strong peristalsis;

(4) when the stomach is excised and kept alive in oxygenated Ringer's solution, the pylorus is opened by acid on the gastric side.

That acid in the duodenum keeps the pylorus closed is shown by the following evidence: (1) acid in the duodenum inhibits gastric discharge (observations of Hirsch, Serdjukow, and Tobler), and as shown above, the effect is not due to stoppage of peristalsis, but to closure of the pylorus; (2) the stomach empties more slowly than normally when the tying of pancreatic and bile ducts prevents alkaline fluids from neutralizing the acid chyme in the duodenum; (3) the discharge of proteid becomes rapid if the pylorus is sutured to the intestine below the duodenum, or if a ring is cut through the muscular coats immediately beyond the pylorus. The effect from the duodenum is thus a local reflex mediated, like movements of the small intestine, by Auerbach's plexus.

Evidence for the acid control of the pylorus is also found in the application of the theory to previous observations on gastric discharge. Proteids leave the stomach only after considerable delay, and then emerge slowly; this fact can be explained (1) by the slow development of a marked acid reaction in the stomach due to the preliminary union of acid with proteid, and (2) by the large amount of acid borne into the duodenum by proteid chyme. Carbohydrates leave the stomach early and rapidly, — a result to be expected, since the acid secreted upon them does not unite with them, and is at once present to open the pylorus. The peculiar rates of discharge of combinations of these food-stuffs are also readily explained on the theory above stated. This fitness of the theory to explain established facts gives it additional support.

The rapid exit of water through the pylorus without change of reaction, and the similar rapid exit of raw egg-white — facts not in accord with the acid control — are accounted for on the assumption that conditions not favoring gastric secretion are attended by a low pyloric tonus, and *vice versa*. Reasons are given for this assumption. The rapid exit of coagulated egg-white, exceptional among proteids, is explained by its slow union with the secreted acid. Fats leave the stomach very slowly. Like water and raw egg-white, they do not stimulate gastric secretion; but they may become acid in the stomach by the separation of fatty acid. Their very slow exit can probably be accounted for largely by the fact that when fats are fed, the pancreatic juice, instead of decreasing, increases the acidity of the duodenal contents.

Strong support for the acid control is found in its relation to other processes in the stomach and duodenum. The retention of food in the stomach until the antrum contents are acid is necessary (1) for the proper continuance of gastric secretion and (2) for the accomplishment of gastric digestion. Such retention is also necessary in order (3) that the chyme emerging into the duodenum may bear with it the acid required to cause the flow of pancreatic juice and bile, and (4) that the pylorus may be held closed until these important secretions are thoroughly mixed with the acid chyme.

The facts presented bring the pyloric mechanism under the "law of the intestine," — the acid when above (in the antrum) causes a relaxation of the sphincter which is below, and the acid when below (in the duodenum) causes a contraction of the sphincter which is above.

## A FURTHER STUDY OF THE ACTION OF MAGNESIUM SULPHATE ON THE HEART.

BY WM. DEB. MACNIDER AND S. A. MATTHEWS.

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IN a previous number of this Journal<sup>1</sup> it was shown by one of us that the salts of magnesium, either the sulphate or the chloride, when injected into the circulation of dogs, in doses of from 2 to 4 c.c. of a 2 *m* 1 solution, had a very depressing effect upon the heart, characterized by a progressive slowing of the heart rhythm and a simultaneous decrease in the amplitude of the contractions, which soon led to a complete standstill from which the heart could be recovered by artificial stimulation.<sup>2</sup>

In this investigation we have attempted to study more closely the condition of the heart poisoned by magnesium sulphate, and, if possible, to arrive at some conclusion as to how and where it affects the heart tissue.

The animals used in this study were medium-sized dogs (6-8 kilos). The magnesium sulphate solution (*m* 1  $\text{MgSO}_4$ ) was injected into the saphenous vein, and the tracings were taken direct from the heart by means of a Roy-Adams myocardiograph;<sup>3</sup> simultaneous tracings being taken from the right auricle and right ventricle.

It was found that doses of from 8 to 12 c.c. of an *m* 1  $\text{MgSO}_4$  solution, if injected in the space of from two to three minutes, were sufficient to bring the heart to a standstill in the condition already described.

A study of the heart thus weakened by the administration of magnesium sulphate showed, even after it had been brought to a complete standstill, that it possessed the following properties:

(1) Light induction shocks about one per second sent through the right ventricle caused contractions, which followed the law of con-

<sup>1</sup> MATTHEWS, S. A., and D. C. JACKSON: This journal, 1907, xix, p. 5.

<sup>2</sup> MATTHEWS, S. A., and D. C. JACKSON: *Ibid*, 1907, xix, p. 9.

<sup>3</sup> ROY and ADAMI: Philosophical transactions, 1853, clxxxiii, p. 207.

traction of heart muscle. The contractions which were thus induced in the ventricle were taken up by the auricle, — apparently propagated to the auricle. After the heart had been brought to a complete rest it generally required from five to eight minutes of continuous stimulation before it resumed its spontaneous rhythm, but after the spon-



FIGURE 1. — Myocardiograph tracing. Dog. One half the original size. At beginning of this tracing 8 c.c.  $m/1$   $MgSO_4$  was injected. Shows complete standstill and effect of accelerator stimulation both on auricle and ventricle.

taneous beat had once been established the contractions were even more complete than before the administration of magnesium sulphate (Fig. 1).

(2) After the heart had been somewhat slowed, the amplitude of the contractions being very much diminished, and the heart dilated to such a degree as to place it almost on the verge of a standstill, stimulation of the accelerators had the same beneficial effect in re-establishing the spontaneous beat as did stimulation of the heart directly,

with the exception that when the heart muscle was stimulated directly, it responded immediately, while stimulation of the accelerators induced contractions after a certain latent period.<sup>1</sup> Stimulation of the motor nerves had the same effect on the irritability of the heart muscle as direct stimulation. The influence of the accelerator stimulation continued for some time after cessation of the stimuli; not only the rate but the force of the heart beat was augmented.<sup>2</sup> The beat having once been obtained by a certain strength of electrical stimulus, the current may be gradually decreased and yet the heart muscle will respond by a contraction to each stimulus. In fact, if the heart responds to a certain strength of current by a normal contraction, unless the current be gradually weakened there is danger of throwing the heart into delirium cordis. This is in accord with other well-known observations that electric stimulation of the heart muscle has a tendency to increase its irritability.<sup>3</sup>

<sup>1</sup> HUNT, REID: This journal, 1899, ii, p. 469.

<sup>2</sup> HUNT, REID: *Ibid.*

<sup>3</sup> CUSHNY and MATTHEWS: Journal of physiology, 1897, xxi, p. 213. W. H. GASKELL: SCHAFER'S Text Book of Physiology, 1898, ii, p. 217.

(3) After the heart has been brought under the influence of magnesium sulphate, section of the vagi tended to increase the diminished tone, and thus to slightly antagonize the magnesium action (Fig. 2). It was noticed also that as the magnesium sulphate action increased, the inhibitory action of the vagi lessened, but stimulation of these nerves always gave some response until just before the heart action ceased.<sup>1</sup> At this point vagus stimulation would first show some slight inhibition followed by quite a marked acceleration. This would indicate, as is probably the case, the presence of accelerator fibres in the vagi. The diminished activity of the vagi gave rise to an apparent increase in the accelerator nerves. A like result was obtained after the administration of 2 mg. atropine.

As soon as the increased accelerator influence manifested itself, whether it was due to electric stimulation, to the use of certain chemicals, or to the throwing out of the vagi by sectioning or by atropine, the heart would withstand from two to three times the ordinary lethal dose of magnesium sulphate.

Electric stimulation of the auricle, the current being passed between the points of contact of a Roy-Adams myocardiograph, always caused a normal contraction which was generally propagated to the ventricle apparently in a normal manner. In two cases there were partial blocks, in one of which the ventricle responded to every third beat of the auricle and in the other to every fourth auricular contraction. In another instance there was a complete block between the auricle and the ventricle; the auricle responded, but the ventricle remained inactive and intensely dilated.

While in all probability there is more or less of a delayed passage

<sup>1</sup> It was stated by one of us in the previous article, already referred to, that the vagi remained unaffected. This is not the case, as stated in this paper.

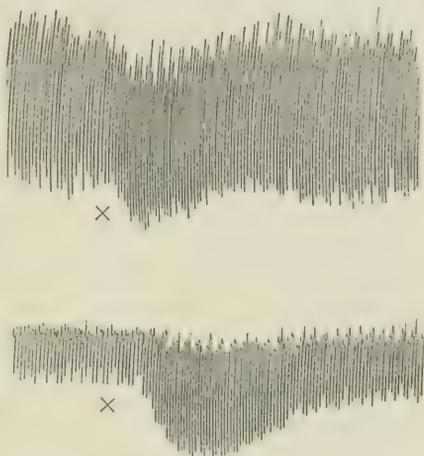


FIGURE 2. — Myocardiograph tracing. Dog. Two thirds the original size. Upper ventricle, lower auricle. Vagi sectioned at X. Increase in contraction resembling accelerator stimulation.

of impulses from the auricle to the ventricle in all stages of magnesium sulphate action upon the heart, the partial and complete blocking only became apparent late in the experiment, and after the animal had been subjected to large doses. The blocks would apparently indicate a lowering of the conducting power of the elements in the auriculo-ventricular junction, namely, the "neuro-muscular bundles (His)."<sup>1</sup>

It will readily be seen from the foregoing description that the heart is placed in a rather interesting condition in that it will respond to all external stimuli with the exception of a lowered vagus irritability, but is bereft of its power to originate a beat.

At the present time there are two theories as to the origin and maintenance of the heart beat. Each has some supporters. One school maintains that the heart, consisting as it does of contractile tissue and therefore bearing an analogy to other tissues of this type, can be thrown into activity only when the normal physiological stimulus reaches it through the intervention of some nervous mechanism.

The other school insists that the heart muscle has the distinguishing property of automatic rhythmicity independent of any nervous element, and that the nervous mechanism is for the purpose of regulating and not inaugurating a beat.

Without any attempt at a solution of these theories, we can say that after the heart has been robbed of automaticity by magnesium sulphate and rendered quiescent, it can be made to beat rhythmically and apparently in a normal manner by stimuli reaching it through a nervous mechanism (accelerator nerves). This would suggest that impulses capable of originating auricular contractions might normally reach the heart over the accelerator nerves.

In considering the probable points upon which magnesium sulphate might act to bring about these changes in the heart we hardly need to consider its central action, although a central depression of the accelerator centre might give rise to a lessened tone of the heart. But if the vagus centre should also be depressed, as it probably is, an apparent increase of accelerator action might appear. Also vagus depression either centrally or peripherally would naturally tend to increase the heart tone. Although the accelerators are active, this does not preclude the possibility that magnesium has some effect through this medium, for these nerves might be made to functionate in a normal manner by strong electrical stimulation; yet at the same

<sup>1</sup> ERLANGËR, J. : This journal, 1906, xv, p. 170.

time they might be so depressed that the normal physiological stimulus would have little or no effect. This assumption is strengthened by the known fact that removal of the vagus action by sectioning or by the use of atropine increases the functional activity of the accelerators and will enable them to overcome in some degree the magnesium sulphate depression. Also direct stimulation (electrical) of the heart muscle increases its irritability and contractility, and renders it more responsive to impulses reaching it over the accelerators and seems to enable it to more readily resume its normal rhythm after magnesium sulphate poisoning. Further, certain chemicals, as calcium chloride, barium chloride, and strophanthus, all of which have as their predominate action an increased tone and irritability of the heart, exert such a powerful action on the heart muscle or on its motor nerves that a tracing may be obtained after magnesium sulphate poisoning almost identical to one produced when the accelerators are stimulated electrically. After the use of these drugs, just as after electrical stimulation, the heart will continue to beat in a normal manner, but with increased efficiency, even when under the influence of from four to five times the lethal dose of magnesium sulphate.

The logical conclusion arrived at from such reasoning would seem to be that magnesium depresses the nervous mechanism in the heart (both accelerator and inhibitory, the latter more than the former) to such an extent that it will not transmit to the contractile tissue impulses of the same degree as are sent in by the usual physiological stimulus, whatever that may be, but that it will transmit the stronger electrical stimulus or the normal motor impulse after the heart muscle has been rendered more irritable.

This conclusion necessitates the conception that the efficiency of a stimulus depends not only upon its strength, but in a large measure upon the receptive power of the object stimulated, — in this case the muscle fibres.

The physiology of the intrinsic nervous mechanism of the heart is so imperfectly understood that it is impossible to say just what rôle it plays in maintaining or restoring the tone of the heart muscle. As before stated, stimulation of the accelerator nerves will restore the heart tone and often cause it to beat after it has been brought to a standstill by magnesium sulphate. Likewise direct stimulation of the heart muscle will do the same. Our experiments seem to suggest a dual action. The inhibitory (vagi) nerves are evidently depressed. The accelerator nerves may be and probably are depressed so as to

destroy, in a measure, their tone-giving influence over the heart. The mechanism by which impulses are propagated from auricle to ventricle is evidently weakened and may in some cases be wholly paralyzed. Finally, the muscle itself may be rendered non-receptive to the influence of stimuli, as in most cases of poisoning certain tissues seem to be more vulnerable than others. Here the depression seems to be more or less localized in the structures which transmit impulses from the auricle to the ventricle. In several cases we noticed when the heart had come to a complete standstill, both the auricle and ventricle were irritable to electric stimulation, yet the auricular contraction was not propagated to the ventricle (Fig. 3).

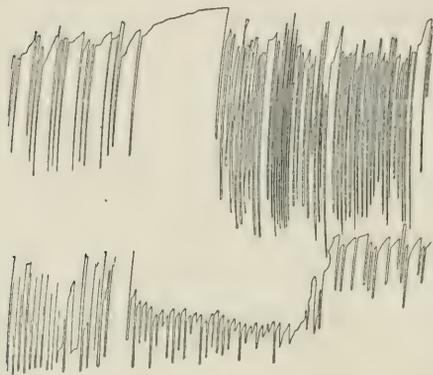


FIGURE 3.—Myocardiograph tracing. Dog. Two thirds the original size. Upper ventricle, lower auricle. Shows complete block. Auricle stimulated electrically. Induction shocks.

While this is evidently one point upon which magnesium sulphate acts, the whole phenomenon of its action cannot be explained in terms of "heart-block." The standstill of both auricle and ventricle can only be accounted for on the assumption that there is present in the cardiac tissue some element capable of being stimulated, and that such stimulation is transmitted to the muscle, resulting in a contraction. In the heart of the *Limulus*, Carlson<sup>1</sup> found such an element

<sup>1</sup> A. J. CARLSON: This journal, 1904, xii, p. 72.

NOTE. Barium chloride used in the dose of 1-2 c.c. of a  $m/50$  solution acts very powerfully on the heart, causing violent and complete contractions.

Calcium chloride in 5 c.c. doses of  $m/8$  solution has a like effect, but to a less degree, and seems to prevent the excessive action of the barium. For this reason we have used in these experiments the following combination:

Calcium chloride $m/8$ . .	230 c.c.
Barium chloride $m/50$ . .	20 c.c.
	250 c.c.

The average dose of this combination was 10 c.c.

Strophanthin, while not so efficient as the above combination in antagonizing the magnesium sulphate action, has a very decided effect, causing forcible contractions and relieving dilatation. The average dose was 1-3 mg.

After the use of barium and calcium and to a less extent strophanthin, very large doses of magnesium sulphate may be given (15-20 c.c.) with little damage to the heart.

in the median nerve cord. Should such a nervous mechanism exist in the mammalian heart, and magnesium sulphate have a like action upon it, as it has upon the tissues which propagate the auricular impulse to the ventricle, it would account for the lost automaticity of the heart while the irritability of the heart muscle still remains.

Whether such chemicals as calcium chloride and barium chloride, or strophanthus, which antagonize magnesium sulphate, do so by acting on the structures which have to do with the propagation of the cardiac impulses or by increasing the irritability of the cardiac muscle, cannot be stated definitely. We can say, however, they relieve the partial heart block produced by the magnesium sulphate and give rise to a heart tracing similar to stimulation of the accelerators.

## ON THE SWELLING OF FIBRIN.

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### INTRODUCTION.

THE forces active in the absorption and secretion of water by animal and vegetable cells have from the earliest periods of modern physiology been the object of active study and research. This is scarcely to be regarded with surprise when the multitude of physiological reactions in which a storage or a movement of water plays a leading rôle is considered. We need, by way of illustration, cite only the maintenance of turgor in plant cells, the often enormous pressures exerted by plants during growth, many of the phenomena of glandular activity, and œdema.<sup>1</sup> Following the original attempts to explain the observed phenomena through specific properties of living protoplasm there arose those in which filtration and (in the higher forms) the pressure of circulating liquids were assumed to play a predominant part. In the course of time the inadequacy of these physical agencies became apparent, so that the more modern attempts to explain the migration of water through changes in osmotic pressure — especially as rendered more generally applicable to biological phenomena through the fundamental work of Overton and Meyer on the lipoids — came as a welcome aid to those workers who seek the solution of physiological problems along purely physical and chemical lines. But the impossibility of explaining more than a comparatively small part of such phenomena as have been alluded to in this paragraph through the laws of osmotic pressure has become more and more apparent as careful physico-chemical observations on living matter have multiplied. It would seem in order, therefore, to cast about for further forces

<sup>1</sup> See FISCHER, MARTIN H.: *The physiology of alimentation*, New York, 1907, pp. 182-187 and 267-269.

capable of influencing the migration of water which we may imagine active in protoplasm. Of such, that which we must call for the present and until it is analyzed physico-chemically *the variable affinity of colloids for water*, seems to stand in the very front rank. Toward a physico-chemical analysis of this affinity of colloids for water, great strides have already been made, and when it is completed we shall, no doubt, be able to deduce from a few simple properties of a colloid its behavior toward water. At present we have still to content ourselves with studies of isolated colloids and their behavior in the presence of water under various external conditions.

The following paragraphs deal with the affinity of beef fibrin for water as influenced by various acids, various salts, and a few non-electrolytes.

#### METHODS.

As our experiments are comparative in character, it was necessary to have at our disposal sufficient quantities of a uniform material. To this end we employed the dried fibrin from blood, prepared by Merck, which we treated as follows. After powdering in a mortar the fibrin was allowed to swell in a 1/10 normal hydrochloric acid solution. When the maximum amount of swelling had been reached, the acid was poured off and distilled water substituted. This was frequently changed until a neutral reaction to phenolphthalein was obtained. To facilitate the washing, an air current was drawn through the wash bottle in such a way as to keep the fibrin in constant motion. After being allowed to dry on filter paper the fibrin was desiccated in a thermostat at 37° C. This dried fibrin was ground a second time in a mortar, and weighed amounts of the resulting powder were used in the experiments. *Unless otherwise noted, the remarks which follow refer only to fibrin prepared in this way.* This fibrin is fairly, but not entirely, free from salts, and swells under proper conditions into a colorless, jelly-like mass.

Our stock acids were one fifth normal (phenolphthalein being used as an indicator). From these were prepared the dilutions mentioned in the experiments.

Our experiments consisted in introducing a weighed quantity (0.25 gm.) of powdered fibrin into a measured quantity (20 c.c.) of various solutions contained in test tubes having a uniform diameter (1.7 cm.), and measuring the height of the swelled fibrin column

in each of the tubes. After a few preliminary experiments we found these quantities of fibrin and solution to give the most satisfactory results. While weighing the fibrin would seem to promise more accurate results than measuring its height, we found that the latter method, except in a few special instances, was less subject to experimental error than the former. The maximum amount of swelling in any of the solutions is usually attained within an hour. When an experiment is long continued, the fibrin slowly goes into solution, and the height of the column may in consequence decrease.

The following paragraphs give a survey of the experiments we have thus far performed.

#### EXPERIMENTS.

I. *Fibrin, when prepared as above, swells more in any acid solution than it does in distilled water, but the amount of this swelling is greater in some acids than in others.* The following table shows the order in which the acids are effective in this regard, and indicates at the same time the striking difference in the height of the fibrin columns between the end members of the series.

TABLE I.

Acids. All 1/10 normal.	Height of fibrin column in mm. after	
	30 min.	4 hrs.
Hydrochloric . . . . .	28	28
Phosphoric . . . . .	27(?)	27(?)
Lactic . . . . .	27	27
Formic . . . . .	24	26
Oxalic . . . . .	24	24
Nitric . . . . .	20	21
Acetic . . . . .	10	15
Citric . . . . .	9	13
Sulphuric . . . . .	8	8
Water . . . . .	6	6

A mere glance at the table is sufficient to show that we are not dealing with the simple effect of hydrogen ions as determined by

their relative concentrations in these acids, for while a "strong" acid (hydrochloric) stands first and another (sulphuric) last in the series, several "weak" organic acids are found between these extremes.

*The arrangement of the acids in the series (Table I) remains the same no matter what the concentration of the acids.* We have employed the above acids in concentrations ranging from one fifth normal to one two-hundredth normal. The arrangement in the series with all of these is the same. While the differences between the end members of the series is very great (except in the more dilute solutions), that between members following each other may be so slight (see Table II) that it falls within the limits of experimental error. We were not surprised in consequence to find neighboring members in the series occasionally changing places. Thus, formic would at times stand ahead of lactic, or phosphoric below it.

*The amount of the swelling is determined by the concentration of the acid, and is the greater, the greater the concentration of the acid.* This can be seen in the following table in which is indicated the maximum amount of swelling (after about six hours) in acids of different concentrations.

TABLE II.

Acid.	1/10 N.	1/20 N.	1/40 N.	1/80 N.	1/160 N.	1 200 N.
Hydrochloric . . .	28	24	17	9	7	
Lactic . . . . .	27	13	10	7		
Formic . . . . .	26	12	10	7		
Oxalic . . . . .	24	10	8	6		
Nitric . . . . .	19	14	8	9(?)	swelling same as in water	swelling same as in water
Acetic . . . . .	18	7	7	8(?)		
Citric . . . . .	11	6	5	6		
Sulphuric . . . . .	9	5	5	6		
Water . . . . .	6	4	5	6		

2. We next studied the effect of different salts on the swelling of fibrin in acid solutions. With the exceptions to be noted, *the addition of any salt to a pure acid solution decreases the amount*

of water absorbed by fibrin in that solution. The exceptions are formed by those salts which are capable of reacting with the acids used in the experiments. The addition of barium chloride to a sulphuric acid solution does not decrease, but increases the amount of swelling of the fibrin. This is because barium sulphate is precipitated, while hydrochloric acid is produced, and fibrin swells more in a hydrochloric acid solution than in a sulphuric acid solution of the same concentration.

*The greater the concentration of the salt in an acid solution the less does fibrin swell in that solution.* Table III illustrates this statement and what has been said in the previous paragraph:

TABLE III.

0.25 gm. fibrin in each tube		KCl.	MgCl <sub>2</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	KI
30 c.c. 1/10 N. hydrochloric acid . .	17				
15 c.c. 1/5 N. hydrochloric acid + 15 c.c. 1/2 M. salt solution . .	..	9	6	5	5
15 c.c. 1/5 N. hydrochloric acid + 15 c.c. 1/4 M. salt solution . .	..	10	8	6	5
15 c.c. 1/5 N. hydrochloric acid + 15 c.c. 1/8 M. salt solution . .	..	13	9	7	6
15 c.c. 1/5 N. hydrochloric acid + 15 c.c. 1/16 M. salt solution . .	..	14	10	10 (?)	10 (?)
15 c.c. 1/5 N. hydrochloric acid + 15 c.c. 1/32 M. salt solution . .	..	15	10	9	9

The effect of any salt upon the swelling of fibrin in an acid solution seems to be made up of the sum of the effects of its constituent ions. To determine this we studied the effect of different equimolecular salt solutions having either a common cation or a common anion upon the swelling of fibrin in hydrochloric acid solutions of different concentrations. With any given base the general arrangement of the anions is the same; with any given anion that of the cations is the same. While the difference between the end members of such two series is very marked (5 to 12 mm. of fibrin column in our experiments), that between members following each other may be only a fraction of a millimetre. As such an amount is within the limits of experimental error, it is not improbable that the placing of any individual ion in the following two series, which

we have constructed, may not be entirely correct, but the general grouping is. Anions arrange themselves in the following order. Fibrin will swell most in an acid solution in which the first named is present, and progressively less if one of the succeeding anions is present:

*Chloride, bromide, nitrate, acetate, tartrate, citrate, sulphate, iodide, ferrocyanide, sulphocyanate.*

Cations arrange themselves in the following order: Fibrin swells most in an acid solution if the first named is present, and progressively less if one of the succeeding members in the series is present:

*Potassium, ammonium, sodium, calcium, magnesium, strontium, barium, copper (?), uranium.*

If the series of anions given above is compared with the series of acids (p. 332) arranged according to their ability to make fibrin swell, it is found that the order of the anions is the same in the two. This, and the fact that the acids do not arrange themselves according to the degrees of their electrolytic dissociation, has led us to suspect that the action of any pure acid on fibrin is an expression of the concentration of the hydrogen ions *minus* the effect of the anion in decreasing the amount of water absorbed.

3. *The difference between the amount our ordinary fibrin swells in distilled water and in pure salt solutions is not great.* It is measurable in our experiments by 3 to 5 mm. A definite difference, however, exists, though it is impossible from our present series of experiments to construct accurate tables for the different salts. It can be said in general that these appear to be very similar to the two tables of ions given above for the action of salts on the swelling of fibrin in acid solutions. We have determined that fibrin swells more in pure potassium solutions than in equimolecular sodium solutions, and in these more than in equimolecular calcium solutions. When fibrin is introduced into equimolecular solutions of salts having a common cation, it is found to swell more in a chloride than in a nitrate, and more in this than in a sulphate. These conclusions have been drawn from measurements of the height of the fibrin column in test tubes containing equal amounts of fibrin (0.25 gm.) in equal volumes (20.0 c.c.) of equimolecular salt solutions varying in the different series from 1/1 molecular to 1/16 molecular; and from weighings of equal amounts of fibrin (2.0 gm.) after having swelled in equal volumes (100 c.c.) of these same salt solutions.

4. When fibrin has swelled to its maximum in a pure acid solution, it will swell still more if distilled water is substituted for the acid.<sup>1</sup> After 0.25 gm. fibrin had been left for an hour in each of three test tubes containing 20.0 c.c. 1/10 N. hydrochloric acid, the protein columns were each 16 mm. in height. One tube was kept as a control. From the second the hydrochloric acid was poured off, and after several rinsings water was substituted. From the third the hydrochloric acid was poured off, and a 1/10 N. sulphuric acid solution was substituted after the fibrin had first been rinsed in this fluid. At the end of an hour the fibrin column in the hydrochloric acid solution still stood at 16 mm.; that in the distilled water had risen to 25 mm.; that in the sulphuric acid had contracted to 9 mm. In a second similarly arranged experiment the fibrin columns measured 15, 14, and 16 mm., after standing an hour in pure hydrochloric acid solutions. Water was substituted in the first tube, sulphuric acid in the second, while the third was kept as a control. At the end of an hour in these solutions the columns measured 26, 9, and 16 mm. respectively.

5. *The taking up and giving off of water by fibrin represents, to a large extent, a reversible process.* In the foregoing paragraph the shrinkage of fibrin, when sulphuric acid is substituted for hydrochloric, has been illustrated. The final height of the protein column is the same as would have been attained if the dry fibrin had originally been allowed to swell in a pure sulphuric acid solution. Similar reversions can be obtained with salts. The fibrin column described in the previous paragraph, which measured 16 mm. in a pure 1/10 N. hydrochloric acid solution and 25 mm. in distilled water, shrank to 10 mm. when a mixture of equal parts

<sup>1</sup> These remarks refer only to *fibrin treated as described in the introduction.* As already stated, this fibrin is fairly free from salts, but not entirely so. When special pains are taken to still further reduce the amount of salts present in the fibrin, quite different results are obtained. We allowed some of our ordinary fibrin to swell a second time in 1/10 N. hydrochloric acid, then washed it twenty-four to seventy-two hours in distilled water (kept in constant motion through a stream of air) until the fibrin was neutral in reaction toward phenolphthalein, dried the fibrin on filter paper, first in air and then in a thermostat kept at 37°, powdered the fibrin a second time in a mortar, and then repeated the whole process a third and even a fourth time. *Fibrin when thus prepared swells more in distilled water than in acid.* This observation leads us to believe that the following is most probably true. *Absolutely pure fibrin (free from salts) swells most in pure water. Every electrolyte reduces the amount of swelling, but the acids are much less powerful in this regard than the salts (see paragraph 7, p. 339).*

$1/5$  N. hydrochloric acid and  $1/4$  M. sodium chloride solution was substituted for water. This, too, represents the height to which the fibrin would have swelled if placed directly in this mixture.

Two more series of experiments may be introduced here to further illustrate what has been said. 0.25 gm. of fibrin was introduced into each of seven test tubes containing 20.0 c.c.  $1/10$  N. hydrochloric acid. After fifty minutes the fibrin columns measured

22, 22, 21, 23, 23, 23, 23 mm.

respectively in the series of tubes. The supernatant pure acid solution was now carefully pipetted off each of the fibrin columns, and these were covered with 20.0 c.c. of a mixture of equal parts of a  $1/5$  N. hydrochloric acid and a  $1/4$  M. solution of the following salts (= a  $1/8$  molecular salt solution in a  $1/10$  N. hydrochloric acid solution): KCl,  $\text{NH}_4\text{Cl}$ , NaCl,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{SrCl}_2$ ,  $\text{BaCl}_2$ . After an hour in these acid-salt mixtures the fibrin columns stood,

18.5, 18, 17.5, 17, 15.5, 15.5, 14 mm.

On the following day the columns stood slightly lower (about 1 mm.), but their order remained the same.

In a similarly conducted experiment 0.25 gm. fibrin introduced into each of four test tubes containing 20.0 c.c.  $1/10$  N. hydrochloric acid had swelled to measure after thirty-five minutes,

20, 20, 20, 21 mm.

After pipetting off the pure acid solution, and substituting 20.0 c.c. of mixtures of equal parts of  $1/5$  N. hydrochloric acid with  $1/4$  M. solutions of  $\text{BaCl}_2$ ,  $\text{BaBr}_2$ ,  $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2$ ,  $\text{BaI}_2$ , the columns stood, at the end of fifty minutes,

11, 8, 7, 5 mm.

and twenty-four hours later,

9, 7.5, 6, 4.5 mm.

It will be seen that the order of the cations and of the anions, as outlined in this paragraph, is identical with that given on page 335.

6. *The taking up and giving off of water by fibrin does not, however, represent a completely reversible process* in the sense that if fibrin has swelled to a certain degree in one fluid, and to a greater or less degree in a second, it will (in the time allowed in our experiments) return entirely to the original degree of swelling when again placed under the original external conditions. In other words, a more or less lasting impression is made upon the fibrin by the conditions through which it has previously passed. This well-recognized property of certain colloids is illustrated by the following experiment. 0.25 gm. of fibrin is introduced into each of three test tubes containing 20.0 c.c.  $1/10$  N. hydrochloric acid. After seventeen hours the fibrin measures 21 mm. in each of the tubes. One tube is now refilled with  $1/10$  N. hydrochloric acid and kept as a control. The acid is poured off the second, and after the fibrin is washed in water, it is covered with this fluid. The third tube has the acid poured out of it, and after first rinsing in the fluid, the fibrin is covered with 20.0 c.c. of a mixture of equal parts of a  $1/5$  N. hydrochloric acid with a  $1/4$  M. sodium chloride solution. After twenty-four hours the fibrin in the pure acid still measures 21 mm.; that in the water 51 mm.; that in the acid-salt mixture 16 mm. The solutions are all changed again, this time back to a pure  $1/10$  N. hydrochloric acid solution. At the end of another twenty-four hours the control tube still measures 21 mm.; the fibrin from the water has shrunk to 27 mm.; and that from the acid-salt mixture has swelled to 18 mm. The solutions are now changed a third time. The control is put into another change of  $1/10$  N. hydrochloric acid, and the other two tubes have water and acid-salt mixture, respectively, once more introduced into them. When another twenty-four hours have elapsed, the control measures 22 mm., the fibrin column in the water 65 mm., and that in the acid-salt mixture only 14 mm.

In explanation of these facts it is probably correct to assume that each time fibrin is removed from an acid solution to distilled water a part of the salts existing as impurities in the fibrin diffuse out, so that each time the process is repeated the fibrin approaches more nearly a salt-free condition, and in consequence gets into the state in which it is capable of absorbing the largest amounts of water (see p. 336, footnote). The reverse is true where fibrin is removed from a pure acid solution and placed in one containing salts in addition. With each change the fibrin loads itself with the salt,

and as colloids part only with difficulty from a salt which they have (mechanically?) bound, as much salt is not lost in the pure acid solution as was taken up in the acid-salt mixture. The effect of a new transference into an acid-salt mixture is therefore added to what has remained of the effect previously produced.

7. We have made a few experiments with *non-electrolytes*. We found the *thrice washed fibrin*, described on p. 336 (footnote), *to swell as much in a pure 1/2 M. solution of cane sugar, glycerine, or dextrose, as in pure water.* 0.25 gm. of the fibrin swelled to a height of 27 mm. in each of these fluids. In a 1/10 N. hydrochloric acid solution prepared as a control the same amount of this fibrin swelled only to a height of 24 mm. and in a 1/5 M. sodium chloride solution to 12 mm. In a 1/2 M. urea the fibrin column stood at 20 mm. We are at a loss to understand why urea should be an exception in the series of non-electrolytes, but believe it due to the fact that urea solutions on standing give rise to (electrolytes) ammonium compounds.

*Neither do non-electrolytes share with electrolytes the power of decreasing by their presence the amount fibrin will swell in any acid solution.* 0.25 gm. of thrice washed fibrin was placed into 20.0 c.c. of each of the following solutions. The height of the protein column in each of the tubes at the end of two hours is appended.

Distilled water . . . . .	36 mm.
1/10 N. hydrochloric acid . . . . .	28 "
Equal parts of 1/5 N. HCl and	
1/1 M. glycerine . . . . .	30 "
1/1 M. sucrose . . . . .	28 "
1/1 M. urea . . . . .	27.5 "
1/1 M. dextrose . . . . .	27 "
1/5 M. sodium chloride . . . . .	23 "

The same fact is brought out if, instead of putting dry fibrin into mixtures of hydrochloric acid and these non-electrolytes, we first allow fibrin to swell in a pure hydrochloric acid solution, and then substitute for this an acid non-electrolyte mixture. After 0.25 gm. of fibrin had swelled to its maximum in 20.0 c.c. of a 1/10 N. hydrochloric acid solution, the protein columns in the series of tubes measured

26, 26, 26, 26, 25, 25, 23 mm.

The pure hydrochloric acid was now pipetted off the fibrin, and this covered with 20.0 c.c. of a mixture of equal parts of 1/5 N. hydro-

chloric acid with the following solutions:  $\frac{1}{2}$  M. sucrose,  $\frac{1}{2}$  M. dextrose,  $\frac{1}{2}$  M. urea,  $\frac{1}{4}$  M. uranium nitrate,  $\frac{1}{4}$  M. copper sulphate,  $\frac{1}{4}$  M. sodium sulphate,  $\frac{1}{4}$  M. sodium nitrate. After remaining seventy minutes in these solutions the fibrin columns stood

28, 27, 27, 12, 15, 13, 16 mm.

#### CONCLUDING REMARKS.

We believe that the experimental results outlined above have a bearing upon a number of problems in physiology. One of these concerns the digestion of proteins in the presence of pepsin under the influence of different acids. While the results obtained by different authors are still in a sense contradictory, all are agreed that different acids affect pepsin proteolysis very differently, and such careful studies as those of Pfeleiderer<sup>1</sup> have shown that the mere degree of dissociation of the acids does not determine the order of their arrangement. While the exact placing of the individual acids in a series is not the same with different authors, — a fact not strange when the difficulties of making quantitative determinations of catalysis are considered, — the general grouping is. Sulphuric acid, for example, is usually the end member of the series in spite of its great dissociation, while various organic acids, nitric acid, and hydrochloric acid stand above it when arranged according to the degree in which these acids favor pepsin proteolysis. We wish to emphasize here what has already been pointed out elsewhere,<sup>2</sup> that the general arrangement of the acids according to the way in which they favor proteolysis under the influence of pepsin is in large measure identical with the arrangement of the same acids according to their power of making fibrin swell. This urges upon one the belief that *the medium in which a proteolytic ferment acts serves to determine the rate of proteolysis not only through its effect upon the ferment, as is generally believed, but also through its effect upon the substance undergoing proteolysis.*

Not only can experimental facts from the literature be adduced to support such a conclusion, but a series of preliminary fermentation experiments which we have ourselves carried out do so also.

<sup>1</sup> PFLEIDERER: *Archiv für die gesammte Physiologie*, 1907, lxvi, p. 605.

<sup>2</sup> See FISCHER, MARTIN H.: *Physiology of alimentation*, New York, 1907, pp. 117-118.

Even a superficial glance at the careful determinations of Chittenden<sup>1</sup> and his co-workers, Allen and Hutchinson, suffices to show how the addition of every salt decreases the amount of fibrin digestion in a pepsin-hydrochloric acid mixture. The results obtained by these authors with the use of different salts cannot be directly compared with each other, as they worked with percentage solutions. But a little calculation soon brings out the fact that *those salts which were found above to be most effective in diminishing the amount of swelling of fibrin in hydrochloric acid are those which most powerfully retard the digestion of this substance in pepsin-hydrochloric acid mixtures.*

Our own fermentation experiments consisted in studies of the rate of digestion of fibrin in various pure acid solutions, and in hydrochloric acid solutions containing various amounts of different equimolecular salt solutions. In order to obtain comparable results we followed, in a somewhat modified manner, Grützner's<sup>2</sup> method of determining the rate of proteolysis. We stained our dried fibrin, prepared as described in the introduction, as deeply as possible in a 1/4 per cent carmine solution. When at the end of several days the supernatant carmine solution no longer lost in color, and the fibrin was stained uniformly red, we washed the fibrin in distilled water, and after drying on filter paper in the air, desiccated it in a thermostat kept at 37° C. Weighed amounts of this dried colored fibrin, which control experiments showed to swell just as readily as our ordinary fibrin, were then introduced into our different fermentation mixtures.

We found the arrangement of the acids and the salts to be practically the same in the fermentation experiments as in those dealing simply with the swelling of fibrin. Acetic acid and the acetates constituted with us the most notable exceptions. While our colored fibrin swelled readily enough in these solutions, the degree of digestion in pure acetic acid, or in pepsin-hydrochloric acid solutions containing acetates, was not so great as might have been expected from the degree of swelling. We are now trying to discover the cause for these exceptions.

<sup>1</sup> CHITTENDEN, R. H., and ALLEN, S. E.: Studies from the Laboratory of Physiological Chemistry of Yale College, 1885, i, p. 76; CHITTENDEN, R. H., and HUTCHINSON, M. T.: *Ibid.*, 1887, ii, p. 55.

<sup>2</sup> GRÜTZNER, P.: Archiv für die gesammte Physiologie, 1874, viii, p. 452, and KORN, A.: Ueber Methoden Pepsin quantitativ zu bestimmen. Inaugural Dissertation, Tübingen, 1902.

As we know that living matter is made up in the main of colloidal material, the experiments on the swelling of fibrin are of interest because they give us an insight into the means by which cells and tissues are able to take up and give off large quantities of water. A colloid such as fibrin absorbs under proper conditions from twenty to fifty times its weight of water. This more than covers the largest amount of water that is ever absorbed by any of the ordinary living tissues. When we consider that the amount held by a colloid can be enormously affected through agencies which we can imagine active in the living organism, such as slight changes in the absolute or relative concentrations of any electrolytes (acids, bases, salts) which may be present; and that these variations in the amount of water held may occur independently of any changes in osmotic pressure, we are perhaps justified in believing that in the variable affinity of the colloids for water lies the explanation of much that is obscure in those physiological phenomena that are associated with the taking up or giving off of liquids.

*The absorption of water by the gastrocnemius muscle of the frog is entirely analogous to the absorption of water by our ordinary (not entirely salt-free) fibrin.* Such fibrin swells more in any acid solution than it does in distilled water or any pure salt solution. The same holds true of frog muscle. When fibrin has attained its maximum degree of swelling in an acid solution, it will swell still more if placed in distilled water. So will a muscle. Those non-electrolytes which are incapable of altering the amount of water absorbed by fibrin, are also incapable of altering the amount of water absorbed by frog muscle. Equimolecular salt solutions affect the swelling of fibrin to different degrees. Fibrin swells more in a solution of a potassium salt than in one of a sodium salt, and more in this than in an equimolecular solution containing a calcium salt. The same holds true of frog muscle. The analogy between the absorption of water by muscle and by soaps was pointed out years ago by Jacques Loeb. Since then we have learned that the soaps are colloidal solutions. We seem in consequence to have in this fact and in the results of our experiments on fibrin adequate reasons for believing that the absorption of water by muscles is governed by the same laws which govern the absorption of water by colloids in general.

## ON THE USE OF BONE ASH WITH THE DIET, IN METABOLISM EXPERIMENTS ON DOGS.

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WHEN healthy dogs are fed a diet consisting of moderate quantities of hashed meat, cracker meal, lard, and water, very little of the food remains undigested and nearly all of it is absorbed. On such a diet, or one that may be equally well digested and assimilated, the fecal discharges from a dog are comparatively infrequent, but the consistency of the excrementitious matter that is eliminated is, as a rule, markedly diarrheal. When an animal that receives such food is confined in a cage and the urine is collected automatically in a receiver at the bottom, the elimination of thin stools is very apt to result in mingling of urine and feces. If the animal is used under these conditions for an investigation in which urine and feces are to be analyzed separately, such mixture of the excreta, contingent on the watery consistency of the feces, may entirely vitiate the experiment, or at least will add greatly to its difficulties, and to the annoyance attending accurate analytic work.

In the first experiment in which the senior author made such observations, a bitch weighing 10.9 kilos received daily, in two equal portions, at 8 A. M. and 6 P. M., a total of 250 gm. of hashed meat, 70 gm. of cracker meal, 40 gm. of lard, and 500 c.c. of water. After a preparatory period which ended with defecation, the bitch eliminated brown to black diarrheal stools on the following days only, during the period of twenty-seven days she was under observation: Ninth, fourteenth, eighteenth, twenty-third, and twenty-seventh days.<sup>1</sup> The thoroughness of the assimilation of the food given this particular animal, in spite of

<sup>1</sup> CHITTENDEN and GIES: This journal, 1898, i, p. 1; also Studies in physiological chemistry, Yale University (1897-1900), 1901, p. 1; and GIES and collaborators: Biochemical researches, 1903, i, Reprint No. 16.

dosage with 5 gm. of borax daily from the tenth day to the eighteenth day inclusive, was shown by the fact that the total amount of nitrogen in the feces during this experiment lasting twenty-seven days was only 9.66 gm., whereas the food during the same time contained 268.33 gm., or an average of 9.94 gm. daily. The nitrogen of the feces discharged during the entire experiment of twenty-seven days was less, therefore, than that in one day's ration. When one considers that the nitrogen of feces consists not only of food residues but also of gastro-intestinal secretory contributions, it is obvious that the food was very economically utilized by the dog of this experiment. The fecal matter alluded to was always thin, brownish black in color, extremely offensive in odor, and very difficult to remove completely from the cage for quantitative analytic purposes.

Shortly after the completion of the experiment that gave the data just alluded to, two additional experiments of similar character yielded results that were practically identical with those already mentioned. Thus, in the longer experiment of the two referred to, a bitch weighing 10 kilos received daily, in two portions, at 8 A. M. and 6 P. M., 160 gm. of hashed meat, 40 gm. of cracker meal, 30 gm. of lard, and 430 c.c. of water. The experiment lasted fifty-six days, during twenty-four of which the animal received large doses of borax or boric acid. The total amount of nitrogen in the food during the fifty-six days was 358.82 gm., or a daily average of 6.41 gm., whereas only 13.55 gm. of nitrogen were contained in the total mass of feces eliminated during that time, in spite of the dosage to which the animal had been subjected. Defecation occurred oftener than in the first of these three experiments, but, since the stools were watery, their more frequent elimination increased the difficulties and annoyances attending their complete removal from the cage and their preparation for accurate quantitative analysis.

The senior author and his co-workers made many similar observations in this laboratory on the thorough assimilability in dogs of such food as that referred to above and also on the fluidity, offensiveness, and troublesomeness of feces eliminated by dogs on diets of the same or similar character.<sup>1</sup> From every other standpoint, however, the use of such food in experiments on dogs was always

<sup>1</sup> See MEAD and GIES: *This journal*, 1901, v, p. 104; also GIES and collaborators: *Biochemical researches*, 1903, i, Reprint No. 21.

eminently satisfactory. A special method for the preparation and preservation of meat for use in metabolism experiments was accordingly devised,<sup>1</sup> and the food mixture referred to above, viz., prepared meat, cracker meal, lard and water, has been given regularly since, in all the experiments in this laboratory on dogs, because of the assimilability of that diet as well as the convenience of handling it, and, until recently, in spite of the annoying fecal conditions attending its employment. As the number of workers in metabolic investigation increased here, however, the difficulties and annoyances attending the elimination of diarrheal stools and their preparation for quantitative analysis became more and more prominent, and developed to an extent that concentrated attention upon them.

About five years ago, when the need for relief from this situation became pressing, the senior author, in considering the possible ways and means to that end, recalled his observation that dogs, which had received food containing bone, eliminated solid feces that were gray in color and practically odorless, and which dried quickly at ordinary room temperature to brittle masses that could be very easily crushed to a light calcareous powder. This condition of the feces appeared to be due to the preponderance in them of bone salts. It seemed apparent that in such cases the ingested bone was thoroughly digested and the organic products absorbed, but that the inorganic constituents of the bone, mainly the calcium compounds (unlike the organic constituents), were almost, if not wholly, eliminated per rectum. Might not the addition of bone to the food regularly given our dogs, as stated above, overcome the annoying fecal conditions attending the use of that particularly assimilable diet?

The desirability of adding indigestible matter to the diet of our animals, in order to give solidity to their feces, had frequently come to mind, of course, because of the favorable experiences of others with such materials. It seemed unwise, nevertheless, to subject dogs in any carefully conducted experiments to the continuous mechanical effects of materials that are never contained in food to which the dog is ordinarily accustomed. The above-mentioned observations of the effect of bone, in the diet of dogs, on the character of the feces, made it seem probable that bone could be em-

<sup>1</sup> GIES: This journal, 1901, v, p. 235; also GIES and collaborators: Biochemical researches, 1903, i, Reprint No. 1.

ployed to advantage to regulate the consistency of the feces. It was obvious that no objection could be raised to such a use of bone on the ground that it was foreign matter which the dog's digestive apparatus could not handle without probable detriment to the animal, for it is too well known that bone is a satisfactory food-stuff for dogs.

There were, however, certain practical objections to the use of bone for the purpose indicated. It would be impossible to feed bone in daily masses of uniform composition, unless it were given in the form of bone *dust*<sup>1</sup> from large, uniformly mixed, reserve supplies of the latter. But such pulverized bone would contain considerable organic matter whose removal by digestion from the compact particles might not be uniform, in which event the bone dust would introduce undesirable inequalities into the daily metabolic changes, and would make irregular contributions to the feces. It seemed wise not to run this risk if it could be avoided. From this standpoint, crude bone black<sup>2</sup> appeared to be better adapted for the purpose, because it presumably contains no soluble or digestible organic matter. Nevertheless, poorly burned commercial bone black, possibly with noxious products in it, could not well be distinguished from the best commercial samples, and on that account, even if there were no objection to the mechanical effects of the contained carbon, it seemed wise to reject bone black also as the carrier of the desired inorganic bone matters. As in the case of bone dust, it was preferable to avoid unnecessary risks.

For many reasons the pure white, thoroughly roasted, pulverulent bone *ash* of commerce appeared to be best adapted for the purpose. The material is cheap and easily obtained in large quantities. It seemed probable that a desired consistency of the feces could be obtained with less bone *ash* than either bone *dust* or bone *black*. There was no reason to think that incineration of bone causes undesirable transformation of its inorganic constituents, although carbonate is increased. The absence from bone ash of organic matter and of half-burned noxious products increased the likelihood of its greater availability than the other products mentioned.

Even in the use of bone ash, however, there seemed to be certain undesirable conditions. The somewhat larger proportion of carbonate in bone ash than in either bone dust or bone black suggested possible disturbances from immoderate liberation of carbon dioxide

<sup>1</sup> Pulverized bone.

<sup>2</sup> Pulverized bone charcoal.

in the stomach. It also appeared likely that the constituents of bone ash would be more prone to exhibit direct and rapid chemical influence in the stomach (where introduction of the free bone ash would place the salts in immediate contact with the gastric juice and gastric mucosa) than would practically the same inorganic matters, when given in the form of *slowly digestible* bone. Any such increased chemical effects, due to augmented mass action of the free osseous inorganic matters, might be expected to induce deleterious local results as well as to cause general metabolic influences.

A few preliminary trials with bone ash in the usual food of several dogs made it obvious, however, that all the expected advantages were gained from its use, but none of the suspected disadvantages appeared to follow its ingestion. The first mention of the satisfactory addition of bone ash to the diet of dogs in this laboratory was made casually four years ago,<sup>1</sup> in a report on the investigation in which its systematic use was begun, after the preliminary experiments that assured its successful employment. Shortly afterward we presented a more detailed account of the advantages that accrue from the use of bone ash in the diet of dogs, especially in metabolism experiments.<sup>2</sup>

During the past five years bone ash has been added regularly to the food of practically all the dogs used in this laboratory, and has been employed with signal satisfaction to all concerned. It has even been given advantageously in milk to kittens, to overcome proneness to diarrhea. Diarrheal tendencies, which commonly follow the use, for food, of milk, rice, dog biscuits, etc., can be almost immediately reduced to a minimum, or prevented entirely by the addition to the diet of proper amounts of bone ash. We have never witnessed a single exhibition of unfavorable symptoms that could be ascribed to the ingestion of *reasonable* amounts of bone ash with food. Even when excessive quantities of bone ash were added to the diet of healthy dogs, the only unfavorable result ever noted was a certain degree of distress as defecation was inaugurated. We have ordinarily given about 10 gm. of bone ash daily in the food of dogs of average size. 1 gm. per kilo has been an effective

<sup>1</sup> TALTAVALL and GIES: Proceedings of the American Physiological Society, This journal, 1903, ix, p. xvi; also GIES and collaborators: Biochemical researches, 1903, i, p. 59.

<sup>2</sup> GIES: Proceedings of the American Physiological Society, This journal, 1904, x, p. xxii.

average quantity with the food used in this laboratory. (See footnote, p. 350.)

Besides preventing diarrhea in dogs subsisting on various kinds of food that usually encourage the elimination of watery stools, the addition of bone ash to a diet consisting of hashed meat, cracker meal, lard and water, considerably increases the bulk of the fecal matter, and makes its discharge comparatively frequent and quite regular. A dog such as the one referred to on p. 343, if given daily 10-15 gm. of bone ash with the food, would ordinarily defecate about once a day. The feces under the influence of the bone ash treatment are usually almost odorless, and have the typical consistency and appearance of the fecal elimination commonly passed from dogs after bones have been eaten by them. Intestinal putrefaction is kept at a fairly constant low level when bone ash is administered.

Occasionally caged dogs eat their own feces, especially, it seems, when their discharges are particularly odoriferous. Such an event in a metabolism experiment cannot occur without very disturbing consequences. Since we began the administration of bone ash to dogs five years ago, we have seen a manifestation of this tendency by only one of several hundred dogs that have been under continuous observation. The exceptional dog referred to was receiving daily an excessive amount of protein matter, and the proportion of bone ash had not been kept as high as it should have been. The feces were therefore quite soft, and were ill smelling besides; and the dog disposed of them in the manner indicated. As a rule the feces that are passed after the administration of bone ash are so nearly devoid of offensive odor, and are so chalky in appearance and consistency on drying, that they appear to excite in caged dogs no inclination to ingest the excrement.

The eliminations from dogs on the diet referred to above are passed in the form of light-brown lumps. These lumps do not adhere to the cage, but may, as a rule, be easily lifted and quickly removed completely, without disintegration. They dry quickly at room temperature to fairly hard, yellowish white, brittle masses, and in this condition may be rendered perfectly pulverulent in a mortar with a few strokes of a pestle. Such a powder can be quickly made as homogeneous as any other. Consequently, desiccation on a water bath or by any special means is entirely unnecessary as a prelude to pulverization; and loss of volatile products, such as ammonia, is greatly reduced. The powdered feces from

dogs on the diet indicated are light, yellowish white, and much like the original bone ash in every way. Passing the pulverized product gently through a very minutely meshed sieve is a simple matter, and the isolation of hair and any foreign particles can thus be quickly and satisfactorily attended to. Feces of this character are easily handled, may be readily prepared for analysis without any annoyance to the operator, and give no trouble in analytic procedures.

For some time seven animals in as many cages have been under daily observation in this laboratory in a number of researches now in progress. It takes Mr. Christian Seifert, our efficient laboratory assistant, a little less than three hours to weigh the food for, and feed, all these dogs; to collect and measure all their eliminated urines, and determine the reactions and specific gravities before bottling them; to remove completely the feces to, and break up the larger masses in, flat weighed evaporation dishes preparatory to deposit on shelves for spontaneous desiccation; to brush up and bottle the hair and scurf from the drip pans; to put each cage into condition for the collection of excreta during the succeeding twenty-four hours; to co-operate in meeting all ordinary emergencies; and to keep an accurate record of every detail on these matters pertaining to each animal. If the feces were diarrheal, it would be impossible even for Mr. Seifert, with all his experience and ability, or for any one else, daily to accomplish so much in so short a time. The great advantages accruing from the use of bone ash with the diet of dogs are appreciated most where large numbers of dogs require daily attention in quantitative experiments. In this laboratory we regard the bone ash treatment as a particularly fortunate method for the advancement of metabolism work.

If it is desired to mark off as distinctly as possible the feces of any particular day, the addition to the food or bone ash for that day of a trifling quantity of pure, finely powdered, thoroughly washed charcoal imparts an unmistakable gray color to the feces eliminated during the succeeding twenty-four hours. This color is about the same as that of a mixture of the amount of charcoal taken and the daily portion of bone ash. If charcoal is withheld from the next day's food, the gray color disappears from the fecal mass of the succeeding day, or is present, sharply defined, in only a small portion of it. We have found that if a gram or two of powdered charcoal is given to a dog of ordinary size, fed regularly each morning on the diet commonly used by us in this labora-

tory,<sup>1</sup> the black material put into the food on one morning appears almost wholly in the fecal discharge during the succeeding twenty-four hours. The fecal elimination during a period of twenty-four hours after a meal, under the usual conditions of our experiments, represents, with a fair degree of completeness (75 per cent or more), the fecal formation during the same time. These facts favor very satisfactory division of an experiment into periods of fairly definite excretory conditions.

The fondness of dogs for bones and the digestibility of bone in dogs are well known. Bone ash does not seem to introduce into a diet anything that is injurious to dogs, but appears to behave in them much like an equivalent amount of bone. That bone ash has little, if any, effect on the taste of the usual diet, or on the animal itself, is evident from the total indifference manifested by dogs toward it. When excessive amounts are given, little attention is paid to the bone ash even by well-nourished animals with the usual appetite.

Thus a normal dog, weighing 17 kilos, took daily for a week or more as much as 100 gm. of bone ash admixed with 250 gm. of hashed meat, 70 gm. of cracker meal, 30 gm. of lard, and 500 c.c. of water, without showing any observable effects whatever, except more frequent and abundant defecation. Although such a soupy mixture, containing a great excess of the bone ash, looks much like thick milk of lime, the dog ate it with evident relish. This animal was a well-nourished one, with only an ordinary appetite.

Moderate amounts of bone ash in the food appear to be devoid of harmful effects on both digestion and absorption. We expect before long to inquire especially into the possible effects on fat absorption. So far as we can now judge there is no hindering influence.

The nature and extent of the chemical changes that bone ash undergoes in transit through the gastro-intestinal tract have not yet been determined, but that very little of such bone ash is absorbed was shown by the results of the following special experiment on the influence of bone ash on the excretion of calcium and phosphate in the urine.

A well-nourished dog, weighing 6.5 kilos, and confined in one of Gies' cages,<sup>2</sup> was given daily 97 gm. of prepared meat,<sup>3</sup> 26 gm.

<sup>1</sup> Usual amounts *per kilo*: prepared meat, 15 gm.; cracker meal, 4 gm.; lard, 3 gm.; bone ash, 1 gm.; water, 35 c.c.

<sup>2</sup> GIES: This journal, 1905, xiv, p. 403.

<sup>3</sup> GIES: *Ibid.*, 1901, v, p. 235; also GIES and collaborators: Biochemical researches, 1903, I, Reprint No. 1.

of cracker meal, 20 gm. of lard, and 225 c.c. of water. This diet was fed daily at 10 A.M. After a preparatory period of five days, during which the dog's weight fell to 6.42 kilos, our analytic work was begun, and was continued on the urines of twenty-seven days. Samples of three-day volumes were used for the determinations of calcium and phosphorus.

Phosphorus was determined in 25 c.c. portions by the usual alkali fusion process. It was weighed as magnesium pyrophosphate and expressed in the records as  $P_2O_5$ .

Calcium was determined, by the advice of Professor Sherman, as follows: 50 c.c. of urine were strongly acidified with acetic acid, then nearly neutralized with ammonium hydroxid, and treated with a moderate excess of ammonium oxalate. After standing not less than four hours, the precipitate was filtered off, washed with water, and dissolved in 20 c.c. of hydrochloric acid of 1.04 sp. gr. This solution was then diluted with water to a volume of 150 c.c., heated to 70° C., and, at that temperature, titrated with  $\frac{1}{10}$  solution of potassium permanganate. The calcium found was expressed in the records as CaO.

Our results may be seen at a glance in the table on page 352.

The summary of analytic data on page 352 makes it evident that administration of bone ash had no influence on the urinary excretion of calcium. It is apparent from the same data, also, that the elimination of phosphorus (presumably also of phosphate) was not increased by the ingested bone ash.

The observed gradual diminution of eliminated phosphorus may have been due chiefly, if not wholly, to the metabolic changes responsible for the steady decline of body weight and decrease of urine volume. It is probable that the animal did not receive food enough upon which to maintain weight equilibrium and that, under these conditions, phosphorus catabolism was less pronounced at the close of the experiment than at the beginning. The low tide of phosphorus elimination at the time when most bone ash was given may be more than a coincidence, however, and additional experiments may show that urinary excretion of phosphorus (phosphate) is somewhat diminished by the administration of bone ash. If this should prove to be the case, the decrease in the amount of phosphate in the urine might be due to diminished absorption of alkali phosphate. Such a result might ensue from interaction, in the intestine particularly, between calcium and phosphate (each being

TABLE I.

RESULTS SHOWING EFFECTS OF BONE ASH IN THE DIET ON THE URINARY ELIMINATION OF PHOSPHATE AND CALCIUM FROM A DOG.

No. of day.	W'ght of dog.	URINE.								Feces.	Bone ash.
		Daily volume.	Specific gravity.	Volume.		P <sub>2</sub> O <sub>5</sub> .		CaO.			
				Three-day periods.		Three-day periods.		Three-day periods.			
				Total.	Daily average.	Total.	Daily average.	Total.	Daily average.		
kg.	c.c.	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.		
1	6.34	240	1,018							4	0
2	6.29	200	1,015							5	0
3	6.25	240	1,019	680	227	1.6063	0.5354	0.0340	0.0113	9	0
4	6.25	190	1,021							5	0
5	6.19	220	1,019							0	0
6	6.13	170	1,021	580	193	1.3805	0.4602	0.0325	0.0108	8.5	0
7	6.13	180	1,020							6	2
8	6.10	200	1,018							12	4
9	6.10	170	1,020	550	183	1.4017	0.4673	0.0451	0.0150	10	6
10	6.10	140	1,020							14	8
11	6.10	140	1,022							23	10
12	6.10	155	1,019	435	145	0.9288	0.3096	0.0348	0.0116	18	10
13	6.09	150	1,020							19	10
14	6.09	190	1,027							17	10
15	6.11	115	1,023	455	152	0.9540	0.3180	0.0419	0.0140	32	25
16	6.08	135	1,025							50	50
17	6.09	210	1,016							10	0
18	6.08	190	1,019	535	178	0.7838	0.2613	0.0439	0.0146	55	50
19	6.11	120	1,021							0	0
20	6.05	195	1,020							7	0
21	6.00	200	1,018	515	172	1.1246	0.3748	0.0464	0.0155	0	10
22	6.03	140	1,021							19	10
23	6.00	215	1,017							17	10
24	6.00	205	1,014	560	187	0.8247	0.2749	0.0336	0.0112	22	10
25	6.03	150	1,021							12	10
26	6.02	145	1,020							27	10
27	5.95	125	1,019	420	140	0.7317	0.2439	0.0330	0.0110	9	10

present in both the food and the administered bone ash), with the production of less soluble or precipitated products. Possibly such interactions would occur especially between alkali phosphate and

that portion of the available calcium that had been converted in the stomach into chlorid from carbonate, and which, as 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. Changes of this sort, comparatively slight at best with the quantity of bone ash we use, would doubtless be quite regular and uniform, daily, in the average experiment, and would probably affect none of the essential conclusions on metabolism. 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.

Additional experiments in this connection, as well as on the effect of bone ash on absorption, will shortly be undertaken.

When we first began the use of bone ash in the diet of dogs, it occurred to us that great difficulty might be encountered in any effort to measure accurately the metabolic changes in which phosphorus was involved. It seemed probable that the large amount of preformed and non-significant phosphate in the feces (derived directly from the bone ash) would be particularly difficult to collect to such a degree of quantitative completeness that inevitable mechanical losses and variations would not be regularly great enough to hide metabolic fluctuations. We have found, however, that fecal conditions after the administration of bone ash are so favorable for easy quantitative assemblage of the daily portions of solid excreta, and their preparation for analysis without material loss, that true phosphorus balances may be determined as satisfactorily as those of nitrogen or sulphur. All that is required is constant care and watchfulness at every stage of the process of collecting the excreta.

The above-mentioned facts were first shown in the hemorrhage experiments in this laboratory described by Hawk and Gies.<sup>1</sup> Their experience has been repeated here in a number of metabolism investigations, *e. g.*, in the radium research by Berg and Welker.<sup>2</sup>

The summaries on pages 354 and 355 give conclusive data bearing on these points.

It often happens in metabolism work on animals, when the urine is not removed artificially, that urine fractions come in contact with

<sup>1</sup> HAWK and GIES: This journal, 1904, xi, p. 171.

<sup>2</sup> BERG and WELKER: Journal of biological chemistry, 1906, ii, p. 371.

TABLE II.  
SULPHUR AND PHOSPHORUS BALANCES IN EXPERIMENTS ON DOGS IN WHICH BONE ASH WAS ADDED TO THE DIET.

PERIODS.		SULPHUR.						PHOSPHORUS.						
No.	Days.	Conditions.	Total.		Balance.		Urinary.		Total.		Balance.		Urinary.	
			In-gested.	Ex-creted.	Total.	Daily average.	In-gested.	Ex-creted.	Total.	Daily average.	In-gested.	Ex-creted.	Total.	Daily average.
A. <sup>1</sup> Weight of the dog, 17 kilos. Food: prepared meat, 250 gm.; cracker meal, 70 gm.; lard, 30 gm.; bone ash, 10 gm.; water, 500 c.c.														
I.	12	Normal . . . . .	9.66	8.05	+1.61	+0.135	0.46	29.47	28.92	+0.55	gm. 0.48	37.36	+1.94	gm. 0.54
II.	16	Anæsthes., operation, and 1st hemorrhage (2.93%)	12.88	13.27	-0.39	-0.023	0.59	39.30	37.36	+1.94	+0.123	24.57	+0.15	0.50
III.	10	Anæsthesia alone . . . .	8.44	7.39	+1.05	+0.105	0.53	31.94	29.84	+2.10	+0.163	51.29	+0.43	0.52
IV.	13	Anæsthesia and operation combined	11.47	11.65	-0.18	-0.014	0.67	16.98	16.53	+0.45	+0.065	9.70	-0.73	0.60
V.	21	2d hemorrhage (3.22%) . .	16.83	20.64	-3.81	-0.180	0.74	2.85	10.43	-7.58	-0.180	17.51	-0.27	0.44
VI.	7	3d hemorrhage (3.51%) . .	4.99	6.59	-1.60	-0.229	0.72	5.89	6.38	-0.51	-0.05	17.51	-0.22	0.48
VII.	4	4th hemorrhage (3.26%) . .	2.85	4.24	-1.39	-0.348	0.82	3.27	3.82	-0.55	-0.11	9.73	-0.09	0.50
B. <sup>2</sup> Weight of the dog, 12 kilos. Food: prepared meat, 200 gm.; cracker meal, 52 gm.; lard, 15 gm.; bone ash, 8 gm.; water, 375 c.c.														
I.	9	Normal . . . . .	5.89	5.89	....	....	0.48	17.51	17.78	-0.27	-0.03	17.51	-0.03	0.44
II.	9	Anæsthesia . . . . .	5.89	6.38	-0.51	-0.05	0.54	17.51	17.74	-0.22	-0.02	17.51	-0.02	0.48
III.	5	Hemorrhage . . . . .	3.27	3.82	-0.55	-0.11	0.59	9.73	9.82	-0.09	-0.02	9.82	-0.02	0.50

C. <sup>3</sup> Weight of the dog, 4.5 kilos. Food: prepared meat, 90 gm.; cracker meal, 35 gm.; lard, 20 gm.; bone ash, 3 gm.; water, 210 c.c.												
I.	9	Normal . . . . .	2.22	2.48	-0.26	-0.03	0.22	7.08	7.07	+0.01	....	0.25
II.	19	Barium bromid . . . . .	4.70	5.55	-0.85	-0.04	0.21	14.94	15.39	-0.45	-0.02	0.20
D. <sup>4</sup> Weight of the dog, 6.5 kilos. Food: prepared meat, 117 gm.; cracker meal, 42 gm.; lard, 20 gm.; bone ash, 5 gm.; water, 260 c.c.												
I.	7	Normal . . . . .	2.22	2.21	-0.01	....	0.25	8.51	8.26	+0.25	+0.04	0.30
II.	13	Barium bromid . . . . .	3.81	3.73	-0.08	-0.01	0.24	14.58	14.41	+0.17	+0.01	0.27
E. <sup>5</sup> Weight of the dog, 6.6 kilos. Food: prepared meat, 110 gm.; cracker meal, 40 gm.; lard, 19 gm.; bone ash, 5 gm.; water, 244 c.c.												
I.	5	Normal . . . . .	1.42	1.25	+0.17	+0.03	0.17	6.11	3.30	+2.81	+0.56	0.16
II.	12	Radium bromid . . . . .	3.40	3.22	+0.18	+0.02	0.18	14.67	13.45	+1.22	+0.10	0.20
III.	5	After . . . . .	1.42	1.38	+0.04	+0.01	0.23	6.11	2.95	+3.16	+0.63	0.23
F. <sup>6</sup> Weight of the dog, 5.6 kilos. Food: prepared meat, 100 gm.; cracker meal, 20 gm.; lard, 10 gm.; bone ash, 5 gm.; water, 225 c.c.												
I.	7	Normal . . . . .	1.64	1.72	-0.08	-0.01	0.18	8.20	7.74	+0.46	+0.07	0.23
II.	8	Radium bromid . . . . .	1.87	1.96	-0.09	-0.01	0.18	9.38	8.25	+1.13	+0.14	0.20
<p><sup>1</sup> HAWK and GIES: <i>Loc. cit.</i>, p. 202.      <sup>2</sup> HAWK and GIES: <i>Loc. cit.</i>, p. 217.      <sup>3</sup> BERG and WELKER: <i>Loc. cit.</i>, p. 388.</p> <p><sup>4</sup> BERG and WELKER: <i>Loc. cit.</i>, p. 394.      <sup>5</sup> BERG and WELKER, <i>Loc. cit.</i>, p. 401.      <sup>6</sup> BERG and WELKER, <i>Loc. cit.</i>, p. 406.</p>												

fecal masses. If the animals are confined in cages on suitable wire supports that suspend the feces, this mingling tendency is reduced to a minimum, because the urine fractions immediately run through the wire netting as they are voided. Even in the best cages with wire net supports, however, fecal fragments occasionally pass through the meshes, and are apt to be washed by urine fractions that pass over them to the receiver, and perhaps flush them into the latter. Bone ash in the food of dogs makes their fecal discharges solid, as we have said, and if such eliminations are not removed from the cage soon after passage (and during the night this cannot be done regularly), they speedily dry, also become somewhat brittle as a consequence, and, if the dog tramples on any of the masses (though this happens only occasionally, for the animal usually avoids the material), fragments pass through the wire support, no matter how small the meshes may be. What is the effect of urine on such fecal matter? Is calcium or phosphate dissolved from it by the normally acid urine, and are appreciable changes brought about in the composition of the latter when dog urine washes fecal fragments? Does acid urine decompose any calcium carbonate, for example, in such feces?

We have given this matter attention repeatedly, and have found that acid urine is practically devoid of solvent action on fecal constituents derived from bone ash. The following observation on bone ash itself is conclusive:

Freshly voided strongly acid urine from a well-nourished dog, and which had not been in contact with any fecal matter, was selected for a determination of the solvent action of normal urine on the calcium in bone ash. Two equal portions of the urine, 150 c.c. each, were transferred to covered beakers of equal size. To one portion, 2 gm. of bone ash were added. The other portion was kept as a control. Each was thoroughly stirred repeatedly during a period of four hours, after which both were filtered. Calcium was determined in 50 c.c. samples of each filtrate, by the method referred to on page 351, with the following results:

50 c.c. of filtrate from urine without bone ash contained 0.0067 gm. of CaO.  
50 c.c. of filtrate from urine with 2 gm. of bone ash, contained 0.0070 gm.  
of CaO.

As a rule no greater changes would occur in the course of a metabolism experiment. Because of the greater bulk of the "bone

ash feces," and the consequent attenuation of the soluble substances in them, it is probable that any removal of such substances by urine is ordinarily both absolutely and relatively less from "bone ash feces" than from the excrementitious matter of dogs whose diet does not contain bone ash.

# A SIMPLE ELECTRICAL ANNUNCIATOR FOR USE IN METABOLISM EXPERIMENTS, AND IN CONNECTION WITH FILTRATION, DISTILLATION, AND SIMILAR OPERATIONS.<sup>1</sup>

By WILLIAM H. WELKER.

[From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.]

IN the paper describing his cage for metabolism experiments, Dr. Gies<sup>2</sup> referred to the advantages of the "sliding shelf" devised as a holder for the urine receiver, and in that connection made the following remark: "The shelf also favors the use of electrical apparatus to ring out the time of elimination of urine fractions, in experiments in which fractions of the urine must be examined separately and immediately after their natural excretion" (p. 407). This remark alluded to one of the several additional devices Dr. Gies had contemplated perfecting for use with the cage described.

In his preliminary communication regarding our annunciator Dr. Gies<sup>3</sup> stated that, "in order that an annunciator might be of the greatest service in metabolism work in the way already indicated, and also to insure its usefulness for filtration, distillation, and other operations in which the weight of a product above a certain maximum amount could be relied upon to close an electrical circuit and announce the delivery of the material, it was necessary that it should be delicately responsive to the weight of several grams, and yet be readily adjustable within relatively wide limits in that

<sup>1</sup> This apparatus was exhibited before the Society for Experimental Biology and Medicine, May, 1906, and was shown at the Scientific Exhibition of the New York Academy of Sciences, in the American Museum of Natural History, December, 1906.

<sup>2</sup> GIES: This journal, 1905, xiv, p. 403.

<sup>3</sup> WELKER and GIES: Proceedings of the Society for Experimental Biology and Medicine, 1906, iii, p. 77.

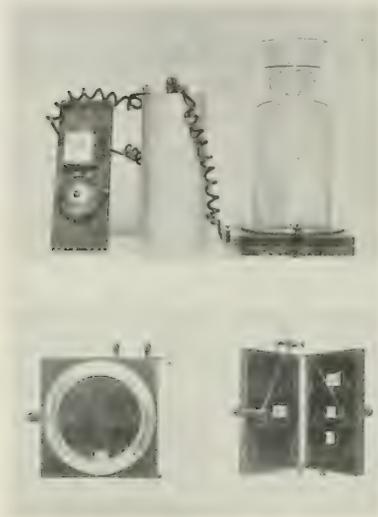
respect; also that it should be light in weight and of small compass, but durable, and resistant to derangement from any cause; and that it should hold, without risk to, or modification of the contents, any suitable vessel placed upon it."

At the request of Dr. Gies, and with his advice, I proceeded to design a working form of apparatus to meet these requirements. The simplest possible device appeared to be two square pieces of board hinged together along one side, and held apart by an adjustable spring on the opposite side, with a bell and appropriate electrical fittings. In the first model the pieces of board, which were of wood, warped somewhat; consequently hard rubber was substituted in the form finally adopted. In the first model a piano hinge was used to connect the boards. In the second, the piano hinge was replaced by a pair of small steel-pivoted brass hinges. Friction in these pivoted hinges was much less than in the long piano hinge, and the substitution of the former for the latter increased the sensibility of the apparatus.

As finally constructed, our annunciator consists of (1) a pair of square pieces of hard rubber ( $4\frac{3}{8} \times 4\frac{3}{8} \times \frac{5}{16}$  in.) united by (2) a pair of small steel-pivoted brass hinges. (3) A post is attached to the middle of the bottom side of the lower rubber board. This post is threaded with a fine screw thread. It also has a key way running its full length. (4) The nut regulating the pressure is a thin circular disk milled on the outside. On top of the disk is (5) a seat for (6) the spring, with a key for the key way in the post. The lower end of the spring is slipped over the thin end of the seat to its rim. To the upper board is fastened (7) a brass plate with a hole in it through which the post attached to the lower board can pass freely. On the under side of this plate is a seat for the top of the spring. When all these parts are in position, a small circular (8) nut is screwed on the post above the plate. With the aid of this nut the distance between the boards may be readily adjusted. Electrical contact is made by means of (9) a platinum plate in the top board and three (10) platinum points in the bottom board. The platinum plate is connected by an (11) inlaid copper wire running to the upper portion of one of the hinges. The lower side of this hinge is connected with one of (12) the binding posts at the side of the bottom board. The points are connected by means of an inlaid copper wire extending to the other binding post.

(13) Four small steel points are fixed at the corners of the bottom board to add stability to the annunciator. (14) The receiver, for urine, distillates, etc., which rests on the upper board, is held in place by means of (15) a large circular washer of rubber packing, which is bound to the board by (16) a circular aluminium washer fastened with four small (17) screws, and is of such size as to leave the edges of the rubber free to oppose any movement of the receiver.

(18) A small dry cell furnishes the current, and (19) a little bell completes the mechanism. The accompanying illustrations make clear the relationships of the parts mentioned.



Electrical annunciator.

The apparatus is very compact. With the dry cell and the bell included, it may be placed on a surface  $5 \times 8\frac{1}{4}$  in. The small bell we have used has a delicate musical sound, and answers all ordinary purposes. When used under a metabolism cage to announce the elimination of excreta or vomit, the ringing of such a bell does not disturb the animal. It is obvious, of course, that the apparatus may be connected with

a bell in a room some distance from that in which the main part of the mechanism is placed.

In its present form the apparatus can be adjusted so sharply that it will announce deposits of liquid as slight in weight as that of 1 c.c. of water. The deposit of fragments of feces, or vomit, or anything equal to that weight, is duly announced. The apparatus may be so delicately sensitized that, when resting on the lightly suspended "sliding shelf" of Gies's metabolism cage, vibration of the cage due to any special movement of the dog effects rapid intermittent contact between the opposing platinum plate and points in the annunciator, thus making and breaking the current, and producing a discontinuous ringing of the bell until the movements of the animal cease and the force of the vibrations falls below the

effective impetus. Such announcements obviously help a busy observer to keep close watch over the animal at significant intervals.<sup>1</sup>

On account of its simplicity, and the ease with which it may be adjusted to ring out different amounts, this apparatus can be used very advantageously in distillation work when certain fractional volumes are desired. It can also be used in filtrations when the filtrates are to be partitioned into certain volumes. The adjustment for any volume is made by placing the weights equivalent to the same on the upper board beside the receiver, and then turning the adjusting screw at the bottom of the post, thus increasing or diminishing the tension on the spring until the contact is *just* made, as indicated by the ringing of the bell. The weights then being removed, an equal weight of liquid must collect in order that contact may again be made and announced by the bell.

This apparatus has been used in this laboratory with particular success on the "sliding shelf" of our metabolism cages to announce the elimination of urine, vomit, or watery stools. It promises to add materially to the equipment of any laboratory whose workers appreciate the time that may be saved, and the accuracy that may be insured, in many operations by the employment of such an automatic device.

<sup>1</sup> HAWK recently made mistaken allusions to GIES's metabolism cage, which BERG and I endeavored immediately to correct (*Journal of biological chemistry*, 1906, ii, p. 410). HAWK did not comprehend the very many advantages accruing from the use of detached receivers, and particularly failed to appreciate the special serviceability of the "sliding shelf" of the cages in this laboratory as a support for a readily removable receiver. The use of an annunciator in the advantageous ways indicated in this paper is made particularly satisfactory by the construction of our cages and the character of the urine receiver used with them.

## AN IMPROVED ANIMAL HOLDER.<sup>1</sup>

BY GUSTAVE M. MEYER.

[*From the Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

WHILE engaged recently in a study of the elimination of various anilin dyes<sup>2</sup> from a dog with a biliary fistula, it was desirable occasionally to suspend the animal in its normal upright position for extended periods. At the same time provision had to be made for the separate collection of the excreta and the bile. As no apparatus possessing all these features was immediately available, a suitable holder was improvised, which served excellently the particular purpose for which it was designed.

Profiting by the suggestions of Professor William J. Gies, I extended the plan of the apparatus so as to make it a dog holder of general serviceability. The finished holder has been employed in this laboratory by Drs. Carter, Cussler, Salant, and by Mr. Welker and myself, in researches now in progress, and has always given satisfaction. As its use during the past year has demonstrated its wide adaptability and usefulness, a description of it is herewith presented.

The particular piece of apparatus referred to will hold easily any dog weighing from about 5 to 13 kilos. The dimensions given below can readily be made to suit if smaller or larger holders are desired. The support constructed for use in this laboratory not only answers practically all purposes for dogs of average size, but can be placed conveniently under an ordinary table out of the way when not in use.

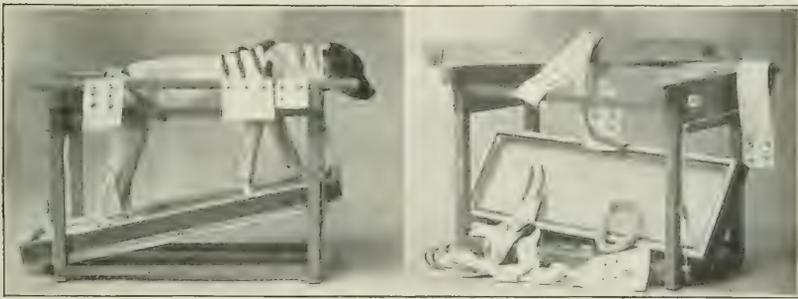
The apparatus consists of four movable parts on a frame: (1) a belt to be fastened around the chest of the animal, with an at-

<sup>1</sup> This apparatus was shown at the Scientific Exhibition under the auspices of the New York Academy of Sciences, held at the American Museum of Natural History, December, 1906.

<sup>2</sup> MEYER: *Journal of the American Chemical Society*, 1907, xxix, p. 906.

tached band to be buckled around the neck, in order to restrain the movements of the animal and to keep the head in a forward position; (2) a support for the hind legs, consisting essentially of two suitable padded rings into each of which a leg can be placed; (3) an adjustable horizontal bar on which parts 1 and 2 may be fastened at various positions and in different degrees of tension; (4) a shelf holding a tray, with wire grating and having an outlet, for the separation and retention of feces and the delivery of urine into a suitable retainer.

The accompanying illustrations give a general idea of the construction of the holder and its appearance when in use. The extreme



Improved animal holder.

dimensions, not including the tray, are: length, 89 centimetres; width, 41 centimetres; height, 50 centimetres. The cross beams (*A*) are 67 centimetres apart. The horizontal bars (*B*) are 89 centimetres long,  $6\frac{1}{2}$  centimetres wide, and  $2\frac{1}{2}$  centimetres thick, with bevelled edges. One of the horizontal bars is firmly fixed on the cross beams, at one side. The other can be moved upon the cross beams, and has a play of 20 centimetres. Two brass window catches, attached to the movable bar, and running in grooves on the cross beams, are used to fasten the adjustable bar in any permissible position. The grooves in the cross beams are reinforced by angle brass ( $\frac{1}{4} \times \frac{1}{8}$  inch).

Free to move between the legs which support the cross beams is a hanging shelf (*C*). This shelf may be raised or lowered into any position by means of a rope and pulley attachment. The shelf is designed to hold the zinc-lined tray, which is also movable, and is held in position on the shelf by means of a window catch. The

tray is provided with a readily removable wire screen. The parts of the tray may be quickly separated and thoroughly cleansed.

The device for keeping the animal in the holder consists of canvas harness specially designed for use in this connection, and referred to briefly above. The canvas (20 oz.), taken double, offers sufficient firmness, with pliability, to be particularly well adapted for this purpose. Leather could of course be substituted. The chest belt (*D*) is 47 centimetres long and 13 centimetres wide. Two flaps, each 20 centimetres long, are attached, one 10 centimetres, the other 15 centimetres, from the ends of the belt. Three straps and buckles fastened on the belt serve to tighten it around the chest of the animal. Extending forward from the chest belt is a strap. This is looped around the chest belt so that it may be readily centred into position or removed at desire. The strap is 53 centimetres long and 6 centimetres wide. On this strap the neck band (*E*) is fitted so as to slide back and forth. The neck band is 30 centimetres long, and is also provided with a set of straps and buckles.

Another attachment, not directly affixed to the harness, but which is placed on the support directly in front of the forelegs, is a plain strap (*F*) 7 centimetres wide and 50 centimetres long. Its purpose is to prevent the dog from placing his forefeet on the wooden support, as well as to offer a comfortable resting-place for the neck and head.

No particular difficulty was experienced in designing suitable harness for the head and shoulders of the animal. On the other hand, it was not an easy matter to hit upon a suitable arrangement for the hind quarters. It was desirable to have as little attachment as possible, but sufficient to keep the animal properly adjusted and supported. A pair of padded canvas rings, 45 centimetres in diameter, suspended on a band (*G*)  $2\frac{1}{2}$  centimetres wide at its central portion and 7 centimetres wide for some distance at its ends, and having a length of 50 centimetres, was found to serve the purpose desired. The rings are detachable, the ends being provided with clasp buttons for this purpose.

To attach the various sections of the harness to the horizontal bars, brass grommets<sup>1</sup> were affixed, 5 centimetres apart from centre to centre, at the ends of the canvas pieces. Several rows of eyelets

<sup>1</sup> Brass grommets (size number one) and coach buttons to match may be obtained at any carriage supply house. A grommet punch is required to attach the grommets. These articles are handy accessories in any laboratory.

make it easy properly to adjust the harness. The horizontal bars are provided along their outer edges with brass coach buttons, with which the eyelets may be engaged.

To fasten the animal in the holder it is best first to buckle the chest and neck bands into position on the animal. The chest belt should be fastened firmly, but of course should not make the animal uncomfortable. The neck band should not be buckled tightly, for the head movements need be only moderately restricted. When the buckles on the straps referred to have been properly adjusted, it is a simple matter to sling the ends over the bars and fasten the grommets on the buttons. The rings for the hind legs can now be put into position. There still remains the adjustment of the horizontal bars, which, when properly effected, tends to give the whole supporting apparatus a proper tension, and holds the animal securely and comfortably. Finally the shelf should be so adjusted that the paws of the animal barely touch the wire screen on the tray.

As already mentioned, the holder is adjustable to dogs of various sizes. The harness encircling the chest and neck is not apt to become soiled if proper care is taken. The holder has served very well for experiments on dogs with gastro-intestinal or biliary fistulas. No undue pressure is exerted in the proximity of the wounds in such animals, and there is no provocation of special exudation. In experiments on dogs with esophageal fistulas the neck band may be discarded, and soiling of the chest strap can be prevented by spreading several layers of cloth on it.

Besides being of use in experiments on animals with fistulas, the holder has proved constantly of service for restraining animals for many other purposes, for example, during injections, feeding by means of a stomach tube, introduction of enemata, etc. The holder is especially convenient for supporting animals firmly in vertical positions. The holder with the animal in it may be turned on either end, in which positions the animal is unable to throw the apparatus over or to change its own posture in it.

# THE ELIMINATION OF RADIUM FROM NORMAL AND NEPHRECTOMIZED ANIMALS.<sup>1</sup>

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## INTRODUCTION.

**D**URING the past fifty years numerous studies of the fate of foreign substances after their introduction into the body have shown conclusively that the kidney is not the sole organ of elimination for such substances. Metallic and other elements, as well as complex organic compounds, if given by mouth or injected into the circulation, pass from the organism not only by way of the kidneys, but may be excreted by the digestive organs, by the skin, in the lachrymal or other secretions, or by various additional routes.

Thus the large number of investigations of the fate of iron in man and other animals, no matter in what form or by what channel it may have been administered, have shown that the large intestine is practically the only channel of elimination for this element. The same was shown for manganese and certain other heavy metals whose elimination has been studied. Somewhat similar data have been obtained for the alkali earth metals. Thus, calcium, strontium, and magnesium, although eliminated in the urine after their injection, are found in much larger quantities in the contents of the intestines. Various other elements, which are excreted chiefly by the kidneys,

<sup>1</sup> The fifth paper in a series of researches with radium inaugurated by Dr. WILLIAM J. GIES in this laboratory several years ago. See (1) BERG and WELKER: *Journal of biological chemistry*, 1906, i, p. 371; (2) BURTON-OPITZ and MEYER: *Journal of experimental medicine*, 1906, viii, p. 245; (3) MEYER: *Journal of biological chemistry*, 1906, ii, p. 461; (4) HUSSAKOF: *Medical record*, 1907, lxxii, p. 89. See also a recent report of an unfinished research in the series, by GAGER: *Science*, 1907, xxv, p. 462.

<sup>2</sup> Fellow of the Rockefeller Institute for Medical Research.

<sup>3</sup> A preliminary report appeared in *Science*, 1907, xxv, p. 459.

are, to some extent, also eliminated by the intestinal epithelium. The work of Good<sup>1</sup> has shown that lithium chloride injected hypodermically into cats and dogs, although largely excreted in the urine, is also found in the saliva as well as in the stomach and in the intestinal contents. Hanford<sup>2</sup> found that cesium is mainly eliminated by the kidneys, but it was also detected, after subcutaneous as well as after intravenous injections, in the saliva, in the contents of the stomach, in the contents of the intestine throughout its entire length, and also in the bile. Likewise, rubidium, which leaves the body mainly in the urine, was found by Mendel and Closson<sup>3</sup> in small quantities in the feces. Such examples might be multiplied.

That the gastro-intestinal wall also serves as an organ for the elimination of complex organic bodies has been demonstrated in the cases of various substances, such as morphin, quinin, atropin, curarin, snake venom, and other toxic compounds of biological origin. These particular substances seem, according to several investigators, to be eliminated by the gastric epithelium as well as by the kidney.

That the bile is an important channel of elimination of various substances introduced into the body has been shown by several workers. After the intravenous injection of albuminate of zinc into cats, dogs, and rabbits, Michaelis<sup>4</sup> found zinc in the bile of these animals. Mosler<sup>5</sup> detected iodine in the bile of dogs eight hours after the administration of potassium iodide. He obtained the same results when he gave potassium iodide to a patient with a biliary fistula. Wichert<sup>6</sup> studied the elimination in the bile of a large number of elements when introduced into the body. Potassium iodide or potassium bromide was given by mouth to cats and dogs. After a short time the bile of these animals showed the presence of iodine or bromine respectively. Similar results were obtained with antimony, nickel, bismuth, lead, silver, and arsenic. In this connection may be mentioned the experiments of Mead and Gies<sup>7</sup> in this laboratory with tellurium. According to these investigators this element is eliminated not only by the kidneys, but also by the liver, and even by the

<sup>1</sup> GOOD: *American journal of the medical sciences*, 1903, cxxv, p. 273.

<sup>2</sup> HANFORD: *This journal*, 1903, ix, p. 235.

<sup>3</sup> MENDEL and CLOSSON: *This journal*, 1906, xvi, p. 152.

<sup>4</sup> MICHAELIS: *Archiv für physiologische Heilkunde*, 1851, x, p. 127.

<sup>5</sup> MOSLER: *VIRCHOW'S Archiv*, 1858, xiii, p. 29.

<sup>6</sup> WICHERT: *Dissertation*, Dorpat, 1860, xiii, p. 29.

<sup>7</sup> MEAD and GIES: *This journal*, 1901, v, p. 104. Also Gies and collaborators: *Biochemical Researches*, 1903, i, Reprint No. 21.

lungs. Organic bodies of complex chemical constitution may likewise be eliminated in the bile. According to Brauer,<sup>1</sup> methylene blue, when given by mouth, is excreted into the bile. Meyer<sup>2</sup> has lately carried out in this laboratory a large number of experiments with various anilin dyes. All of them were eliminated in the bile.

It is evident, therefore, from the foregoing very general *résumé* that foreign substances, if introduced into the body, may be ejected through many exits.

The results of the recent work here with barium by Berg and Welker,<sup>3</sup> and with radium and anilin dyes by Meyer,<sup>4</sup> led us all in this laboratory to believe that various conclusions pertaining to the paths of elimination of certain substances, inorganic compounds particularly, have been jumped at too hastily by a number of workers. Thus, Meyer<sup>5</sup> has lately drawn attention to what appears to be an unwarranted deduction by Mendel and Sicher<sup>6</sup> on the excretion of barium in the urine,—unwarranted because the experimental data offered by Mendel and Sicher failed to support it. With regard to the elimination of foreign substances through the intestinal mucosa, it seems that in many cases the experimental evidence did not support the announced positive conclusion to that effect. In some of the instances alluded to, such a conclusion appears to have hinged on the assumption that the presence of a given substance in the intestinal contents indicates the excretion of that substance through the intestinal wall,—a deduction that is assuredly fallacious in view of the fact that metallic and other elements have been detected in such gastro-intestinal streams as saliva, bile, and pancreatic juice. Hence the excretory function of the intestine can be accurately determined in a particular case only after the passage of all such secretions into the intestines has been prevented. Meyer is at present engaged in such an investigation of the excretion of barium, and the companion study described in this paper is a further outcome of our feeling here that these questions need more thorough experimental study than many of them have received.

Although evidence is not wanting that the intestines are capable

<sup>1</sup> BRAUER: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 182.

<sup>2</sup> MEYER: *Journal of the American Chemical Society*, 1907, xxix, p. 892.

<sup>3</sup> BERG and WELKER: *Journal of biological chemistry*, 1906, i, p. 371.

<sup>4</sup> MEYER: *Loc. cit.* Also, *Journal of biological chemistry*, 1906, ii, p. 461.

<sup>5</sup> MEYER: *Journal of biological chemistry*, 1906, ii, p. 474.

<sup>6</sup> MENDEL and SICHER: *This journal*, 1906, xvi, p. 147.

of eliminating various foreign substances introduced into the body, the need of additional data obtained in the way suggested above is very desirable. Moreover, since therapeutic measures, based on the assumption that the gastro-intestinal mucosa eliminates foreign substances that are of no use to the organism, are frequently resorted to by clinicians, a study of elimination through the stomach as well as through the bile was also desirable. On account of the facility with which very minute amounts may be sharply detected in the tissues and fluids of the body, Professor Gies suggested the use of radium for the study of our problem.

#### EXPERIMENTAL.

**Methods.**— Our experiments were carried out on dogs and rabbits under ether narcosis. A permanent biliary fistula was established in one dog (Experiment 2). In all the other animals bile was obtained from temporary fistulæ. To prevent the passage of saliva into the stomach and intestines during an experiment, a ligature was placed at the cardiac end of the stomach. Ligatures were likewise placed at the pylorus, and below the opening of the duct of Wirsung, also at the junction of the large and small intestines in the dog. In each rabbit the upper and lower ends of the cecum were closed by ligatures.

In each experiment radium bromide (1000 activity), in aqueous solution, was injected *subcutaneously*. Bile and urine were usually collected. The contents of the different sections of the gastro-intestinal canal were carefully removed at the end of an experiment, and examined for radium by the quadrant electrometer. A detailed description of methods for the detection of radium in animal tissues and fluids has already been described by Meyer.<sup>1</sup> The same methods were employed in this investigation.

*Experiment 1.* Dog; weight, about 10 kilos. 10 mg. of radium bromide were injected. About two hours later the dog was killed. The bile collected from the gall bladder was radioactive.

*Experiment 2.* Dog; weight, about 10 kilos. A permanent and complete gall bladder fistula was established on June 11, at 4 P. M. The dog made a very good recovery from the operation. On June 18th the stitches were removed, and the fistula was apparently well healed. During the collection of bile the dog was placed in the holder described in the preceding paper.

<sup>1</sup> Meyer: Journal of biological chemistry, 1906, ii, p. 462.

June 25th, 2.30 P. M. 30 mg. of radium bromide were injected into the left leg. The bile obtained several hours later was radioactive. Urine and feces collected separately were likewise radioactive.

June 27th, 4 P. M. The dog was killed by chloroform narcosis. The stomach was found empty. Small pieces of the gastric wall were removed for examination. The contents of the intestines as well as small pieces of the wall of the intestines were also separately tested for radium. The results of the examination showed that neither the stomach nor the intestines were radioactive. The contents of the intestines, however, were radioactive.

*Experiment 3.* Female dog; weight, 8 kilos. 10.30 P. M.: ether anesthesia. The stomach was ligated at the cardiac and pyloric ends. The small intestine was ligated at some distance below the opening of the common duct. The large intestine was ligated at the junction of cecum and appendix. The neck of the gall bladder was clamped and a cannula placed in the common bile duct. 11.15 A. M.: 20 mg. of radium bromide were injected into the right leg. 11.15-1.15 P. M.: 10 c.c. bile were collected. 1.15-5 P. M.: 7 c.c. bile were collected. Urine was removed from the bladder at 5 P. M. The dog was killed at 6 P. M.

Both samples of bile as well as the urine were radioactive. The stomach and contents separately examined failed to show the presence of radium. The contents of the small intestine included between the pylorus and the opening of the duct of Wirsung were active; the wall of this part of the intestine was inactive. The record of the test for radioactivity of the remaining portion of the small intestine was unfortunately lost. The contents of this portion proved to be radioactive. The large intestine as well as the contents separately examined were both inactive. The blood was also examined and proved to be radioactive.

These experiments show, therefore, that in the dog the liver as well as the kidney is able to eliminate radium. The behavior of the gastro-intestinal tract is especially worthy of remark in this connection. Neither the stomach nor the large intestine excreted radium. Elimination of this element seemed to take place, however, all along the small intestine.

The advisability of studying elimination in the rabbit now suggested itself, since it has been shown by Noel Paton and Bergmann that herbivora behave differently from carnivora in respect to elimination. Thus Paton's<sup>1</sup> experiments with phosphoric acid have shown that none of it is excreted in the urine of goats, whereas

<sup>1</sup> PATON, N., DUNLOP and AITCHISON: *Journal of physiology*, 1900, xxv, p. 212.

Bergmann<sup>1</sup> made the interesting observation that in dogs phosphoric acid is almost entirely excreted by the kidney, while in sheep elimination takes place through the intestines.

*Experiment 4.* Female rabbit; weight, 2.2 kilos. 11 A.M.: ether narcosis.

A cannula was placed in the common bile duct. Ligatures were attached as follows: Above the cardiac and immediately below the pyloric ends of the stomach, below the duct of Wirsung, above and below the cecum. At 12 M., 10 mg. of radium bromide were injected into the right leg. Bile was collected during the following periods:

I	12-1	P. M.	10	C.C.
II	1-4	"	15	"
III	4-8.30	"	14	"
IV	8.30-9	A. M.	15	"
Urine obtained at	1	P. M.	10	"
	4	"	10	"

At 9 A.M. the next morning the rabbit was found dead. 15 c.c. of bile were secreted after 8.30 P.M. the previous day. The stomach and intestines were then removed from the body of the animal and carefully washed free from all adherent blood. The contents of the various portions of the gastro-intestinal canal were washed into clean casseroles, and in each case thoroughly mixed before portions of them were placed in trays in preparation for the tests for radium. The results of the examination were the following:

Bile, sample I,	Radioactive
" II,	"
" III,	"
" IV,	"
Urine, sample I,	Not "
" II,	Radioactive
Stomach,	Radioactive
Contents of " ,	Not radioactive

The part of the small intestine included between ligatures placed at the pylorus and beyond the duct of Wirsung was not radioactive. The contents of this part were radioactive. The rest of the small intestine as well as the contents separately examined were radioactive. Neither cecum nor contents were radioactive. The large intestine was inactive, while the contents were radioactive.

<sup>1</sup>BERGMANN: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvii, p. 77.

*Experiment 5.* Male rabbit (gray); weight, 2 kilos. Ether narcosis. Cannula in the common bile duct. Ligatures were placed as follows: At the cardiac and pyloric ends of the stomach, and above and below the cecum. The bladder was emptied. 15 mg. radium bromide were then injected into the left leg at 1 P. M. Bile was collected as follows:

1-2	P. M.	8 c.c.
2-5.15	"	11 "

At 9 A. M. next day the rabbit was found dead. 12 c.c. of bile were secreted after 5.15 P. M. of the previous day. The stomach and intestines were removed from the body of the animal and treated as described above.

The bile as well as the urine collected at the end of the first hour after the injection of radium bromide was only slightly radioactive. Radioactivity of the bile and urine secreted during the next three hours was, however, very marked. Another sample of each of these secretions obtained before the death of the animal was only slightly radioactive. The stomach as well as its contents, separately examined, failed to show any radioactivity. Examination of the small intestine for radium likewise proved negative, but its contents were markedly radioactive. Neither cecum nor large intestine showed radioactivity, while the contents in both cases were only slightly radioactive. The blood which was obtained from the heart was also tested. The results were negative.

Analysis of the data obtained in our experiments with rabbits indicates that in these animals elimination of radium invariably takes place through the liver, the kidneys, and the small intestines, after its introduction into the circulation. Moreover it is worthy of remark in this connection, that the liver and the kidneys are apparently equally efficient as organs for the excretion of radium, since, as was shown in experiment 5, the elimination of this element began in both organs at about the same time and probably continued for equal periods. Quite different was the behavior of the stomach, the cecum, and the large intestine with regard to the excretion of radium. In neither of the two rabbits experimented on was there any indication that radium had been eliminated through the stomach. Although the wall of the stomach was radioactive, the contents of the stomach failed to show the presence of this element. The cecum presented some variation in this respect. Radioactivity of the contents in one rabbit was slight, but was altogether absent in the other,—an indication that this part of the intestine, at least in normal rabbits, is variable in its activity as an organ for the elimination of radium. The large intestine of the

rabbit seems likewise to vary somewhat in this regard in different individuals. Thus, while radioactivity of the contents of this part of the intestines was very marked in one rabbit, it was slight in the other, suggesting that this part of the gut does not eliminate radium with equal facility in all rabbits. If the results of our observations on dogs and rabbits are now compared, the following interesting resemblances and differences in the manner of elimination of radium in these animals may be seen.

By referring to the table on page 376 we see that the liver as well as the kidney participated in the elimination of radium in the dogs and rabbits. The gastro-intestinal canal, however, exhibited differences in this connection. While the stomach in each of the animals failed to excrete radium, elimination invariably took place through the wall of the small intestine. The large intestine, on the other hand, acted dissimilarly in the dog and rabbits. Thus, in the dog the portion of the intestine which, it will be remembered, is the organ for elimination of iron, manganese, and certain other elements, did not excrete radium. In the rabbits, on the contrary, elimination through the large intestine did take place, although the rate of elimination seemed to vary in different individuals. If deductions based on the evidence presented on the foregoing pages are warranted, we may conclude that the elimination of radium takes place chiefly through the liver, the kidneys, and the small intestine, and, to a lesser extent also, through the large intestine in some of the herbivora.

At this stage of the investigation it occurred to us that a study of the elimination of radium from nephrectomized rabbits would be very desirable. Although considerable experimental evidence has accumulated to indicate that even when the function of the kidney is unimpaired, the elimination of foreign materials through the digestive tract and the fluids entering it, may take place, the study of the passage of substances through these channels in disease of the kidney or when the urinary passages have been blocked, has made but little progress. The few available experimental data on this subject suggest, however, that organs other than the kidney may by way of compensation eliminate foreign substances from the body under such conditions. Claude Bernard<sup>1</sup> in his experiments with potassium ferrocyanide has shown that this substance which is normally excreted through the kidney may pass into the saliva if the renal arteries or the ureter

<sup>1</sup> BERNARD, CLAUDE (quoted by ACHARD et LOPEZ): Séances et memoires de la Société Biologique, March 15, 1902.

have been ligated. In this connection may also be mentioned the fact that in advanced cases of nephritis, urea may be eliminated through the digestive tract, the lungs, the skin, and in the lachrymal and other secretions. It was of interest, therefore, to study whether those parts of the gastro-intestinal canal, such as the stomach and cecum, which fail to excrete radium in normal rabbits, might eliminate this element if the kidneys were removed. Our experiments were carried out on two healthy, full-grown rabbits on which the operation for double nephrectomy was performed under ether narcosis and elimination of radium studied in the way indicated in the previous experiments.

*Experiment 6.* Male rabbit; weight, 2.24 kilos. Both kidneys were removed by the abdominal route, and a cannula was introduced into the common bile duct immediately afterward. Ligatures were placed at the cardiac end of the stomach, around the duodenum, immediately below the pylorus, at the junction of the cecum and small intestine, and at the junction of cecum and large intestine. 10 mg. of radium bromide were then injected into the left leg. The bile collected during a period of thirty minutes after the injection of radium was not radioactive. The next sample collected during the succeeding hour was active. A third sample, obtained nine hours after the administration of radium bromide, was also active. The rabbit died ten hours after double nephrectomy had been performed. The stomach and the various parts of the intestines were carefully taken from the body of the animal, washed free from adherent blood, the contents removed and put into zinc trays, which were treated as previously described. In each case the organ and its contents were separately examined for radium.

The tests of the various parts of the digestive tract for radium have shown the following results: Stomach as well as contents were slightly radioactive. Both the small intestine and its contents were also radioactive, whereas the cecum as well as its contents failed to show the presence of radium. The large intestine and contents were both radioactive. The results obtained show therefore that elimination through the liver is not accelerated as a result of removal of both kidneys, for the bile obtained during a period of thirty minutes after radium was administered was not yet radioactive. The results obtained with stomach contents, on the other hand, show that there is a tendency to vicarious elimination, for the contents were slightly radioactive. In this regard the intestines behaved the same as those of normal rabbits. The contents of both the large and small intestines showed the presence of radium, whereas the contents of the cecum, as in both normal rabbits, were free from radium.

*Experiment 7.* Female rabbit; weight, about 2.5 kilos. Double nephrectomy was performed by the abdominal route. Ligatures were placed at the cardiac and pyloric ends of the stomach, at the junction of small intestines and cecum, and at the junction of large intestines and cecum. A cannula was introduced into the common bile duct. At 12.35 P. M. 10 mg. of radium bromide were injected. Bile removed at 1.10 P. M. was not radioactive. Another sample obtained between 1.10 and 2.10 P. M. was radioactive. At 5.30 P. M. the rabbit was bled to death. The bile removed shortly before the death of the animal was still radioactive. The various sections of the gastro-intestinal canal were removed and treated as in the other experiments. Tests for radium showed that neither stomach nor contents were radioactive. The small intestine was likewise inactive, but its contents were radioactive. Examination of the large intestine as well as the contents gave in both instances negative results. In this experiment, therefore, the stomach failed to assume any vicarious function. Moreover, the large intestine, which in all the other rabbits eliminated radium, has in this case behaved in a rather anomalous manner. Contrary to expectations, the excretion of radium was entirely inhibited.

The results of our experiments with nephrectomized rabbits do not answer the question whether the vicarious elimination of radium might be expected to occur in most rabbits. The recent work of Meltzer and Lucas<sup>1</sup> furnishes strong evidence, however, that the gastro-intestinal canal fails to eliminate more readily, after the removal of the kidney, certain substances introduced into the body. Thus, when magnesium sulphate was administered to nephrectomized rabbits, in much smaller doses than those given without special effect to normal rabbits, deep anesthesia was produced. It is probable, therefore, that while vicarious elimination may take place through the walls of the alimentary canal after extirpation, or in diseases, of the kidneys, in the cases of various organic substances produced within the body as a result of metabolism, or when introduced from without, it apparently fails to occur in the cases of certain inorganic substances, which normally leave by other channels. It is quite possible that the liver and small intestine, which normally excrete radium, suffice to meet this need of the body after removal of the kidney.

Finally, we desire to call attention to the study of elimination in the urinary bladder. Although some experimental evidence on absorption from the bladder exists, we could not find any reference in the litera-

<sup>1</sup> MELTZER and LUCAS: Proceedings of the Society for Experimental Biology and Medicine, 1906, iv, p. 10.

ture to an investigation bearing on excretion through this organ. It seemed to us, therefore, that the question ought to be put to an experimental test. Our results indicate that radium was not excreted through the wall of the urinary bladder.

A summary of our results for radioactivity is appended.

TABLE GIVING THE RESULTS FOR RADIOACTIVITY OF VARIOUS PARTS IN EXPERIMENTS 2-7.

Experiment No.	Normal dogs.		Normal rabbits.		Double nephrectomized rabbits.	
	2	3	4	5	6	7
Stomach . . .	Inactive	Inactive	Active	Inactive	Slightly active	Inactive
Stomach contents	....	Inactive	Inactive	Inactive	Slightly active	Inactive
Intestine . . .	Inactive					
Contents of the intestine . .	Active					
Bile . . . . .	Active	Active	Active	Active	Active	Active
Feces . . . . .	Active					
Blood . . . . .	Active	Active	....	Inactive	Slightly active	
Kidney . . . .	Active					
Urine . . . . .	Active	Active	Active	Active		
Small intestine .	....	Inactive	Active	Inactive	Active	Inactive
Contents small intestine . .	....	Active	Active	Active	Active	Active
Large intestine .	....	Inactive	Inactive	Inactive	Active	Inactive
Contents large intestine . .	....	Inactive	Active	Slightly Active	Active	Inactive
Cecum . . . . .	....	....	Inactive	Inactive	Inactive	Inactive
Contents of cecum . . . . .	....	....	Inactive	Slightly active	Inactive	Inactive

#### SUMMARY OF CONCLUSIONS.

In dogs and rabbits the kidney, the liver, and the small intestine eliminate radium. In normal rabbits elimination also takes place through the large intestine. The passage of radium through the wall of the large intestine is probably slower than through the wall of the small intestine. Elimination of radium into the cecum of the rabbit is slight and in some individuals may fail altogether.

In a nephrectomized rabbit the elimination of radium takes place through the small intestine and through the liver. The rate of radium excretion from the livers of rabbits is not affected by removal of the kidneys. In a nephrectomized rabbit elimination of radium through the large intestine is uncertain; in such a rabbit radium is not eliminated through the cecum, but may pass through the stomach wall.

The channels of elimination for radium appear to vary in different species and in individuals of the same species.

After removal of both kidneys from a rabbit there is no compensatory elimination of radium through those parts of the digestive tract which are not concerned in its elimination when the kidneys are intact.

We are indebted to Prof. William J. Gies for numerous suggestions.

# ON THE CHEMICAL NATURE OF PARANUCLEO- PROTAGON, A NEW PRODUCT FROM BRAIN.

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## INTRODUCTION.

TWO years ago Posner and Gies published numerous results of an extended study of brain protagon.<sup>1</sup> They concluded that protagon, whether prepared by the classical methods or by Cramer's coagulation process, was without physical or chemical definiteness, *i. e.*, that it was merely a *mixture* of substances. They made it evident, also, that, even with the use of exactly the same method of preparation, products of particularly divergent composition were

<sup>1</sup> POSNER and GIES: Journal of biological chemistry, 1905, i, p. 59.

obtained, when the physical conditions attending the brain extractions were slightly modified.<sup>1</sup>

Among the deductions drawn by Posner and Gies from their proof of the heterogeneity of protagon was one pertaining to Ulpiani and Lelli's paranucleoprotagon.<sup>2</sup> They said regarding the latter:<sup>3</sup>

<sup>1</sup> Shortly after the conclusion of the experiments described in this paper, LOCHHEAD and CRAMER published a few results of a study "On the phosphorus percentage of various samples of protagon," which led them to conclude, contrary to the recent deductions by POSNER and GIES (*loc. cit.*), "that protagon is an individual substance of a well-defined chemical composition" [Bio-chemical journal, 1907, ii, p. 350 (June 20)]. One looks in vain, however, through the paper by LOCHHEAD and CRAMER, for evidence warranting the conclusion that protagon is *not* a mixture. On the contrary, their results seem to support unmistakably the position taken by POSNER and GIES. Thus, on recrystallization, all the products except one that LOCHHEAD and CRAMER referred to, lost phosphorus in marked degree after each such treatment. When the phosphorus contents of their protagon products were lowered by recrystallization to the percentage amount that appeared to them to be about right, however, they arbitrarily discontinued in each case the recrystallization process, in spite of the fact that repetition of it promised to decrease further the proportionate contents of phosphorus. Furthermore, they used the process of recrystallization from *glacial acetic acid* as a method of purifying one of the protagon products that seemed to be among their best, apparently without being aware of the very significant fact that KOCH has employed the same method to remove from crude protagon every trace of phosphorus-containing substance or substances, prior to the ultimate isolation of one of the leading compounds contained in the protagon mixture, viz., phrenosin (pseudocerebrin, cerebrin, cerebrin). (See GIES: Journal of biological chemistry, 1906, ii, p. 159.)

The foregoing remarks in this footnote are made by us on the assumption that the products analyzed by LOCHHEAD and CRAMER were sufficiently like typical protagon to warrant the term protagon as the designation of them. LOCHHEAD and CRAMER appear to be satisfied on this point, but the reader of their paper will find no evidence in it of any effort on their part to prove the validity of their new methods for the preparation of protagon. Thus, the only data that were presented regarding "protagon F," for example, and which LOCHHEAD and CRAMER appeared to think showed that it was protagon, were the facts that it was white, that it contained 1.18 per cent of phosphorus, and that it had been obtained from brain by extraction with ether, not one of which characters is a *differential* quality of protagon. We believe the paper by LOCHHEAD and CRAMER has confused the situation without adding anything material to the facts in the case. The results of a critical examination of LOCHHEAD and CRAMER's paper was published by GIES in the last number of the Journal of biological chemistry (iii, p. 339.)

<sup>2</sup> ULPIANI and LELLI: *Gazetta chimica italiana*, 1902, xxxii, p. 466.

<sup>3</sup> POSNER and GIES: *Loc. cit.*, p. 109.

"The new proteid, paranucleo-protagon, that was described by Ulpiani and Lelli (1902) as a definite substance, must be regarded as a mixture of products. It is our intention to begin at once an inquiry into this matter."

The intention to make an experimental study of paranucleoprotagon could not be gratified until quite recently. Meanwhile no one appears to have confirmed or contradicted the conclusions of Ulpiani and Lelli, nor have the discoverers of paranucleoprotagon recorded any further observations regarding it that we know of. The following digest of Ulpiani and Lelli's paper<sup>1</sup> presents the essentials of the recorded data pertaining to paranucleoprotagon.

#### REVIEW OF THE REMARKS AND THE WORK OF ULPIANI AND LELLI ON PROTAGON AND PARANUCLEOPROTAGON.

**Ulpiani and Lelli on protagon.** — Ulpiani and Lelli stated that they made extensive studies of protagon, during the course of which they came to the conclusion that protagon did not exist in the brain in a free state, but occurred there united with a protein substance, which complex compound, after long and arduous endeavor, they finally isolated and separated by cleavage with alcohol into its (chief?) components, paranuclein and protagon, and accordingly named paranucleoprotagon.<sup>2</sup> They referred to Walter's<sup>3</sup> supposition that ichthulin, obtained by him from carp eggs, was a protein-protagon compound, but also alluded to Walter's inability to show that protagon was contained in it.

Ulpiani and Lelli stated that in their studies of protagon they prepared that material by the Gamgee and Blankenhorn process, and also remarked that they obtained results in that connection which were in close agreement with those published by Gamgee and Blankenhorn.<sup>4</sup> It was their study of protagon obtained by this method and

<sup>1</sup> ULPIANI and LELLI's paper was published in Italian (*loc. cit.*), and no complete review of it has ever appeared. For this reason, probably, their important paper has received comparatively little attention. For the same reason our review of it here will be more detailed than would be desirable otherwise.

<sup>2</sup> Their paper was issued in 1902, a long time after the publication of WÖRNER and THIERFELDER'S (*Zeitschrift für physiologische Chemie*, 1900, xxx, p. 542), but shortly before the appearance of the paper by LESEM and GIES (*This journal*, 1902, viii, p. 183; also GIES and collaborators: *Biochemical researches*, 1903, i, Reprint No. 11).

<sup>3</sup> WALTER: *Zeitschrift für physiologische Chemie*, 1891, xv, p. 477.

<sup>4</sup> No data were given. They said nothing about phrenosin (pseudocerebrin) in this connection, and did not appear to be acquainted with GAMGEE'S second

the observation that their protagon products dissolved in chloroform, and could be precipitated<sup>1</sup> therefrom with ethyl acetate, acetone, or alcohol, that led them to use chloroform for the purpose of extracting protagon from brain, and that resulted finally in their unexpected discovery of paranucleoprotagon, the compound in which, according to Ulpiani and Lelli, all the protagon that occurs in the brain is combined.

Ulpiani and Lelli's remarks on protagon, and on their experiments with it, lead one to think that they simply skimmed over the surface of the history and facts relating to protagon<sup>2</sup> and accepted without question the accumulated mass of superficial statements regarding it. As usual, there was the conventional allusion to Thudichum for his obstinacy in adhering to his well-founded conviction, on the basis of the results of his well-ordered experiments, that protagon was a mixture of substances. The important work of Wörner and Thierfelder on phrenosin (cerebron), which gave strong evidence of the heterogenous nature of protagon, was ignored. Ulpiani and Lelli stated that protagon was without doubt a definite chemical individual, but they offered no new facts bearing on the question.

contribution on the subject. Evidently, like GAMGEE and BLANKENHORN'S products, their protagons contained phrenosin (pseudocerebrin). See GIES: *Journal of biological chemistry*, 1906, ii, p. 168.

<sup>1</sup> Whether completely or not does not appear to have been determined.

<sup>2</sup> Thus, they laid special stress on their observation that protagon is soluble in chloroform, something they declare had not been noted before. Evidently they were not familiar with the well-known prior work of WÖRNER and THIERFELDER (*Zeitschrift für physiologische Chemie*, 1900, xxx, p. 542), in which chloroform was employed with alcohol to dissolve from protagon the constituents associated in it with phrenosin (cerebron) (GIES: *Journal of biological chemistry*, 1906, ii, p. 159). Phrenosin itself was found by WÖRNER and THIERFELDER to be soluble in hot chloroform, and it seems very probable that phrenosin is appreciably soluble, also, in *cold* chloroform solutions of the substances which, with phrenosin, compose protagon. ULPIANI and LELLI stated that protagon is precipitated from its chloroform solution by alcohol or acetone. WÖRNER and THIERFELDER used mixtures of alcohol-chloroform (50 per cent) to separate phrenosin from protagon by fractional precipitation from cooled extracts (made at 45-50° C., but in which not all the protagon dissolved). Chloroform-acetone solution was used for *purification* by recrystallization. That the significance of these and further observations by WÖRNER and THIERFELDER and others was not appreciated by ULPIANI and LELLI is apparent not only from the failure on their part to note the above-mentioned use to which the former investigators put chloroform, alcohol, and acetone in perfecting the mechanical separation of phrenosin from protagon, but also from various other oversights that become evident as one reads their paper.

**Ulpiani and Lelli's preparation of paranucleoprotagon.**—Paranucleoprotagon was prepared by Ulpiani and Lelli as follows: Horse brains,<sup>1</sup> mechanically freed from extraneous matter, were extracted<sup>2</sup> with chloroform.<sup>3</sup> Extraction at 45° C. favored stratification of the chloroform, without detrimental effect on the paranucleoprotagon;<sup>4</sup> at room temperature, however, the brain-chloroform mixture was too intimate to permit of ready mechanical separation.<sup>5</sup> The filtered extract<sup>6</sup> was treated with an equal volume of acetic ether, which caused the separation of an abundant precipitate (paranucleoprotagon, with impurities). This precipitate<sup>7</sup> was filtered off, extracted with ether,<sup>8</sup> filtered off again, and then extracted in a Soxhlet apparatus, first with ether and finally with chloroform.<sup>9</sup> The remaining product was dried *in vacuo* over sulphuric acid, pulverized and analyzed.<sup>10</sup> Single determinations of the percentage proportions of four elements

<sup>1</sup> Whether macerated or not was not made clear.

<sup>2</sup> Length of time was not indicated.

<sup>3</sup> Proportion was not mentioned.

<sup>4</sup> How this was ascertained the reader is obliged to guess.

<sup>5</sup> In the unstated proportions employed.

<sup>6</sup> Whether freed from the water layer or not was not specified.

<sup>7</sup> Its qualities were not referred to.

<sup>8</sup> Conditions were not stated.

<sup>9</sup> No explicit statement was made as to the exact purpose of this treatment, how long it was maintained, nor how thoroughly it was effected. Presumably it was intended to remove adherent cholesterin, etc., and was continued until no more substance could be dissolved out, but the reader finds nothing to reassure him upon either of these points.

The insolubility of paranucleoprotagon in chloroform at this point in the process is in marked contrast to the solubility of the product in chloroform at the beginning. ULPIANI and LELLI explain this by the assumption that the product was soluble in the original chloroform extract because of the presence there of lecithin, cholesterin, etc.,—in short, that paranucleoprotagon is soluble in chloroform solutions of lecithin, cholesterin, and similar bodies extractable from brain by chloroform, but is insoluble in pure chloroform. No experimental data were offered to substantiate this very plausible explanation, however.

<sup>10</sup> The physical properties of the substance, moist or dry, were passed over in complete silence by the authors. Whether it was white or black, pasty or fibrous, for example, appeared to be of no significance to ULPIANI and LELLI. That it could be pulverized when dry was the only hint as to its consistency. No statement was made by them regarding either the absolute or the proportionate quantity of paranucleoprotagon obtained from brain,—a particularly striking omission, in view of the assumption by ULPIANI and LELLI that all the protagon of the brain occurs there in the form of paranucleoprotagon.

were made in but one preparation of paranucleoprotagon,<sup>1</sup> the results of which are appended:

C	H	N	P
60.79	8.74	6.20	1.62

The product prepared in the manner indicated was called paranucleoprotagon, because Ulpiani and Lelli believed that it was a compound that yielded paranuclein and protagon, by cleavage with alcohol. Their method of preparing protagon from their paranucleoprotagon product is given briefly below.

Ulpiani and Lelli's cleavage process for the separation of paranuclein and protagon from paranucleoprotagon, and their new method for the isolation of protagon. — The dry paranucleoprotagon was treated with 85 per cent alcohol at 45° C., for the purpose of effecting cleavage of the product.<sup>2</sup> After cooling to 0° C., the mixture was filtered.<sup>3</sup> "After this treatment," said Ulpiani and Lelli, "the substance which at first was insoluble in chloroform became in large part soluble in that medium." Next they treated in a Soxhlet apparatus with chloroform, the matter that was insoluble in the cold (0° C.) alcohol, and obtained, in the cumulative chloroform extract,<sup>4</sup> the major part of the substance thus handled.<sup>5</sup> On treating the chloroform extract with acetic ether, a white substance was precipitated<sup>6</sup> which physically and chemically was regarded as being identical with protagon.<sup>7</sup> Analysis of the only product mentioned<sup>8</sup> by Ulpiani and Lelli gave the following percentage results:

C	H	N	P
66.67	10.45	2.64	1.38
66.67	10.54	2.51	1.24
Average . . 66.67	10.50	2.57	1.31

<sup>1</sup> There is no recorded evidence that ULPIANI and LELLI made more than one preparation of paranucleoprotagon.

<sup>2</sup> Nothing was said of the proportions of alcohol used, of the length of the period of treatment, or of the solvent effect.

<sup>3</sup> No statement was made as to the length of time the refrigeration process was maintained. The filtrate received no attention, and was apparently discarded; at least no information was given regarding anything contained in it.

<sup>4</sup> The portion in the receiver was kept hot, of course. The extraction was continued apparently as long as anything dissolved.

<sup>5</sup> The insoluble portion, "nuclein," is referred to farther on.

<sup>6</sup> Precipitates were also obtained with alcohol and acetone.

<sup>7</sup> What the filtrate contained was not suggested. There was no intimation that the filtrate received any attention.

<sup>8</sup> Whether dried to constant weight and how were not indicated.

The only additional chemical inquiry into the protagon-like qualities of this product was the subjection of the latter to hydrolysis for twenty hours in 7.5 per cent hydrochloric acid, with a determination of the presence of reducing material<sup>1</sup> among the cleavage products, — a reaction given by phrenosin or any cerebroside or cerebroside mixture, and not at all characteristic of protagon among brain educts.

Ulpiani and Lelli appear to have done nothing to assure themselves and the reader that protagon can be prepared by the method employed in isolating this product from what they call paranucleoprotagon.<sup>2</sup>

What is the percentage proportion of phosphorus in protagon?— The “protagon” obtained by Ulpiani and Lelli contained a comparatively large proportion of phosphorus, as may be seen from the figures in the summary on pages 386 and 387.

The general average of the figures on pages 386 and 387, excluding Ulpiani and Lelli's, is 1.07 per cent. It might be inferred from this that Ulpiani and Lelli's substance with 1.31 per cent of phosphorus could not have been protagon. A glance at the data given in the footnotes pertaining to the summary referred to makes it evident, however, that our average value for phosphorus content (1.07 per cent), although it agrees perfectly with the classical Gamgee and Blankenhorn figure, was derived arbitrarily by excluding all results for phosphorus content in protagon that, for one reason or another, departed fairly far from 1 per cent.<sup>3</sup> If, as Kossel and Freytag suggested, and as Cramer also thought, there were *many protagons* (homoprotagons, as Cramer proposed that they be designated) differing widely in composition, the average figure referred to, *i. e.*, 1.07 per cent, would be of little or no differential value; protagons might then contain much more or much less phosphorus than that (per cent) according to circum-

<sup>1</sup> Tested with Fehling's solution.

<sup>2</sup> The method employed by ULPIANI and LELLI for the isolation of protagon at this point is analogous to LOCHHEAD and CRAMER'S process for the preparation of their “protagon F,” which was extracted from brain with *boiling* chloroform, and, after cooling the extract, was precipitated from the latter by the addition of three volumes of ether: LOCHHEAD and CRAMER found, however, that their product contained 1.18 per cent of phosphorus, but learned nothing else chemically about it. See footnote, p. 379.

<sup>3</sup> We have done this, against our sense of the fitness of things, merely to repeat the method of CRAMER (and other supporters of the hypothesis that protagon is something definite), and then to show how misleading is such a one-sided presentation of the case, and how meaningless is the conclusion, based on it, that LOCHHEAD and CRAMER have recently drawn regarding the chemical individuality of their products. See footnote, p. 379 of this paper.

stances, and Ulpiani and Lelli's product might be (a) protagon, despite the decided disagreement of their figure for its phosphorus content with the average for the same in most protagons as already given (1.07 per cent). If, however, there is only one protagon, then it is obvious that we cannot tell who ever had a pure sample of it or what its percentage content of phosphorus is, for the data in the footnotes below the summary on the next two pages are quite as significant as the purely arbitrarily selected data to which preference has been shown by their placement in the table as particularly representative figures (following the fashion of most indiscriminating writers on subjects pertaining to protagon), in which event Ulpiani and Lelli have no evidence whatever, from their data for percentage phosphorus content, upon which to base their claim that their product with 1.31 per cent of phosphorus was protagon. But if protagon is always merely a mechanical mixture of substances, as we think must be conceded, the remarkable discordance among the figures given in the summary and corresponding footnotes (pp. 386-387), is an inevitable result of that state of invariable heterogeneity, and, under such conditions, Ulpiani and Lelli's figure for phosphorus content (1.31 per cent) implies as much or as little as any one else's, so far as protagon characters are concerned. It seems to us that, in the present state of our knowledge in this relation, *i. e.*, that mechanical fractions of protagons may contain from 0.1 per cent to about 2 per cent of phosphorus, an analytic result, within that range, for percentage content of phosphorus in a product is altogether too uncertain in significance to base upon it with confidence a claim that the product in question is or is not protagon.

It is significant that Kossel and Freytag obtained from an ether extract (containing considerably more lecithin and similar material than an alcoholic extract) a protagon product having a phosphorus content equal to 1.35 per cent — one apparently having a greater admixture of lecithins than that commonly present in the protagon obtained from an alcoholic extract. In their use of chloroform and ethyl acetate, for the isolation of protagon from paranucleoprotagon, Ulpiani and Lelli probably brought about a similar result.

**Ulpiani and Lelli's paranuclein obtained from paranucleoprotagon.**— Having separated completely what they thought was protagon from what they termed paranucleoprotagon, Ulpiani and Lelli concluded that that portion of their product which had been filtered from cold (0° C.) alcohol, and which likewise remained insoluble in the chloroform that dissolved away the liberated protagon, was a paranuclein.

## PERCENTAGE AMOUNTS OF PHOSPHORUS IN TYPICAL PROTAGON PRODUCTS.

Liebreich <sup>1</sup> . . . . .	1.10		Ruppel <sup>6</sup> . . . . .	1.13
Gamgee and Blankenhorn <sup>2</sup>	1.07		Chittenden and Frissell <sup>7</sup>	1.12
Thudichum <sup>3</sup> . . . . .	1.06		Zuelzer <sup>8</sup> . . . . .	1.01
Baumstark <sup>4</sup> . . . . .	1.05		Noll <sup>9</sup> . . . . .	1.16
Kossel and Freytag <sup>5</sup> . .	0.97		Gulewitsch <sup>10</sup> . . . . .	1.06

<sup>1</sup> LIEBREICH: *Annalen der Chemie und Pharmacie*, 1865, cxxiv, p. 29. One product obtained from human brain contained 1.5 per cent of phosphorus. Comparable results obtained by FRÉMY and others before LIEBREICH's publication are purposely omitted from the above table.

<sup>2</sup> GAMGEE and BLANKENHORN: *Zeitschrift für physiologische Chemie*, 1879, iii, p. 279. The authors obtained by recrystallization, after continuous warming in ether, one product containing only 0.72 per cent of phosphorus. This fall in percentage content was ascribed erroneously to decomposition, but was certainly due merely to physical fractionation of the mixture of products of which protagon is composed. Those who favor the hypothesis that protagon is a chemical individual never mention these points.

<sup>3</sup> THUDICHUM: *Annals of chemical medicine*, 1879, i, p. 258. THUDICHUM obtained by simple fractional recrystallization from 85 per cent alcohol and washing with ether, protagon products having as much as 2 per cent of phosphorus, and as little as 0.1 per cent of that element. The range of fluctuation in the proportions of phosphorus in the products obtained from protagon by THUDICHUM in his fractional recrystallization process, including those in mother liquors, was 0.1 per cent to 2.9 per cent. GAMGEE obtained from "impure" protagon, by repeated recrystallization from 80 per cent alcohol, a fraction containing only 0.08 per cent of phosphorus. This product was called pseudocerebrin (Gamgee: *A textbook of the physiological chemistry of the animal body*, 1880, i, p. 441).

<sup>4</sup> BAUMSTARK: *Zeitschrift für physiologische Chemie*, 1885, ix, p. 145.

<sup>5</sup> KOSSEL and FREYTAG: *Ibid.*, 1893, xvii, p. 431. The figure given above for phosphorus content is that commonly quoted for their "best" product, which also contained 0.51 per cent of sulphur. Another product had 1.06 per cent of phosphorus. A third contained 1.35 per cent of phosphorus and 0.88 per cent of sulphur. Those who support the idea that protagon is something definite chemically always ignore this evidence against that conception.

<sup>6</sup> RUPPEL: *Zeitschrift für Biologie*, 1895, xxxi, p. 86.

<sup>7</sup> CHITTENDEN and FRISSELL: *Science*, 1897, v (N. S.), p. 901 (Proceedings of the American Physiological Society, May, 1897). Frissell obtained results on subjecting his protagons to fractional recrystallization from 85 per cent alcohol at 45° C. that were similar to THUDICHUM's. CHITTENDEN assumed, however, that FRISSELL's results were due to "decomposition" of the protagon instead of to its mechanical partition, regardless of GAMGEE's previous statement that "pure protagon is remarkably rebellious to the action of even boiling alcohol, though that action be continued for hours." (GAMGEE: *A textbook of the physiological chemistry of the animal body*, 1880, i, p. 429.)

<sup>8</sup> ZUELZER: *Zeitschrift für physiologische Chemie*, 1899, xxvii, p. 255. One of the products had a phosphorus content of 0.72 per cent. The figure above was the only one for the product of the two referred to. ZUELZER regarded the product containing only 0.72 per cent of phosphorus as that of a "decomposed" protagon — merely a guess. Cramer might have mentioned this as one of the hypothetical "homoprotagons." See footnote 13, next page.

<sup>9</sup> NOLL: *Zeitschrift für physiologische Chemie*, 1899, xxvii, p. 376. The carbon content of NOLL's product was unusually high — 67.15 per cent. See footnote 13, next page.

<sup>10</sup> GULEWITSCH, cited by NOLL: *Ibid.*

PERCENTAGE AMOUNTS (*continued*).

Ulpiani and Lelli <sup>11</sup> . . . . .	1.31		Posner and Gies <sup>14</sup> . . . . .	0.93
Lesem and Gies <sup>12</sup> . . . . .	1.22		Lochhead and Cramer <sup>15</sup> . . . . .	1.01
Cramer <sup>13</sup> . . . . .	1.04			

<sup>11</sup> ULPIANI and LELLI: *Gazetta chimica italiana*, 1902, xxxii, p. 466.

<sup>12</sup> LESEM and GIES: This journal, 1902, viii, p. 183. Fractional recrystallization from 85 per cent alcohol at 45° C. yielded protagon products with phosphorus contents ranging from 0.12 per cent to 1.23 per cent. Fractional products in the mother liquors contained proportions of phosphorus ranging between 0.85 per cent and 2.59 per cent. The authors agreed with THUDICHUM and disagreed with CHITTENDEN in their interpretation of these results. (GIES and collaborators: *Biochemical researches*, 1903, i, Reprint No. 11.)

<sup>13</sup> CRAMER: *Journal of physiology* (English), 1904, xxxi, p. 31. CRAMER stated (p. 34) that in one specimen, which was prepared *in the same way as the others*, he got "different analytic results with regard to the percentage of carbon." Its carbon content was 64.7 per cent, that of the normal product was 66.3 per cent; its phosphorus content was 1.16 per cent, that of the normal product is given above. CRAMER added: "It is probable that this substance is another representative of the group of *protagon*s (!) assumed to exist by KOSSEL (see footnote 5, p. 386), which, in order to distinguish them from the protagon usually described and analyzed, might well be called homoprotagon<sub>s</sub>. . . . A substance belonging to this group has *only once been isolated and described*, by NOLL (*Loc. cit.*). It contained 67.15 per cent of carbon." In short, CRAMER would have it that, whether the product under examination has much more or much less carbon (per cent) than that in protagon, it is a "homoprotagon." On the other hand, the greater probability that protagon is nothing definite chemically, and that his "homoprotagon" was evidence in that direction, were ignored by CRAMER. If CRAMER's suggestion were accepted, the protagon products prepared by WÖRNER and THIERFELDER (*Zeitschrift für physiologische Chemie*, 1900, xxx, p. 543) and having carbon contents ranging from 62.37 per cent to 64.62 per cent, were "homoprotagon<sub>s</sub>." But each of WÖRNER and THIERFELDER's products yielded much phrenosin (cerebron) by simple fractional recrystallization. The proportion of phosphorus in their protagon<sub>s</sub> was not determined. However, a crystalline product was obtained from the mother liquor that remained after the isolation of some of the phosphorus-free phrenosin (cerebron), which contained an average of about 0.9 per cent of phosphorus, but the preparations of which showed wide fluctuations in composition. The melting-point was about 190° C. CRAMER's normal protagon melted at 192.5° C.

<sup>14</sup> POSNER and GIES: *Journal of biological chemistry*, 1905, ii, p. 59. The figure quoted above pertains to the phosphorus content of a product that had been recrystallized ten times from 85 per cent alcohol at 45° C. Two protagon products were obtained having contents of phosphorus equal to 1.7 per cent. On recrystallization, products were isolated from each having contents of phosphorus as low as 0.15 per cent, whereas products from the mother liquors ranged in phosphorus content between 0.8 per cent and 2.44 per cent. These results confirmed the conclusions of THUDICHUM and of LESEM and GIES, that protagon is a mechanical mixture.

<sup>15</sup> LOCHHEAD and CRAMER: *Bio-chemical journal*, 1907, ii, p. 350. The figure given above for phosphorus content represents the average of LOCHHEAD and CRAMER's four products of "highest purity." Recrystallization from the second to the third time resulted in reductions of phosphorus contents equal, for three products, to 0.17 per cent, 0.2 per cent, and 0.29 per cent, respectively. Fractional recrystallization of a protagon containing 1.22 per cent of phosphorus, yielded two products with 1.14 per cent and 1.05 per cent of phosphorus respectively. See footnote, page 379 of this paper.

This residue from which the supposed protagon had been separated by continuous extraction with chloroform was found to be insoluble in alcohol, ether, chloroform, and similar reagents, but was soluble in dilute alkali, from which it could be precipitated by acid added to excess. Dried over sulphuric acid *in vacuo* and analyzed, the recorded data for percentage elementary composition were the following:

	C	H	N	P
	54.19	7.71	11.57	1.86
	54.66	7.72	11.25	1.90
Average . .	54.43	7.71	11.41	1.88

The physical properties noted above for this residue, *vis.*, insolubility in chloroform, alcohol, etc., solubility in dilute alkali and precipitability from the latter by addition of excess of acid, together with the proportions of phosphorus and nitrogen revealed by analysis, led Ulpiani and Lelli to believe that the material in question was a nuclein or a paranuclein. In following up this idea they found that 1.6256 gm. of the product, kept for forty-eight hours in artificial gastric juice,<sup>1</sup> remained "unaltered."<sup>2</sup> Tested for purin bases by Kossel's method after cleavage with 10 per cent sulphuric acid, the results were negative, and Ulpiani and Lelli concluded that the material was paranuclein. It gave the biuret and Millon tests for protein, and yielded phosphate on cleavage with alkali.

**General deductions pertaining to Ulpiani and Lelli's paper.**—Ulpiani and Lelli concluded their paper with the general deduction that protagon is not found in the brain in a free state, but is combined there with a paranuclein as paranucleoprotagon. They dwelt on the important point that, whereas their paranucleoprotagon failed to yield protagon to chloroform during the first washing with chloroform,<sup>3</sup> it did yield it to chloroform, they thought, *after* the treatment with alcohol. They alluded to the lecitho-proteins in egg yolk, discovered by Hoppe-Seyler<sup>4</sup> and studied in some detail by Osborne and Campbell,<sup>5</sup> and concluded that the cleavage of paranucleoprotagon into paranuclein and protagon by alcohol was analogous to the similar severing influ-

<sup>1</sup> Temperature and strength were not indicated.

<sup>2</sup> Presumably it was undiminished in amount.

<sup>3</sup> No observations were recorded in the paper in this connection. Whether this was merely assumed or not is not clear.

<sup>4</sup> HOPPE-SEYLER: *Medicinischem-chemische Untersuchungen*, 1865, ii, p. 215.

<sup>5</sup> OSBORNE and CAMPBELL: *Journal of the American Chemical Society*, 1900, xxii, p. 416.

ence of alcohol on lecitho-nucleovitellins, with the consequent partition of the latter into lecithin and nucleovitellin. Ulpiani and Lelli stated that they repeated Hoppe-Seyler's experiments in this connection with egg yolk and obtained confirmatory results. They also tested the effect of preliminary extraction with chloroform in place of ether. They thoroughly extracted egg yolk in a Soxhlet apparatus two days with chloroform, in which lecithin dissolved readily. The residue was then treated with alcohol, which immediately removed much lecithin, *i. e.*, split off lecithin, and dissolved it.<sup>1</sup>

Before proceeding to the description of our own work in this connection the reader's attention should be concentrated upon the following general references to the statements and data in Ulpiani and Lelli's paper.

Their description of the method by which paranucleoprotagon was prepared is lacking in important details of procedure, so that it may be impossible, by following them as closely as their *general* directions permit, to obtain their quantitative yield of paranucleoprotagon (whatever it was), although *qualitatively* the product would probably be approximately the same, if paranucleoprotagon was a definite chemical individual and not a mechanical mixture.

The ordinary physical qualities of paranucleoprotagon were not mentioned. In repeating the work, as well as the meagre description allows, there is little or nothing in the remarks by Ulpiani and Lelli that would enable one to be sure that a perfect duplicate of their product had been obtained, or that would lead to its recognition from any special physical characters attributed to it.

They regarded protagon as being without doubt a single definite substance instead of the mixture it is now considered to be.<sup>2</sup>

In their effort to isolate the protagon-like cleavage product from paranucleoprotagon they used a new method, — extraction with chloroform and precipitation with acetic ether, after preliminary treatment with alcohol, — which they failed to show could be used satisfactorily to prepare protagon.

<sup>1</sup> The assumptions the reader is apparently expected by ULPIANI and LELLI to make here are that all free lecithin was removed before the treatment with alcohol and that the chloroform did not effect cleavage. These points were not established by evidence given in their paper.

<sup>2</sup> HALLIBURTON: *British medical journal*, May 4 and 11, 1907 (Oliver-Sharpey Lectures, April 29 and 30, 1907); also GIES: *Journal of biological chemistry*, 1907, iii, p. 339.

The protagon-like product that was isolated by them held more than the usual proportion of material containing much phosphorus, as the high figure for percentage amount of phosphorus in their "protagon" indicated. They did not show definitely that the product they called protagon was sufficiently like the classical protagons to deserve the name.

The statement by Ulpiani and Lelli that protagon does not occur free in the brain, but exists there combined with paranuclein, is not supported by any experimental evidence whatever. If correct, the statement implies that all the constituents of protagon are thus contained in the brain, in which event the yield of paranucleoprotagon must be relatively very large. Ulpiani and Lelli failed to mention the quantity obtained by them. Noll stated that about 20 per cent of the solid matter of the brain consists of protagon.<sup>1</sup>

Our own study of paranucleoprotagon was conducted in the way described below, where, it is hoped, sufficient details of procedure are given, without redundancy, to enable future students of this subject to repeat our work easily and exactly.

#### EXPERIMENTAL. PREPARATION OF PARANUCLEOPROTAGON.

**Method of extraction.** — Fifty sheep brains were finely minced in a hashing-machine, and the resultant hash treated in a large jar with about 10 litres of chloroform, which was added gradually. Intimate mixture was effected by thorough stirring frequently. The material was kept covered with a glass lid so as to prevent undue evaporation of chloroform. For thirty hours the mixture stood at room temperature, during which period the chloroform solution showed relatively little tendency to stratify. At the end of the time stated, the mass was thoroughly stirred once more, transferred to glass-stoppered bottles and kept in the latter at 45° C. for about twenty-four hours. This treatment caused perceptible flocculation of the previously viscid mixture and favored more decided separation of the chloroform solution. The mixture was filtered under cover over night in the ordinary way, but into closed vessels, and the residual fluid was expressed, filtered also, and added to the original filtrate. The liquid consisting of the combined filtrates is designated farther on as "the first chloroform extract."

The brain residue was returned to the bottles, and again treated with about 5 litres of chloroform, at 45° C., for sixty hours. Filtration, expression of the residual fluid, and filtration of the latter were effected as for

<sup>1</sup> NOLL: *Zeitschrift für physiologische Chemie*, 1899, xxvii, p. 384.

the first chloroform extract. The liquids comprising the combined filtrates at this point are designated below as "the second chloroform extract."

**Separation of crude paranucleoprotagon from the first chloroform extract.** — The first chloroform extract filtered readily, was clear, and, as it filtered, it stratified promptly into two layers. The chloroform portion, which was very slightly yellowish, was isolated with a separatory funnel, and treated gradually with a little more than an equal volume of acetic ether,<sup>1</sup> whereupon bulky flakes of a spongy, sticky, yellowish mass were immediately precipitated, which speedily sought the surface of the solution and collected there in a slightly tenacious layer. After about twenty-four hours the precipitate was skimmed from the solution, placed on a hard-paper filter, washed with chloroform-acetic ether (50 per cent each), transferred to a stoppered bottle, covered there with ether and thoroughly washed in fresh portions of the latter occasionally for several days. The substance did not harden particularly as a result of this treatment, and was apparently a mixture of grayish and yellowish materials. This crude product is referred to below as paranucleoprotagon A.

The watery portion of the first chloroform extract, from which the chloroform layer has been separated, did not yield a precipitate on treatment with acetic ether.

**Separation of crude paranucleoprotagon from the second chloroform extract.** — The second chloroform extract was like the first in general appearance. The watery proportion was less. Separation and treatment of this chloroform portion were conducted as they were for the first extract. Only an insignificant amount of substance was precipitated from the chloroform portion by addition of acetic ether even in large excess.<sup>2</sup>

The watery layer, as in the first instance, failed to yield a precipitate when treated with acetic ether.

The precipitate (paranucleoprotagon B) obtained from the chloroform portion of the second extract, while comparatively slight in amount, was apparently like that from the first chloroform extract. It was subjected to the same treatment as that accorded paranucleoprotagon A and was added to the latter in ether.

**Purification of the crude paranucleoprotagon products.** — Mixed paranucleoprotagon A and B, after the preliminary washing with ether, were subjected to continuous extraction with ether in a Soxhlet apparatus for two days. At the end of that time the last portion of ether that bathed the material yielded a very slight residue on evaporation, but all of it was readily soluble in chloroform. Continuous extraction with chloroform

<sup>1</sup> A larger proportion of the ether caused no further precipitation.

<sup>2</sup> If the brain mass had been merely washed with chloroform after the removal of the first extract, the second extract would have contained still less, probably none.

was next effected in the Soxhlet apparatus, for twenty-four hours, at the end of which time the last portion of distilled chloroform in contact with the substance left no appreciable residue on evaporation. The material was then washed free from chloroform with ether on a hard-paper filter and dried in a desiccator over sulphuric acid.

**Qualities of purified paranucleoprotagon.**— At the conclusion of this treatment the product consisted of fairly soft, somewhat tenacious, grayish and yellowish particles. In spite of the fact that no appreciable mechanical losses occurred, only about 10 gm. of this paranucleoprotagon were separated from the fifty sheep brains taken, obviously not enough to warrant Ulpiani and Lelli's guess that all the protagon of the brain occurs in that organ as paranucleoprotagon nor to support their assumption that protagon may be completely separated from brain with chloroform in the form of paranucleoprotagon. Fifty sheep brains will readily yield much more than 10 grams of protagon.<sup>1</sup>

That the product possessed protein qualities was shown by its response to various protein color tests. It was found to contain only 0.75 per cent of phosphorus.<sup>2</sup> Ulpiani and Lelli's product contained 1.62 per cent of phosphorus.

In view of the fact that our product was small in amount, we made no other qualitative tests with it, but used practically all of the rest for Ulpiani and Lelli's cleavage operation in alcohol (p. 383), as follows:

#### PREPARATION OF PROTAGON AND PARANUCLEIN FROM PARANUCLEOPROTAGON.

**Cleavage in alcohol.**— The remaining quantity of paranucleoprotagon (approximately 5 gm.<sup>3</sup>) was treated for about twenty hours with a litre of 85 per cent alcohol at 45° C. Little change was noticed, although the bulk of the solid mass appeared gradually to diminish somewhat.

Ulpiani and Lelli's purpose, in carrying out this treatment, was to effect a division of paranucleoprotagon into paranuclein and protagon,

<sup>1</sup> POSNER and GIES: *Loc. cit.* See also NOLL: *Loc. cit.*

<sup>2</sup> Our washing operations were probably more thorough than theirs. We have already called attention (p. 382) to the inadequacy of ULPIANI and LELLI'S description of their procedures, and the consequent impossibility of following them closely.

<sup>3</sup> The material was inadvertently used for this purpose before its exact weight had been taken.

after the manner of cleavage, by alcohol, of lecitho-nucleovitellin into lecithin and nucleovitellin.<sup>1</sup> After the treatment with alcohol, they cooled the *unfiltered* mixture to 0° C., apparently with the special purpose of precipitating whatever protagon had been broken off by the alcohol, and of keeping it associated with the non-protagon part. They filtered the cold mixture (0° C.), and apparently made no examination of the filtrate. From the solid matter filtered off at the low temperature, however, they extracted, in a Soxhlet apparatus, with chloroform,<sup>2</sup> what they thought was protagon, although they presented no evidence that protagon could be prepared from such a chloroform extract in the manner described by them.<sup>3</sup>

It seemed to us that a better way of determining the presence of protagon in, or its absence from, the alcoholic liquid would have been the employment of the *classical method* for its isolation. Accordingly we proceeded from this point as follows:

**Special treatment of the warm alcoholic filtrate containing the protagon.**

After the paranucleoprotagon had been in the 85 per cent alcohol at 45° C. for about twenty hours, and the product had perceptibly diminished in bulk, as indicated on p. 392, we quickly *filtered* the warm extract and washed the residue with 85 per cent alcohol at 45° C. The washings, which were small in volume, were added to the alcoholic filtrate. (The insoluble part is referred to on p. 395 as "residue in warm alcohol.")

The alcoholic filtrate was then quickly cooled to 0° C. and kept at that temperature about five hours. As the temperature fell, the liquid soon became opalescent, then milky, and finally white flakes began to form. They consisted of amorphous matter. Separation of the white precipitate was effected very rapidly by pressure filtration. The resultant "protagon" was washed with a small quantity of cold alcohol (0° C.). The washings were added to what is called, on p. 394, the "protagon filtrate."<sup>4</sup>

**The protagon.**— The precipitate (protagon) showed no crystalline characters. On drying it formed waxy lumps, which could easily be powdered. The general resemblance to protagon was quite marked, but its phosphorus content was only 0.73 per cent. That of Ulpiani and Lelli's protagon, obtained by a different process, how-

<sup>1</sup> OSBORNE and CAMPBELL: *Loc. cit.*

<sup>2</sup> The extract in the receiver was kept boiling, of course.

<sup>3</sup> They ignored RUPPEL's statement that protagon is decomposed by boiling chloroform. RUPPEL: *Zeitschrift für Biologie*, 1895, xxxi, p. 86.

<sup>4</sup> Under the general circumstances attending preliminary preparation we thought it well not to wash with ether, but to get the effects of treatment with alcohol alone.

ever, was practically twice as much, *i. e.*, 1.31 per cent. Only 0.095 gm. of protagon-like product was obtained, and it was necessary to use all of it for the phosphorus determination, so that no other tests could be applied to it. That the material resembled protagon was very obvious. That it was chemically very unlike Ulpiani and Lelli's product, however, was made unmistakable by our result for phosphorus content.

That more than one substance was present in the *original warm alcoholic filtrate* was made evident by the following results of our examination of the alcoholic filtrate *obtained at 0° C.*, which may be conveniently called the "protagon filtrate."

**The protagon filtrate.**— The alcoholic filtrate, from the protagon-like precipitate that was obtained at 0° C., was evaporated gradually to dryness on a water bath. As the bulk of the solution diminished and the percentage of water in it increased, the liquid became yellowish and very viscid. On drying it was yellowish brown and had an odor similar to that of lecithin preparations. In these respects it resembled, under similar conditions, the products of high phosphorus contents obtained by Thudichum, by Lesem and Gies, and by Posner and Gies, in the filtrates from their fractionally recrystallized protagons.

The amount of substance obtained was 0.955 gm., practically ten times the quantity of the protagon-like product. Its phosphorus content amounted to 2.25 per cent. It appeared to be a mixture containing lecithin-like material. It was readily soluble in alcohol, in ether, and in chloroform, and was partly, perhaps wholly, precipitated from its chloroform solution by ethyl acetate or acetone.<sup>1</sup> The available quantity of the product was insufficient for further study of its qualities.

Material like this must have been present in the corresponding filtrate obtained by Ulpiani and Lelli, but they failed to look for it. Possibly they precipitated some of it by their method of isolating their "protagon." That the latter was a mixture seems equally probable. By reason of its greater proportion, the unprecipitated product obtained by evaporation of our alcoholic filtrate must be regarded as of more significance than the corresponding protagon-like material. At least two substances were present in the original *warm alcoholic filtrate*. Probably there were more of them. It is not very likely that both or all of these were liberated by alcoholic

<sup>1</sup> See p. 383 for ULPIANI and LELLI'S method of precipitating their protagon.

cleavage of only one primary substance. We have already referred to the heterogeneous appearance of our paranucleoprotagon.

**Residue in warm alcohol, extracted with chloroform.** — The residual matter, which was filtered from the warm alcohol at the conclusion of the treatment that had been intended to separate protagon, was subjected in a Soxhlet apparatus to continuous extraction with chloroform for about twenty-four hours. The dried residue, corresponding to Ulpiani and Lelli's paranuclein, will be referred to below.

The *chloroform extract* at this point, which, in Ulpiani and Lelli's work, doubtless contained material similar to our protagon-like product (and possibly also some of the substance or substances of high phosphorus contents present in our "protagon filtrate"), was treated by the Ulpiani and Lelli method for the precipitation of protagon, *i. e.*, by the addition of ethyl acetate, but no precipitate could be obtained in any proportions of mixed chloroform extract and ethyl acetate. Acetone likewise failed to produce a precipitate. It was apparent that everything obtained by Ulpiani and Lelli at this point had already been separated by us by the method already described. We finally evaporated the unprecipitable chloroform-ethyl acetate solution to dryness.

The residue amounted to 0.3 gm., and contained only 0.03 per cent of phosphorus. Our reagents were phosphorus free, and yielded only exceedingly slight residues on evaporation.

Ulpiani and Lelli paid no more attention to the chloroform-acetic ether filtrate than they did to the cold alcoholic filtrate. They were obviously very arbitrary, therefore, in calling their product *paranucleo-protagon*, even if protagon had certainly been an individual substance with the well-defined physical and chemical qualities they attributed to it.

**Paranuclein.** — The portion of the paranucleoprotagon that resisted solution, first in the warm alcohol and then during the continuous treatment with chloroform, corresponded with Ulpiani and Lelli's paranuclein fraction of the paranucleoprotagon. It was hard and brittle, — quite different in these respects from the original paranucleoprotagon. The particles continued to show grayish or yellowish coloration, as they did from the beginning. A little more than 3 gm. was recovered (3.3). The phosphorus content was only 0.38 per cent. Ulpiani and Lelli's product contained 1.88 per cent. Although their product and ours were the outcome of essentially the same treatment,<sup>1</sup> they differed to this surprising extent in phosphorus con-

<sup>1</sup> So far as their meagre description permits of near approach to duplication of procedure.

tent. This outcome agrees, however, with our other observations in pointing to heterogeneity as the chief characteristic of both Ulpiani and Lelli's paranucleoprotagon and our own.

This "paranuclein" gave sharp responses to the protein color reactions. Strongly peptolytic artificial gastric juice appeared to have no effect on it. After standing for three days in 0.5 per cent potassium hydroxid at room temperature, the appearance of the substance seemed to be unchanged and its amount undiminished. The filtrate, on neutralization and cautious acidification, became only faintly turbid. It required considerable heating to bring about appreciable solution promptly in 0.5 per cent potassium hydroxid.

**Summary of quantitative data.**—The foregoing quantitative data pertaining to our paranucleoprotagon and its cleavage products, with some corresponding data from Ulpiani and Lelli's paper, are summarized below:

	Fractional product.	Weight in grams.	Percentage of phosphorus.	
			Steel and Gies.	Ulpiani and Lelli.
I.	1. Protagon . . . . .	0.095	0.73	1.31
	2. Solid matter in the alcoholic filtrate from the protagon . . . . .	0.955	2.25	... <sup>1</sup>
II.	1. Paranuclein . . . . .	3.313	0.38	1.88
	2. Solid matter in the chloroform washings of the paranuclein . . . . .	0.314 <sup>2</sup>	0.03	... <sup>1</sup>
III.	1. Substance recovered . . . . .	4.677 <sup>3</sup>	...	...
	2. Paranucleoprotagon taken . . . . .	... <sup>4</sup>	0.75	1.62

It will require extended study to determine more definitely and finally the chemical and physical qualities of paranucleoprotagon. The above data show clearly, however, that the term paranucleoprotagon is a misnomer. The material designated by that name is, nevertheless, an important product, and although it is apparently a mixture of substances, it seems to contain at least one combination which, like lecitho-nucleovitellin, may be severed by cleavage with alcohol. It is our purpose to investigate further in this laboratory the qual-

<sup>1</sup> ULPIANI and LELLI failed to examine the corresponding material.

<sup>2</sup> A very small proportion was derived from the reagents used. See p. 395.

<sup>3</sup> This does not exactly represent the total, because small portions of some of the fractional products were used for qualitative tests before the materials were dried and the weights taken.

<sup>4</sup> Approximately 5 gm. of anhydrous material. By an oversight the exact weight was not taken (see the footnote on p. 392). The figures for phosphorus contents are none the less significant, however.

ities of paranucleoprotagon, and to study also the substance or substances in it that resemble the lecithoproteins in the feature indicated.

#### SUMMARY OF GENERAL CONCLUSIONS.

The brain product called paranucleoprotagon, as Ulpiani and Lelli stated, resembles lecithoproteins in undergoing a certain degree of cleavage when treated with warm alcohol, although resisting such cleavage by ether and by chloroform. The extent of this chemical cleavage as distinguished from mechanical partition has not been determined.

The substances produced from paranucleoprotagon by 85 per cent alcohol at 45° C are not simply paranuclein and protagon, as stated by Ulpiani and Lelli, but one or more additional products are separated by such treatment. The term paranucleoprotagon, as applied by Ulpiani and Lelli, is a misnomer therefore.

Ulpiani and Lelli failed to show that paranucleoprotagon is a definite, individual substance. The difficulty of getting a product exactly like theirs by careful repetition of their own method (so far as that is possible from their inadequate description of it), together with the observation that several products are obtainable from paranucleoprotagon by their prescribed treatment with alcohol, indicate that paranucleoprotagon, like protagon itself, is a mixture of substances. Further, like protagon, paranucleoprotagon yields different products when acted on by 85 per cent alcohol at 45°C. It is not very probable that all these products are derived by cleavage from a single, very complex molecule. If more than one of them is a cleavage product, it is also quite probable that some of them result simply from mechanical fractionation.

Ulpiani and Lelli have not proved that their paranuclein is a typical nuclein. They failed to show that it is not a mixture of residual substances.

The facts pertaining to paranucleoprotagon lend no support whatever to the assumption that protagon is anything definite, physically or chemically. On the contrary, the observations recorded in this paper agree with those previously reported from this laboratory, in showing that protagon has always been a mechanical mixture of substances. We have indicated reasons for concluding that the data published recently in dissent by Lochhead and Cramer actually support this view of the nature of protagon.

The amount of paranucleoprotagon that is obtainable from brain by the Ulpiani and Lelli process is much less than the quantity of protagon that may be extracted from an equal amount of brain by the classical method for the isolation of protagon. Consequently, Ulpiani and Lelli's conclusion that *all* the protagon that is separable from brain occurs there combined with paranuclein in the form of paranucleoprotagon is incorrect. Ulpiani and Lelli have indeed not even shown definitely that *any* protagon (*i. e.*, the constituents of protagon) exists in the brain in the form of the product they named paranucleoprotagon.

# THE EFFECT OF UNIFORM AFFERENT IMPULSES UPON THE BLOOD PRESSURE AT DIFFERENT LEVELS.

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## I.

IN 1903 it was shown<sup>1</sup> that the stimulation of the depressor nerve in rabbits in which the blood pressure had been lowered by injuries to the abdominal viscera produced a percentage change in the blood pressure as great as that produced by a stimulus of equal strength applied to the nerve when the blood pressure was at its normal level. In 1907 similar results were published<sup>2</sup> regarding the sciatic and the brachial nerves in rabbits, cats, and dogs, in which uniform stimuli were applied to these nerves when the blood pressure was normal, and when it had been lowered by injuries to the brain.

The present investigation is an inquiry into the effect of the uniform stimulation of the depressor, brachial, and sciatic nerves at various levels from the normal down to about 9 mm.

## II.

The animals employed were rabbits, cats, and dogs. They were invariably etherized during the operations performed upon them. Before etherization a small quantity of morphia was injected beneath the skin of some of the dogs. Tracheotomy was done and cannulas were placed in the crural or carotid artery and, when necessary, in the crural or jugular vein. Afferent impulses were obtained by the electrical stimulation of the depressor, sciatic, and

<sup>1</sup> W. T. PORTER and W. C. QUINBY: Boston medical and surgical journal, 1903, cxlix, pp. 455, 456; see also This journal, 1903-04, x, pp. xii, xiii.

<sup>2</sup> W. T. PORTER and T. A. STOREY: This journal, 1907, xviii, pp. 181-199.

branches of the brachial plexus in or near the axilla. For convenience, these branches will be called in the text the brachial nerve. Precautions were taken to prepare the nerves with the least possible injury and to protect them against drying. The nerves were always severed and the proximal end lifted into the air when the electrodes were applied. A constant current from Daniell or "gravity" cells supplied the inductorium, and the secondary coil was kept at a uniform distance from the primary coil.

Before stimulating the brachial or sciatic nerve curare mixed with from 15 to 25 c.c. of warm normal saline solution was very slowly injected into the crural or jugular vein. From time to time the nerves were stimulated to determine whether just enough curare had been given. By these precautions it was possible to operate with the least injurious dose. Efforts were made to have the curarization uniform throughout each experiment. Care was also used to equalize as far as possible the stimulating action of the ether. It is important in such experiments to prevent the magnesium sulphate, used to prevent clotting, from being drawn through the arterial cannula into the circulation as the blood pressure falls. To this end the membrane manometer was employed in almost all the measurements, as its liquid displacement is so small that the movement of about 1 c.mm. of magnesium sulphate solution out of the manometer chamber, and thus out of the cannula, would record a fall of almost 100 mm. in blood pressure. In some experiments the mercury manometer was used as a control, but the stopcock between the cannula and the manometer was closed during every considerable fall in blood pressure, and the connection re-established only after the pressure in the manometer had been reduced by opening the proximal limb to the air. This precaution ought on no account to be neglected, though, unless care be taken, not enough magnesium sulphate may be left in the cannula to prevent clotting.

In all the graphic records the blood pressure was measured from the atmospheric pressure line to the lowest point in the blood pressure curve. This point can of course be easily determined and is little exposed to inertia errors, whereas it is much more difficult to determine the mean or the systolic pressure. The use of the lowest point in the curve makes all readings lower than if the mean or the systolic pressure had been chosen, and this is particularly to be noted with regard to the very numerous cases

in which the usual difference between the systolic and the diastolic pressure was increased by a lessening of arterial tension not compensated by an equivalent lessening in the force or frequency of the ventricular stroke.<sup>1</sup>

In recording the effect of stimulating afferent nerves on the blood pressure at various levels, advantage was taken of the spontaneous changes in blood pressure seen in many experiments, but for the lower levels it was necessary to resort to agencies leading to what surgeons would call "shock." These agencies were section of the spinal cord, exposure and mechanical injury of the abdominal viscera, application of nitric acid or zinc sulphate to the peritoneum, cauterization of the skin of the limbs, hæmorrhage, injuries to the brain, and section of the splanchnic nerves.

### III.

Graphic records were secured from thirty-eight rabbits, twenty-seven cats, and four dogs. The total number of measurements was 765, of which the brachial nerves supplied 196, the sciatic 248, and the depressor 321.

Measurements of this order group themselves about a central value or type. Thus the central end of the sciatic nerve of the rabbit was stimulated in seven individuals, while the blood pressure was at 60 mm. Hg. The blood pressure rose to 81, 88, 92, 92, 92, 110, and 110 mm., respectively. If the arithmetical mean be taken, 95 mm. Hg will be the typical value which the blood pressure may be expected to attain when the afferent fibres of the sciatic nerve are stimulated in the rabbit with an initial blood pressure of 60 mm.

The individual observations in the above series are distributed about the central value through the operation of constant or accidental errors.

Excessive curarization would be a constant error, tending to make all the readings in the over-curarized rabbit too high. The precautions against over-curarization have already been mentioned. So far as can be judged, there were no constant errors in the observations.

<sup>1</sup> Some of these points have been mentioned in the paper of PORTER and STOREY (*loc. cit.*), but it was necessary to repeat them here, partly for the convenience of the reader and partly because the observations collected by PORTER and STOREY are included in the material analyzed in the present investigation.

An accidental error is one that is just as likely to fall on one side of the central value as on the other. It follows that if the number of observations be sufficiently large, those that fall at a certain distance on one side of the central value will be balanced by those that fall at the same distance on the other side. There should therefore be no choice between the individual observations that are thus by mutual compensation to reveal the hidden type. Every observation made has accordingly been included in the material here presented.<sup>1</sup>

It is evident that by the operation of accidental errors the individual observations will be distributed symmetrically about the central value, which can then be calculated with an exactness proportionate to the number of observations. Where the number of observations at any one unit of measurement is small, the accidental errors will correspondingly be few in number and compensation will be very imperfect. It will be, then, of advantage to treat the observations in larger groups. For example, instead of analyzing the results of stimulation at each millimetre of blood pressure between 60 and 70, all the observations between these levels may be treated as one group. This has been done in the present investigation. Table I presents the arithmetical mean of all observations recorded when the blood pressure at the beginning of stimulation was between 11 and 20, 21 and 30, 31 and 40 mm. Hg, and thus by increments of 10 up to 180 mm. Hg. The difference between the mean of the blood pressures at the beginning of stimulation and the mean of the highest or, in the case of the depressor nerve, the lowest values reached in consequence of stimulation was recorded as the typical change produced by stimulation. For example, the central end of the sciatic nerve of the rabbit was stimulated seventeen times while the blood pressure was between 41 and 50 mm. The average height to which the blood pressure rose on stimulation was 82 mm. The difference between 82 and 45, the mean level before stimulation, is 37 mm. Hg, which is therefore the typical value obtained by stimulating the sciatic nerve of the rabbit at the mean level of 45 mm. Hg.

The figure just obtained is the absolute change in blood pressure upon stimulation of an afferent nerve. But for purposes of comparison it is clear that the percentile value should be employed.

<sup>1</sup> This material was collected during four years. None of the experiments was made for the present investigation.

In one series the stimulation of the sciatic nerve in the rabbit while the blood pressure was 100 mm. Hg caused a rise of 35 mm., and when the blood pressure was 50 mm. a stimulus of equal intensity still caused a rise of 35 mm. The absolute change was the

TABLE I.

THE ABSOLUTE AND PERCENTILE CHANGE IN BLOOD PRESSURE UPON STIMULATION OF THE CENTRAL END OF THE SCIATIC, BRACHIAL, AND DEPRESSOR NERVES.

Blood pressure before stimulation. mm.Hg	Number of observations.			Absolute change on stimulation.			Percentile change on stimulation.		
	Sciatic.	Brachial.	Depressor.	Sciatic.	Brachial.	Depressor.	Sciatic.	Brachial.	Depressor.
				mm.Hg rise. 34	mm.Hg rise. 37	mm.Hg fall. ..	p. c. rise.	p. c. rise.	p. c. fall.
151 to 160	2	6	..				22	24	..
141 to 150	2	7	1	34	26	33	23	18	23
131 to 140	10	9	3	35	32	40	26	24	30
121 to 130	9	7	7	37	29	41	30	23	33
111 to 120	5	13	4	38	32	29	33	28	25
101 to 110	10	8	11	39	39	33	37	37	31
91 to 100	12	7	15	44	46	38	46	48	40
81 to 90	15	6	20	42	33	36	49	39	42
71 to 80	15	10	47	44	45	26	59	60	35
61 to 70	23	14	74	35	44	24	54	68	37
51 to 60	28	23	48	32	26	16	58	47	29
41 to 50	34	11	32	33	20	12	73	44	27
31 to 40	25	18	26	25	17	8	71	49	23
21 to 30	41	32	19	13	9	4	52	36	16
11 to 20	17	25	14	6	9	1	40	60	7

same in both, but in the first instance this change was 35 per cent, while in the second it was 70 per cent. The effect of the same stimulus was only half as great in the first as in the second instance.

The values gained by the above methods show the irregularities inseparable from the statistically small number of observations, but they suffice for some instructive conclusions.

## IV.

In Table I are shown the absolute and the percentile change in blood pressure upon stimulation of the central ends of the sciatic, brachial, and depressor nerves, together with the number of observa-

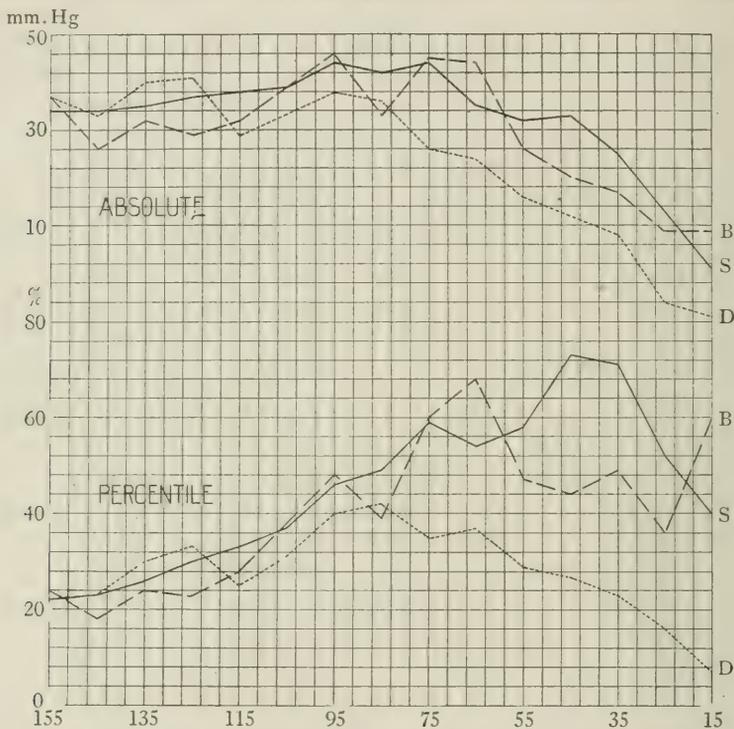


FIGURE 1.—The absolute and percentile change in blood pressure upon stimulation of the central ends of the sciatic (unbroken line), brachial (line of dashes), and depressor nerves (dotted line). The abscissæ give blood pressure in millimetres of mercury. The ordinates for the absolute curves give blood pressure in millimetres of mercury, ordinates for the percentile curves give per cent.

tions upon which each result is based. The alterations in blood pressure are shown graphically in Fig. 1.

Table I and Fig. 1 justify the following statements:

(1) The absolute change in blood pressure upon stimulating these afferent nerves remains almost unchanged until the blood pressure has fallen to about one-third its normal height.

(2) The relative or percentile change in blood pressure, which is the true index of the condition of the vasomotor cells, increases as

the blood pressure falls. In the case of the sciatic nerve, this increase persists until the blood pressure is 30 mm., which is about the level reached when the spinal cord and bulbar vasomotor centre are destroyed. In the case of the brachial nerve the increase lessens when the blood pressure has fallen to 65 mm. Hg, but with both the brachial and the sciatic nerves the percentile rise is greater even when the blood pressure has fallen below 30 mm. Hg than at the normal level of about 150 mm. Hg. The percentile change on stimulation of the depressor nerve increases until the blood pressure falls to 85 mm. Hg, whence it diminishes slowly to the 35 mm. level, and then more rapidly to the 15 mm. level. But it should be particularly noted that the depressor reflex is as great with the blood pressure at 30 mm. Hg as with the blood pressure at 145 mm.

(3) The data presented are wholly opposed to the hypothesis that would explain surgical shock by the exhaustion of the vasomotor centres. The effect of the afferent impulses upon the vasomotor cells is as great at 35 mm. Hg as when the blood pressure preceding stimulation is at the normal level. Exhaustion is always preceded by fatigue, and fatigue is a gradual process. These measurements give no evidence of a gradual fall from normal power. The reflex fails only when the blood pressure sinks to a level at which anæmia of the vasomotor cells is certain. Indeed, all we know regarding these and similar cells strengthens the belief that their endurance under stimulation is very great. On the other hand, they are extraordinarily sensitive to variations in their blood supply.

(4) The course of the absolute and percentile curves in Fig. 1 is not the same for the bulbar and spinal vasomotor reflexes. The curves suggest a specific difference between the bulbar and the spinal vasomotor cells. The probability of this difference is increased by the effect of certain drugs.<sup>1</sup>

(5) It will be noted in Fig. 1 that as the blood pressure falls, the power of the brachial and sciatic fibres increases. The brachial and sciatic nerves here display a protective action. The same stimulus produces relatively a larger increase in the blood pressure as the danger of bulbar and spinal anæmia increases. The greater the danger, the greater the reflex.

<sup>1</sup> The action of these drugs upon the vasomotor reflexes is now being studied in this laboratory.



# SOME OBSERVATIONS ON THE BEHAVIOR OF THE AUTOMATIC RESPIRATORY AND CARDIAC MECHANISMS AFTER COMPLETE AND PARTIAL ISOLATION FROM EXTRINSIC NERVE IMPULSES.

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MORE than ten years ago I began a series of observations on the consequences of simultaneous and successive division of the vagi. Although the theme is a somewhat hackneyed one, the contradictory statements of fact, and especially the conflicting interpretations of the observed facts in the literature, seemed to justify a renewed investigation. Only very brief references to some of the results have hitherto been published.<sup>1</sup> In the meantime experiments made in conjunction with some of my pupils<sup>2</sup> on the partial or complete isolation of the respiratory and other bulbar centres by anæmia, sometimes combined with section of both vagi and of the "upper paths," have given these observations a new interest. Therefore I desire to put them on record in greater detail and to discuss their bearing on our more recent work, some further points in which may advantageously be considered in this connection.

<sup>1</sup> STEWART, G. N. : American year book of medicine, 1901, p. 548; Science, 1905, xxi, p. 889.

<sup>2</sup> STEWART, GUTHRIE, BURNS and PIKE, Journal of experimental medicine, 1906, viii, p. 289. STEWART and PIKE: This journal, 1907, xix, p. 328; xx, p. 61.

The extensive literature of bilateral vagotomy has been well summarized by Pierre Herzen<sup>3</sup> and Boruttau,<sup>4</sup> and only such papers as bear upon the observations now communicated will be referred to here. The classical clinical picture presented by such animals as the dog, cat, and rabbit, when they have suffered division of both vagi in the neck, is too well known to need description. The slow, deep respiration, in which the duration of inspiration greatly exceeds that of expiration, the rapid heart beat, the frequent vomiting or regurgitation, the progressive emaciation in animals which survive for more than a day or two, as dogs often do, and the terminal double pneumonia which so frequently cuts off the animal, are very familiar phenomena. It is the way in which these symptoms are produced, their permanence, their relative importance in bringing about the fatal result, and the constancy of the latter, particularly when an interval has been allowed to elapse between the division of the two vagi, which have chiefly been subjects of discussion and dispute. Practically everybody is agreed that in animals like the dog which not infrequently survive for a considerable time (several weeks, or occasionally months) the pulse rate tends to return towards the normal. But there is by no means the same unanimity as regards the respiration, some writers asserting that it gradually tends to increase in rate, while others maintain that the slow, deep respiration established immediately on the elimination of the pulmonary vagus fibres is not essentially modified as time goes on. It may at once be stated that in a series of more than fifty dogs, except in those rare instances where the animals survive for an indefinite period the section of both vagi, I have seen no such tendency to increase in the respiratory rate as has been described, for instance, by Nikolaides,<sup>5</sup> in dogs when an interval was allowed to elapse between the division of the first and second vagi, even when the animals eventually died from the consequences of the vagotomy. For instance, in the

*Experiment of January 9, 1897*, a fox terrier, whose vago-sympathetics were simultaneously divided in the neck, lived 31 days, being fed at first by rectal enemata of eggs beaten up in milk. It received food by the mouth for the first time 8 days after the operation. As shown in Table I, the pulse rate, which before the section, with the animal under morphia, was 65

<sup>3</sup> HERZEN, PIERRE: Thèse, Lausanne, 1897.

<sup>4</sup> BORUTTAU: Archiv für die gesammte Physiologie, 1895, lxi, p. 39.

<sup>5</sup> NIKOLAIDES: Archiv für Physiologie, 1905, p. 465.

a minute and after the section 154, had diminished by the fourteenth day to 110. The rate of respiration, which was 15 a minute before section (under morphia) and 11 immediately after section, never on succeeding days rose higher than 10, and was usually lower, except on one occasion when the animal was excited by seeing a mouse, when it reached 12½.

TABLE I.

Days since operation.	Pulse.	Respiration.	Ratio.	Days since operation.	Pulse.	Respiration.	Ratio.
2	145	10	14.5	25	110	6.2	17.7
3	132	8	16.5	"	145 <sup>1</sup>	12.5 <sup>1</sup>	11.6
7	135	10	13.5	26	136	7.0	19.4
9	120	9	13.3	"	128	8.0	16.0
13	120	10	12.0	27	128	8.0	16.0
14	110	10	11.0	28	94	8.0	11.7
16	114	8	14.2	29	147	6.3	23.7
18	136	9	15.1	"	148	..	....
20	136	10	13.6	"	154	..	....
21	134	10	13.4	30 <sup>2</sup>	158	7.5	21.0
23	130	10	13.0	31	144	8.5	16.9
24	134	..	....	"	137	8.5	16.1
"	128	7	18.3	..	..	..	....

<sup>1</sup> In excitement caused by sight of mouse. Some of the respirations are shallow. Counting only the deep respirations, the respiratory rate is not increased.

<sup>2</sup> The dog is very weak.

The ratio of the heart rate to the respiratory rate, which was 4.3 to 1 before section was never less than 11 to 1 afterwards and nearly always considerably more. In another fox terrier operated on at the same time and kept under the same conditions, except that it ate for the first time on the third day, the pulse rate soon after the operation was 160 and the rate of respiration 10 (ratio, 16 : 1). The pulse rate on the ninth day had sunk to 118, but the respiration was only 8 (ratio, 16 : 2.1). On the twelfth day the numbers were 120 and 7 (ratio, 17.1 : 1). It died on the thirteenth day.

In a collie on the evening before operation the pulse and respiratory rates were 78 and 22 respectively (3.5 : 1); on the morning before operation 82 and 28 (2.9 : 1); under morphia and ether 96 and 16 (6 : 1); after section of the first vagus 126 and 20 (6.3 : 1); after section of the second vagus 170 and 18 (soon 14); on the day after the operation 158 and 11.6 (13.6 : 1); on the second day 160 and 16 (10 : 1), on the fifth day 143 and 9.3 (15.4 : 1), on the seventh day 140 and 11.4 (12.2 : 1), on the twelfth day 128 and 16 (8 : 1), on the thirteenth day 117 and 12 (9.7 : 1). The animal died on the fourteenth day.

In a spaniel the pulse and respiratory rates on the evening before operation were 94 and 16 respectively (5.9 : 1); on the morning before operation 84 and 18 (4.6 : 1); under morphia and ether 68 and 18 (3.8 : 1); after section of the first vagus 140 and 10 (14 : 1); immediately after section of the second vagus 160 and 15 (10.6 : 1); on the first day after the operation 188 and 6.7 (28 : 1); on the second day 170 and 9 (19 : 1); on the fifth day 152 and 6.8 (22.3 : 1); on the sixth day 144, and 6.6 (21.8 : 1); on the seventh day 146 and 7.8 (18.7 : 1); on the ninth day 154 and 9 (17.1 : 1); on the day of its death (the eleventh day) 151 and 7 (21.5 : 1).

Not only does the rate of respiration show a relatively high degree of stability in one and the same dog after bilateral vagotomy, but there is a far smaller range than normal in the frequency in different dogs in the absence of the pulmonary regulating impulses. This is well illustrated in Table II, which gives the pulse rates, the respiratory rates, and the ratios between them before section, after section of one vagus, and after section of both in twenty-three animals, all of which died. In only one case was the rate of respiration more than 12, after section of both vagi, when the immediate excitation caused by the section had passed off. In eighteen cases it was 9 or less; in four cases 4 or 5. In 50 per cent of all the cases the rate was 6 to 8. The ratio of pulse to respiratory rate was invariably much greater than the normal, although, owing to the tendency of the pulse rate to diminish in animals which survived more than a few days, the ratio tended towards the normal as time went on, but without ever reaching it, except in those rare cases of complete recovery already mentioned.

It is an interesting question how this relative stability in the respiratory frequency, which is notoriously susceptible to so many influences in the normal animal, is maintained after elimination of the vagi. Some have laid stress on the vicarious control of the

TABLE II.  
SECTION OF BOTH VAGI IN DOGS.

No. of experiment.	Pulse rate.			Respiratory rate.			Ratio of pulse to respiration.		
	Before section.	After section of one vagus.	After section of second vagus.	Before section.	After section of one vagus.	After section of second vagus.	Before section.	After section of one vagus.	After section of second vagus.
1	79	140	160 188 <sup>1</sup>	15	10	15 6.7 <sup>1</sup>	5.2	14	10.6 25.0 <sup>1</sup>
2	96	126	170 158 <sup>1</sup>	14	20	18 11.6 <sup>1</sup>	6.8	6.3	9.4 13.6 <sup>1</sup>
3	65		154	15		11	4.3		14.0
4	118		164	20		4	5.9		41.0
5	51		152	14.2		5.8	3.6		26.0
6	50		144	8.4		7	5.9		20.5
7	78	90	151	24	15	6	3.2	6	25.1
8	36	58	160	22	16	5	1.6	3.6	32
9 <sup>2</sup>	45		190	15		12	3.0		15.8
10			205			5			41.0
11 <sup>3</sup>	92	147	191	14	17	10	6.5	8.6	19.1
12 <sup>3</sup>	69	130	165	11	19	8	6.2	6.8	20.6
13	80	120	145	18	8	8	4.4	15	18.1
14 <sup>4</sup>	68		172	10		7	6.8		24.5
15	70	72	104	16	12	9	4.4	6	11.5
16	108	72	116	29	24	13.5	3.3	3	8.6
17	77	86	150	13	10.5	9	5.9	8.2	21.4
18	46		92 156 <sup>1</sup>	18		10 7 <sup>1</sup>	2.5		9.2 22.3 <sup>1</sup>
19	60	88	140	16	11	8	3.7	8	17.5
20	70	72	104 154 <sup>5</sup>	16	12	9 8 <sup>5</sup>	4.4	6	11.5 19.2 <sup>5</sup>
21	56	135	180	14	10	6	4	13.5	30.0
22	66	72	80 120 <sup>6</sup>	33	10	4 3 <sup>6</sup>	2	7.2	20 40 <sup>6</sup>
23 <sup>4</sup>	36	68	160	8	19	9	4.5	3.6	17.8

<sup>1</sup> After one day.

<sup>2</sup> Vagi crushed thoroughly without being divided.

<sup>3</sup> Pup three months old.

<sup>4</sup> Second vagus divided fifty-nine days after the first. The counts were made during anæsthesia with morphia and ACE mixture.

<sup>5</sup> Seven hours after operation.

<sup>6</sup> After five or six minutes.

“higher paths.” We have seen good reason to believe not only that these paths from parts of the brain lying higher than the bulbar respiratory centre do exert an influence upon that centre after section of the vagi (in cats), but that they are normally active since section of them, the vagi being intact, produces some diminution in the frequency of the respiratory movements, contrary to Marckwald’s observations (on rabbits).<sup>6</sup> Yet the stability in the rate of the respiratory discharge is mainly due, we believe, not to the development of an effective extrinsic regulation which takes the place of the regulation normally exercised by the Hering-Breuer fibres, but to the relative isolation of the bulbar centre from extrinsic impulses, which permits its “native” automatic or autochthonous rhythmical discharge to dominate the situation. For nothing is more striking than the diminished susceptibility of the respiratory rhythm of the vagotomized dog to such influences, including psychical disturbances, as powerfully affect the respiration of the normal animal, although experimental stimulation of the central end of the vagus or of the sciatic still produces a great effect. In the experiment of January 9, 1897, for instance, already quoted, the intense psychical excitation produced by the sight of a mouse, which obviously aroused the keenest interest on the part of the dog, only increased the respirations to 12½ a minute, and this at a time when the animal was in fairly good condition, on the twenty-fifth day after the operation. It would almost seem as if the bulbar centre in the absence of vagus control developed its own rhythm to such a degree that it remained stable in the presence of extrinsic excitations which in its normal condition would have caused a marked alteration in it.

In conjunction with Dr. F. H. Pike<sup>7</sup> I have recently brought forward fresh evidence that the bulbar respiratory centre is not only capable of such automatic rhythmical discharge at a point in the resuscitation of the brain and cervical cord after a period of anæmia when as yet all the afferent paths leading to it, including the vagi, are still incapable of conduction, but that the initial rate of discharge under these circumstances is remarkably constant. The character of the respiration at this time irresistibly reminds one of the respiration after double vagotomy. Although these resuscitation experiments were made mainly on cats, with a few observations

<sup>6</sup> MARCKWALD: *Zeitschrift für Biologie*, 1887, xxiii, p. 149; 1890, xxvi, p. 260.

<sup>7</sup> STEWART and PIKE: *This journal (loc. cit.)*.

on rabbits and very few on dogs, which are not suitable on account of the free collateral circulation to the brain through the intra-vertebral vessels, it cannot be doubted that in some points they throw light on each other. I have the less hesitation in so applying them that the practically constant initial rate of discharge (about 4 a minute) of the resuscitated respiratory centre of the cat agrees extremely well with the rate observed by Loewy<sup>8</sup> in rabbits, after section of the vagi and the brain stem above the bulb, and with the rate observed by Katschkowsky<sup>9</sup> and by myself (Table II) in a certain number of vagotomized dogs. The constancy of this rhythm points to its dependence upon a fundamental property of the nervous elements in which it originates. A qualitative similarity between the respiratory centres of animals even of widely distant groups is universally admitted. There is nothing improbable in the existence of a quantitative similarity between not very widely separated mammals. It is a very natural supposition that in a certain number of animals, especially when fully anæsthetized, the higher parts of the brain should be exerting so little influence that section of the vagi alone is sufficient to bring about that degree of isolation of the respiratory centre from afferent impulses which is associated with the development of its fundamental rate of discharge, although in the majority the influence of the higher paths must still be removed before the necessary isolation is attained.

The relative constancy of the discharge of the respiratory centre (in cats) so long as it is isolated from the afferent impulses is well illustrated in the experiments cited in Table III, where the isolation was accomplished by subjecting the brain and cervical cord to a period of anæmia, and in those of which Table IV gives some specimens, where, in addition to the anæmia, the brain stem was divided at the posterior boundary of the posterior corpora quadrigemina or farther forward. In some cases both vagi were also cut, and occasionally the spinal cord as well in the lower cervical region. In determining the initial rate it must be remembered that when the return of function of the respiratory centre is very rapid, as after relatively short occlusions, the rate obtained by counting several respirations or even by measuring the interval between the first two may be greater than the true initial rate, since the afferent paths may begin to be opened up even in this short interval.

<sup>8</sup> LOEWY: *Archiv für die gesammte Physiologie*, 1888, xlii, p. 245.

<sup>9</sup> KATSKHKOWSKY: *Archiv für die gesammte Physiologie*, 1901, lxxxiv, p. 6.

TABLE III.  
OCCLUSION OF HEAD ARTERIES IN CATS.

Date of experiment.	Length of occlusion in minutes.	Time after release in minutes.	Rate of respiration per minute.	Date of experiment.	Length of occlusion in minutes.	Time after release in minutes.	Rate of respiration per minute.
1905.				1907.			
April 19	16	30 <sup>1</sup> 34 37 44	4 12 12 <sup>2</sup> 16	Feb. 23	8	6 <sup>1</sup> 7 11 13 27	5.80 8 20 41.90
April 29	15	8 <sup>1</sup> 11 74 183	16 208 224	2d occlusion	7	3.30 <sup>1</sup> 8.30 19	7.20 14.50 49.10
May 2	30 <sup>3</sup>	14.15 <sup>1</sup> 18.15 21.15 22.15 360	4 3.75 6.30 13.30 84	Feb. 27	18.30	13 <sup>1</sup> 16 53	3.50 3.50 7.50 <sup>4</sup>
May 23	51	31 <sup>1</sup> 41 45	4 24 <sup>2</sup> 30	March 12	9	3.15 <sup>1</sup> 3.35	6 10.90
May 27	45	40.30 <sup>1</sup> 42.30 50.30 57.30 67.30 75.30	3 to 4 3.30 7 7.30 <sup>2</sup> 6.80 7.70	March 13		3.45 <sup>1</sup>	16.80
June 7	31	9 <sup>1</sup> 26 27 32 370	4 13.50 <sup>2</sup> 18 28 112	2d occlusion	7	10 <sup>1</sup>	6.20
1907.				March 18	7	+ 3.30 <sup>1</sup> 6	5 7
Feb. 20	12	6 <sup>1</sup>	4.30	March 19	15.15	7 <sup>1</sup>	6 <sup>5</sup>
Feb. 22	7	17 <sup>1</sup> 23.30	6	March 20	15	11 <sup>1</sup>	7.50
				April 24	8.20	7.40 <sup>1</sup> 9 10	3.20 6.80 9.10 <sup>6</sup>
				March 30	16.10	4	
				2d occlusion	11	11.30 <sup>1</sup> 12.30	4.10 4.10
						15 22 25	4 7 7.20

<sup>1</sup> First respiration.<sup>2</sup> After stopping the artificial respiration.<sup>3</sup> Imperfect occlusion.<sup>4</sup> Both vagi had been cut.<sup>5</sup> Exactly 6 large respirations a minute with apparently an additional small movement between each pair of large ones.<sup>6</sup> This is not the initial rate, as the respiration had returned earlier, at a time not definitely noted.

The constancy of the initial rate in resuscitation does not depend on a constant stimulation of the centre connected with such physical factors of the circulation as the blood pressure, since it is equally observed in animals during resuscitation after a period of cerebral anæmia when the general arterial pressure is much lower than

TABLE IV.  
SECTION OF THE BRAIN ABOVE THE BULB AND OCCLUSION OF HEAD  
ARTERIES IN CATS.

Date of experiment.	Length of occlusion in minutes.	Time after release in minutes.	Rate of respiration.	Remarks.
1907. June 26	13	9 <sup>1</sup> 11	.. 5.5	One minute later stimulation of the brachial causes distinct acceleration of respiration.
July 3	10	33 <sup>1</sup> 39 41 43 47	.. 4.3 4.3 4.3 4.5	Exactly the same rate during stimulation of the brachial or the vagus.
July 10	10	4 <sup>1</sup> 8 11 13 15 20 32 75	.. <sup>2</sup> 4.6 7.0 6.0 6.6 11.0 .. 9.0	After stopping the artificial respiration. Spinal cord divided and aorta clamped below the diaphragm.
<p><sup>1</sup> First respiration. <sup>2</sup> Section of both vagi produces no effect on the respiration.</p>				

normal (Table III), in animals after section of the vagi and the brain stem above the bulb without cerebral anæmia when the blood pressure may be normal, and in animals after cerebral anæmia when the blood pressure has been kept abnormally high by ligation of the aorta just distal to the origin of the head arteries or at any point between this and the lower surface of the diaphragm (Table V). Nor is it necessarily dependent on any particular chemical condition of the blood, its content of carbon dioxide or oxygen for example, since in resuscitation after cerebral anæmia, with artificial respiration of constant rate and depth, the spontaneous respiratory movements return in different experiments at very different intervals after the restoration of the circulation, and the

time of their return and their initial frequency are quite independent of the rate of the artificial respiration or of the total ventilation produced by it. It is easy to see that if the respiratory discharge depends on stimulation by the carbon dioxide of the blood the "excitability" of the centre must be restored before discharge can take place. Perhaps the tension of the carbon dioxide in the centre must be reduced to a certain point before this excitability is restored. In any case the initial rate must, as has already been remarked, be connected with some internal property of the respiratory centre, and the fact that whether the respiration returns early or late in resuscitation, after a long or short period of anæmia, its rate is at first the same, suggests that the property on which this rhythm depends is a very fundamental one. For it is impossible to suppose that in every particular the chemical or physical condition of the respiratory centre is the same at the moment when it first resumes its discharge, whether the occlusion has been long or short, single or repeated, the return of respiration tardy or prompt, the animal deeply or lightly narcotized before the occlusion, the blood at or near the normal temperature or decidedly below it, the animal young or old, a rodent or a carnivore.

An interesting observation illustrating the relation of the circulation to the respiratory centre during resuscitation may generally be made in experiments where the aorta is clamped below the origin of the head arteries, so that a relatively high pressure is restored in the head immediately on release of the head arteries. Respiration, having returned, ceases after a time, as is very often the case also where the aorta has not been clamped, a state of apnœa being induced. When the aorta is now released so as to lower the blood pressure in the head, the respiratory movements soon return, as in the experiment of March 30, 1905 (Table V). It would be of interest to determine whether heat dyspnœa can be obtained immediately after the return of respiration in resuscitation, and whether the rate of discharge of the centre is affected by increase of temperature before the afferent paths are opened up; also whether the time of appearance of the first respiration is influenced by the temperature of the blood. But hitherto I have not been able to test these questions. As the afferent paths, especially the pulmonary vagus paths, begin to open up in resuscitation, there is an increase, often abrupt, in the mean rate of discharge (Table VI in addition to Tables III and V). There is an equally significant diminution

in its stability, stimulation of the afferent fibres not only in the vagi but in the ordinary peripheral nerves affecting it more and more. At a certain stage in resuscitation the influence of the incoming

TABLE V.

OCCCLUSION OF THE HEAD ARTERIES IN CATS AFTER LIGATION OR CLAMPING OF AORTA DISTAL TO ORIGIN OF LEFT SUBCLAVIAN.

Date of exp.	Length of occlusion in minutes.	Time after release in minutes.	Rate of respiration.	Remarks.
1905.				
March 29 (I)	10	5.10 <sup>1</sup> 11.30 21.30	4 4 ..	Half-grown cat. Internal mammaries tied. Jaws, tongue, forelimbs, and ribs move strongly in resp., but not the diaphragm. Stim. of left phrenic causes no cont'n of the diaphragm, nor does direct stim. Resp. now mainly head and jaw move'ts. Released aorta.
		25.30 46.30 48.30	20 .. 20	
March 29 (II)	20	5 <sup>1</sup> 7 11	3 to 5 6 8	
		13 29	12 33.3	
		79 27.30 <sup>1</sup> 30.30 39.30 44.30 76.30 92.30	.. 5 (exactly) 11.2 12 .. ..	
March 30	24.30	95.30 97.30 67.30 <sup>1</sup> 70.30 108.30 119.30	.. 18 .. about 3 5 26	Diaphragm contracts on direct stimulation but not on stimulation of phrenic. The diaphragm does not participate in the movements, though direct or indirect stimulation of it causes contraction. Respiration has ceased. Diaphragm still contracts on stimulation of phrenic. Released aorta. Resp. soon started.
April 3	50	67.30 <sup>1</sup> 70.30 108.30 119.30	.. .. 5 26	Prepared cat.
1907				
March 7	7.15	1.45 <sup>1</sup> +	3.30 6	
Second occlusion ..	..	26.30 <sup>1</sup> 28.30 35	6.2 7.9	
March 29	7.45	+ 1 <sup>1</sup>	4.3	
April 2	5	+ 1.30 <sup>1</sup> +	3.6	After 1½ minutes asphyxia produced by clamping the trachea, respiration is 5 a minute. After 2½ minutes asphyxia, 6.5 a minute.
Second occlusion ..	6	- 4 <sup>1</sup>	..	

<sup>1</sup> First respiration.

impulses from the vagi comes so to dominate the automatic rhythm that in some experiments the natural respiratory movements have been seen to acquire precisely the same frequency as the artificial

TABLE VI.  
EXPERIMENT MAY 13, 1905. CAT. TIME OF OCCLUSION, 60½ MINUTES.

Time after release.	Rate of respiration per minute.	Time after release.	Rate of respiration per minute.
33½ m. <sup>1</sup>	2 to 4	7 h. 47 m.	16
37½ m.	9 <sup>2</sup>	8 h. 19 m.	16
44½ m.	8 <sup>2</sup>	9 h. 15 m.	19
52½ m.	4.3 <sup>3</sup>	9 h. 21 m.	22
3 h. 24 m.	10.7	9 h. 26 m.	18
3 h. 30 m.	14	9 h. 47 m.	30
4 h. 7 m.	14	10 h. 4 m.	30
5 h. 37 m.	14	10 h. 32 m.	19
6 h.	14	11 h. 12 m.	23
6 h. 12 m.	13	11 h. 57 m.	23.5
6 h. 29 m.	14.5	12 h. 47 m.	6.5
6 h. 51 m.	16		

<sup>1</sup> First respiration. The second respiration followed 30 seconds after the first, the next five at intervals of 15, 30, 15, 10, and 12 seconds respectively. Then the rate became nine a minute, half of the respirations being weak and the rest very strong. Each weak respiration began in 7 seconds, and each strong one in 12 seconds, from the beginning of the last respiration.

<sup>2</sup> Strong and weak alternately as before.

<sup>3</sup> Very strong, without any weak respirations interposed.

At 3 h. 12½ m. artificial respiration was stopped. From 9 h. 15 m. till 10 h. 31 m. ether was administered to control the spasms. At 11 h. 12 m. ether was again begun and continued till 15 h. 22 m. after release, when the animal died of ether poisoning.

respiration and to persist at exactly this rate for some time after the stoppage of the artificial respiration, an artificial rhythm having been, so to speak, impressed upon the respiratory centre by the rhythmical excitation of the pulmonary vagus fibres. For example, in the

*Experiment of March 24, 1905*, after an (imperfect) occlusion of 30 minutes in a young female cat, respiration had returned for several minutes,

when it was noticed that the natural respiratory movements were exactly synchronous with the artificial respiration, which was at the rate of 42 a minute. Artificial respiration was now stopped, and the animal went on breathing at precisely the same rate for about 25 seconds. This was 14 minutes after the release of the head arteries. Seven minutes later the spontaneous respiration was going on at the rate of 32.4 a minute; 52 minutes after release it was 75; and 80 minutes after release, 171 a minute. In another cat (a large adult male), after a perfect occlusion of 5 minutes, respiration returned 35 seconds after release. On stopping the artificial respiration,  $8\frac{1}{2}$  minutes after release, the cat went on breathing for  $3\frac{1}{2}$  minutes at exactly the same rate (42 a minute). The respirations then became quicker, increasing to 54 a minute 14 minutes after release, and to 128 a minute  $38\frac{1}{2}$  minutes later. In the experiment of March 22, 1905, in a very large adult male cat, after an imperfect occlusion of  $10\frac{5}{8}$  minutes, spontaneous respirations did not disappear, and soon after release it was observed that they were proceeding at exactly the same rate as the artificial respiration (48 a minute). In the first minute or two after release the spontaneous respiration ceased if the artificial was stopped. This was also the case for the last minute or two of occlusion, as if the excitation of the respiratory centre depended upon the afferent impulses set up in the vagi by the movements of the lungs. When the artificial respiration was stopped,  $5\frac{3}{8}$  minutes after release, the natural respiration went on with exactly the same rhythm for a little time, and then, in less than a minute, began to get quicker.

Much evidence has been obtained in the course of this work that, great as the influence of afferent impulses on the rate and character of the bulbar respiratory discharges is, it is, as a rule, only capable of being exerted when the respiratory centre is already discharging itself automatically. There are, however, conditions in which a perfectly quiescent respiratory centre can be roused to discharge by the excitation of afferent nerves, as in the following experiment.

*Experiment of November 5, 1890.*— Dog weighing 13 kilos received 0.16 gm. morphia hydrochlorate subcutaneously. Five hours later, stimulation of the central end of the anterior crural nerve caused violent respiratory movements. Chloroform was now administered, and the respiration stopped. Stimulation of the anterior crural always causes respiration to begin and to go on again violently for a little time. The animal struggles also, but respiration soon ceases. The pulse is much accelerated. The corneal reflex is well marked, and there are occasional swallowing movements. Obviously the bulbar centres are not all paralyzed. Yet, if left

alone, respiration soon ceases. Air enters the lungs freely, and there is no obstruction in the trachea. If the condition is one of apnoea, it has not been induced by artificial respiration, since none has been employed. The animal was kept alive for more than an hour by occasional stimulation of the anterior crural, a burst of respirations following each stimulation, but spontaneous breathing never returned, although no chloroform was given from the time the respiration first stopped. At last the animal was killed by bleeding. After blood had ceased to flow, respiratory movements began spontaneously and continued for a minute or two.

This seems to show that the respiratory centre, injured too much by the chloroform and morphia to act spontaneously and only discharged itself when stirred up by the arrival of afferent impulses, recovered its natural automatic power when the blood was drawn off, either because some of the still circulating poison was withdrawn, or because the anæmia increased temporarily the excitability of the centre or its power of developing autochthonous stimuli, or the intensity of the "blood stimulus."

S. J. Meltzer and J. Auer<sup>10</sup> have found that magnesium salts produce such an effect on the respiratory centre that the respiration stops, although if the dose is not too great stimulation of the sciatic may cause respirations to be discharged. The fact that in such conditions the respiratory centre can be roused to activity by afferent impulses is, of course, no more an argument against its normal automaticity than the existence of conditions in which a perfectly quiescent heart can be caused to beat by stimulating its augmentor nerves is an argument against the automaticity of the heart's beat. I showed long ago<sup>11</sup> that the frog's heart when reduced to complete standstill by cautiously heating it in salt solution, can be caused to beat by stimulating the cervical sympathetic, even while the temperature necessary for standstill continues to be maintained. A long series of beats ensues which much outlasts the stimulation. This, as well as the distance of the sympathetic from the heart, eliminates the possibility of escape of current on to the heart being the cause of the contractions, — a criticism to which Schelske's observations<sup>12</sup> on the effect of stimulation of the frog's vagus during

<sup>10</sup> MELTZER, S. J., and AUER, J. : This journal, 1905, xiv, p. 366 ; 1906, xv, p. 387.

<sup>11</sup> STEWART, G. N. : Journal of physiology, 1892, xiii, p. 93.

<sup>12</sup> SCHELSKE: Über die Veränderungen der Erregbarkeit durch die Wärme, Heidelberg, 1860.

heat standstill of the heart are justly exposed, since he states that the stimulation causes a tetanic condition and that single induction shocks are followed by single contractions. My observations constitute, I believe, the first satisfactory demonstration that the augmentor nerves of the heart have the power of causing beats when the heart is entirely at rest, although they seem to be unknown to H. E. Hering, who long afterwards<sup>13</sup> demonstrated a similar action of the accelerantes in mammals on hearts reduced to standstill in various ways. He incidentally observed a similar rousing of auricles and ventricles to activity in a dog, some years earlier,<sup>14</sup> but specifically states that he could not tell whether the veins might not still have been pulsating at the time of the stimulation. Hering rightly considers that Schelske's vague statements do not constitute a demonstration of this action for the frog's vagus.

S. A. Matthews<sup>15</sup> has shown that, in the standstill of the dog's heart produced by magnesium salts, stimulation of the accelerantes causes it temporarily to resume beating.

Another analogy, and an interesting one, between the automatic respiratory and the automatic cardiac mechanism is the sudden and strong return of respiration in resuscitation after cerebral anæmia and the sudden and strong return of the ventricular beat under the influence of direct massage in resuscitation after cardiac failure induced by asphyxia, ether poisoning, and in other ways. For example, in the experiment of May 16, 1905, after an occlusion of 81 minutes the heart was found to have stopped about 16 minutes after release. The chest was opened, the aorta clamped below the origin of the head arteries, direct massage of the heart started, and the animal put into a hot-water box, artificial respiration, of course, being kept up all the time. The auricles began to beat very soon after massage was begun, as usually happens, but the ventricles beat very poorly and intermittently. About 20 minutes after the stoppage of the heart and 15 minutes after the starting of massage, the ventricles quite suddenly began to beat well. No further massage was necessary throughout the experiment. More than five hours later the heart was beating excellently, when the experiment had to be stopped. Here it would seem that the nervous

<sup>13</sup> HERING, H. E.: *Zentralblatt für Physiologie*, 1905, xix, p. 129; *Archiv für die gesammte Physiologie*, 1906, cxv, p. 354.

<sup>14</sup> *Ibid.*, 1901, lxxxvi, p. 578; *Zentralblatt für Physiologie*, 1901, xv, p. 683.

<sup>15</sup> MATTHEWS, S. A.: *This journal*, 1907, xx, p. 323.

mechanism, if it is nervous, which conducts the impulses from the auricles to the ventricles when its resuscitation reaches a certain point suddenly begins to conduct, just as the respiratory centre when its resuscitation reaches a certain point suddenly begins to discharge. Under the conditions of our experiments the "all or nothing" law appears to apply to the respiratory centre when isolated from afferent impulses as it normally does to the heart.

We might also consider the fact that the respiratory centre, isolated as described, discharges itself not continuously but with a constant rhythm, — a token that, as regards such excitation as is exerted on it by the blood, it possesses a refractory period.

The relative constancy of the heart rate in the first hours or days after double vagotomy in different individuals of the same species and even in the different mammalian species studied, whatever the rate may have been before section, may also be considered analogous to the relative but more marked constancy of the respiratory frequency after isolation of the respiratory centre either from all afferent impulses or from that most influential contingent which reaches it through the vagi. Of course, greater variations are to be expected in the rate of the heart, which is still in connection with the greater part of its accelerator supply and probably even with a certain portion of its inhibitory supply after section of both vagi, than in the rate of respiration after complete elimination of the afferent paths to the bulbar centre. But still the range is singularly narrow after the immediate effects of the section and of the anæsthetic have passed off. What the respective shares of the accelerator mechanism, whose tone has been investigated by Tschirjew,<sup>16</sup> Friedenthal,<sup>17</sup> Hunt,<sup>18</sup> and others, and of the local regulating mechanism in the heart which many writers assume, may be in bringing about the gradual return of the heart rate towards the normal in dogs which survive for some time, cannot be settled at present. There is some evidence that inhibitory fibres may reach the heart by other paths than the vagi, perhaps issuing from the upper thoracic cord with the accelerator fibres,<sup>19</sup> and these, although perhaps normally of comparatively slight importance, may come to play a more important rôle when the main cardio-inhibitory path has been severed.

<sup>16</sup> TSCHIRJEW: *Archiv für Physiologie*, 1877, p. 116.

<sup>17</sup> FRIEDENTHAL: *Archiv für Physiologie*, 1902, p. 135.

<sup>18</sup> HUNT: *This journal*, 1899, ii, p. 395.

<sup>19</sup> Cf. FRIEDENTHAL: *Loc. cit.*

The rate of the heart isolated from all extrinsic innervation and under given conditions of temperature, coronary pressure, and nutrition, appears to be still more constant in individuals of the same species and in different mammalian groups than the rate after elimination of the vagi alone. Taking the point in resuscitation in

TABLE VII.

Date.	Length of occlusion in minutes.	Time after release in minutes.	Blood pressure in mm. of Hg.	Pulse rate.	Date.	Length of occlusion in minutes.	Time after release in minutes.	Blood pressure in mm. of Hg.	Pulse rate.			
1907. Feb. 27 (cat)	18 30	32 60.30 74	158 124 130	179 163 171	1907. April 5 (rabbit)	6.45 (1)	0 6.45 12 <sup>2</sup> 16.45 18.15	84 58 112 119 120	221 <sup>3</sup> 210 <sup>3</sup> 161 174 170			
Mar. 5 (cat)	4 45 (1)	14.15 <sup>1</sup> 19.15 <sup>1</sup>	124 120	166 161	April 20 (rabbit)		19.30 (1) (imperf.) 6 (2)	8.30 7.45 21 26 <sup>2</sup>	94 110 48 110	182 <sup>3</sup> 166 148 163 163		
Mar. 7 (cat)	7.15 (1)	7.45 18.45 21.15 24.45 38.45 <sup>2</sup>	140 124 120 119 121	150 151 150 144				Mar. 29 (cat I)	7.45	5	184 124	146 <sup>3</sup> 165
(cat II)	9	3.15 6.30	158 60	180 <sup>3</sup> 164								

<sup>1</sup> No reflex inhibition of the heart can yet be obtained.  
<sup>2</sup> Reflex inhibition first obtained.  
<sup>3</sup> Before occlusion.  
 A number in parentheses in the occlusion column indicates whether the occlusion was the first or second in experiments in which there was more than one occlusion.

about forty cats (specimens of the data are given in Table VII) at which both the inhibitory and the accelerator mechanisms have not yet recovered their tone while the heart muscle is still in a tolerably normal condition and the pressure and temperature of the blood are not far from normal, the average pulse rate is between 155 and 160. In the few rabbits which yielded results suitable for this determination the average was between 160 and 165. In an experiment on a dog by Tschirjew after elimination of the vagi and the accelerators with the knife I find the average of all the observations is about 151. But if one selects observations where the blood

pressure was within the normal range, the average is higher (155 to 160). The average rate for the twenty-two dogs referred to in Table II after section of the vagi alone is 163, when only observations made after the primary excitation effect of dividing the vagi has passed off are included. The rate for the dog's heart isolated completely from extrinsic impulses must be less than this on account of the accelerator tone. The average of all the observations on a rabbit quoted by Tschirjew I find to be a little over 165. The suggestion is that, under precisely similar conditions, the hearts of the rabbit, cat, and dog when isolated from the central nervous system beat very much at the same rate, just as the respiratory centres of these animals when isolated from all afferent impulses discharge themselves very much at the same rate. Of course such calculations are open to a certain amount of error, and it is possible that careful experiments specially directed to this point, in which the temperature and blood pressure were exactly controlled might show constant differences even in individuals of the same species. But it can be safely predicted that the range would not be found a wide one even between not too distant groups. And without pressing the analogy too much, it may be considered, perhaps, an additional argument in favor of the idea that the mechanism which originates the cardiac rhythm is of the same nature as the mechanism which originates the respiratory rhythm, namely, a nervous one. That is to say, if the relative constancy of the fundamental respiratory rhythm, that of the isolated respiratory centre, is a deep-seated attribute of nervous structures, the existence of a similar constancy in the fundamental cardiac rhythm, that of the heart isolated from extrinsic impulses, is an indication that the seat of that rhythm may be of nervous nature too.

It has been mentioned that as a rare phenomenon a dog both of whose vagi are cut in the neck at the same time may recover and live for an indefinitely long period, comporting himself to all intents and purposes as a normal dog. I have had one, perhaps two, such cases among more than fifty dogs subjected to simultaneous bilateral vagotomy. One of these unfortunately escaped, owing to the carelessness of an attendant, when all danger seemed to be passed and it was gaining weight rapidly, 24 days after the excision of about an inch of each vago-sympathetic. The other was kept for 300 days and then sacrificed for autopsy. The ends of the left vago-sympathetic were found separated by about  $\frac{1}{2}$  inch. In the right

vago-sympathetic the seat of the section was indicated by a fine transverse scar visible to the eye, and seen on cutting longitudinal sections after hardening in Muller's fluid embedding in celloidin, and staining by Berkley's "rapid" modification of the Weigert-Pal method<sup>20</sup> to be occupied by scar tissue. Scar tissue free from fat cells occupied the part of the sheath opposite the transverse lesion. The section was complete except for a strand of sheath at the outer side of the nerve, which perhaps was responsible for keeping the two portions of the severed nerve so well in line. Some regeneration appeared to have taken place, since stimulation of the nerve with induction shocks, before death, caused slowing and weakening of the heart. This, however, does not account for the recovery of the animal, since all the serious symptoms had disappeared long before any regeneration could have occurred. For the same reason it cannot explain the rapid return of the pulse rate, the respiratory rate and the ratio between them to the normal (Table VIII). When the animal vomited, as it did repeatedly from the eleventh day after the operation till the end of the third month, it always made the sound associated with that act in the normal dog. The other vagotomized dogs made no sound while vomiting, indicating that apparently in this exceptional dog a portion of the innervation of the glottis had escaped. Slapping the dog's sides elicited the sound of vomiting although he did not actually vomit, and he not infrequently made this sound spontaneously without vomiting. When he did vomit, it was usually only frothy liquid and not more or less digested solid food, as in the case of ordinary dogs. These cases of complete recovery were mentioned in an article written in November, 1900.<sup>21</sup> At the Madrid International Medical Congress of Medicine, in April, 1903, Ocaña showed a dog which, ten weeks after simultaneous section of the vagi in the neck, was in perfect health and remained so for more than six months. After death, he states, the complete division of both vago-sympathetics was verified.

Several observers have supposed that when a considerable interval is allowed to elapse between the section of the two vagi the fatal result is postponed. Nikolaides<sup>22</sup> states that two dogs out of three operated recovered completely when the second vagus was cut 47 days and 57 days respectively after the first. He seems to assert

<sup>20</sup> LEE, BOLLES: *Vade-Mecum*, 5th edition, p. 403.

<sup>21</sup> STEWART, G. N.: *American year book of medicine*, *loc. cit.*

<sup>22</sup> NIKOLAIDES: *Zentralblatt für Physiologie*, xiv, p. 197; xv, p. 482.

that as a general rule large strong adult dogs will survive the operation and recover if it be performed in two stages with such an interval between. Whether the comparatively genial climate of

TABLE VIII.

February 5, 1897. Skye terrier. Pulse in the evening before operation 120, respiration 38 (ratio 3.1); morning before operation, pulse 90, respiration 38 (ratio 2.3); after morphia and ether, pulse 63, respiration 23 (ratio 2.7); after exposure of the vagi, pulse 85, respiration 24 (ratio 3.5); after division of first vagus, pulse 86, respiration 19 (ratio 4.5); after division of second vagus, pulse 86, respiration 19.

Days since operation.	Pulse.	Respiration.	Ratio.	Days since operation.	Pulse.	Respiration.	Ratio.
1	{ 153 157 165	12 4 14.3 9.7	12.6 10.9 16.2	25	112	24	4.6
2	{ 130 140 126 128	24.0 28.0 22.0	5.4 5.8	28	80	22	3.6
3	{ 135	30.0	4.5	32	100	20	5.0
4	{ 160 144*	48.0 19.0*	3.3 7.5	35	100	22	4.5
5	{ 119 130	19.8	6.0	40	105	22	4.7
6	{ 146 † 107	25.0 †	5.2	42	112	24	4.6
7	{ 163 † 140	19.0 †	8.5	45	108	26	4.1
9	115	45.0	2.5	49	82	18	4.5
10	{ 150 140	30.0	4.7	56	94	18	5.2
11	140	34.0	4.1	63	102	28	3.6
12	128	28.0	4.6	70	96	20	4.8
13	100	26.0	3.8	77	106	20	5.3
14	114	50.0	2.3	84	104	22	4.7
17	114	50.0	2.3	91	112	24	4.6
18	85	30.0	2.8	124	86	20	4.3
19	112	38.0	3.0	161	90	19	4.7
21	110	30.0	3.6	300	130 †		

\* A large abscess due to infection by the syringe used to inject morphia was opened in the morning. The counts indicated by the asterisk were taken in the afternoon. Before the abscess was opened the pulse, and especially the respiration, were accelerated. In ordinary dogs after double vagotomy no such extraordinary acceleration of the respiration was ever seen.

† Animal excited so that the respiration could not be counted.

Athens may have had something to do with this result I forbear to speculate. Undoubtedly in my experience, as in that of others, a sudden drop in the external temperature or other unfavorable climatic change does sometimes appear to determine the onset of the fatal terminal pneumonia, for example in the experiment of January 9, 1897 (Table I). But I have seen no reason to believe that the result is in any essential respect modified by the lapse of even a long time between the division of the two nerves, provided, of course, it is not long enough for effective regeneration to have occurred. I agree with Friedenthal<sup>23</sup> that such a substitution of the influence of one vagus by the other paths as will render the elimination of the second vagus compatible with long life is not realized, at least under our conditions. For instance, in Experiments 14 and 23 (Table II), 59 days elapsed between the first and the second operation, yet the animals died on the second and fourth days respectively after division of the second vagus. In a bitch whose right vago-sympathetic was cut on January 23, 1901, and the left vago-sympathetic on June 8th of the same year (interval of 136 days), the animal was found dead and in rigor on June 10th. The pulse rate before the anæsthetic was given for the second operation was 88, and the rate of respiration 20 (ratio, 4.4 : 1). Under the anæsthetic before section of the left vago-sympathetic, the rates were 60 and 10 respectively; after section of the nerve 180 and 7 (ratio, 25.7 : 1).

I do not propose to discuss here the somewhat discordant views which have been taken by different observers of the cause or causes of death after double vagotomy. Two main groups may be distinguished, — those who lay stress on the pulmonary changes (so-called "section pneumonia"), so frequently seen at autopsy, and those who emphasize the digestive troubles. There can be no doubt that the immediate cause of death is often the pulmonary lesion, and Pawlow<sup>24</sup> and others have shown that when infection of the lungs by the entrance of swallowed or vomited matter is avoided, as by leaving one recurrent laryngeal intact to innervate the muscles that provide for closure of the glottis, or by first making a double œsophageal and gastric fistula, dogs may survive indefinitely. It may be quite clearly demonstrated, however, in animals which live for some weeks after complete section of the vagi in the neck, that notwith-

<sup>23</sup> FRIEDENTHAL: *Archiv für Physiologie*, 1902, p. 137.

<sup>24</sup> For the literature see KATSKOWSKY: *Archiv für die gesammte Physiologie*, *loc. cit.*

standing the paralysis of the glottis associated with aphonia, no pulmonary symptoms may be present till a day or two before death. The picture presented in these cases is that of an animal suffering above all from alimentary disturbances, difficult deglutition, frequent and persistent vomiting, and progressive emaciation in spite of an appetite which is sometimes ravenous. The animal appears to be slowly starving to death because of the difficulty of swallowing, retaining, and digesting its food. The respiration is, to be sure, very different from the normal in frequency, depth, and type, but there is nothing to suggest that the lungs are the seat of any pathological process. Suddenly the picture changes. Pulmonary symptoms obtrude themselves. The physical signs of consolidation may be detected, and in a short time the animal is inevitably dead. Sometimes, as has been mentioned, the determining cause of the pulmonary lesion seems to be some external circumstance, as a sudden fall of temperature. The idea is exceedingly apt to present itself to the observer that the pneumonia is an accident, an acute intercurrent affection breaking the course of a chronic malnutrition, which in any case must have ended in death, unless the disturbances in the functions of the alimentary canal could have been met, as in Pawlow's experiments, for instance, by direct gastric feeding through a fistula. Of course the vagotomized animal is predisposed to this accident, but there is no definite time after section of the nerves at which it must take place.

The cause of the regurgitation of partially swallowed food which is present in addition to true vomiting has generally been supposed to be the accumulation of the food in the lower part of the paralyzed œsophagus. Cannon<sup>25</sup> states that in the cat elimination of the vagus fibres for the œsophagus only causes a temporary paralysis of the peristaltic movements of the lower part of the tube which is provided with unstriped muscular tissue, although the upper part remains permanently paralyzed. There is no doubt that in the dog swallowing remains difficult, although food can be forced into the stomach after the first few days. The muscles of the mouth and pharynx concerned in deglutition can be observed to contract more persistently than normal, as if struggling with the morsel. There is a good deal of difference in different vagotomized dogs in the case with which food is swallowed. In the exceptional "immune" cases the power of satisfactory deglutition returns early.

<sup>25</sup> CANNON: This journal, 1907, xix, p. 436.

As to the explanation of the existence of such cases, little that is satisfactory can be said. Their rarity is great, and the absence of any sign by which they can be distinguished beforehand are obstacles to their study which one has been unable to overcome. The possibility, a slender one, that exceptionally early regeneration might take place where the severed ends of the nerves or of one of them were specially well situated with regard to each other, led to a series of experiments on twelve dogs, in which one vagus or vago-sympathetic was divided wholly or partially with as little disturbance of the relations as possible and with the sharpest possible edge. In some of the experiments where the nerve was totally divided, the head was kept fixed during and for some time after the operation by mechanical arrangements, so as to prevent retraction of the fragments. The following extracts from the protocols will illustrate the results:

*February 25, 1901.* — Total section of left vago-sympathetic with small, narrow knife, without disturbing relations.

*March 2.* — Killed dog. The ends of the nerve were separated by not more than 6 mm., and lay well in line with each other and in contact with carotid. Stimulation of upper end before death caused no effect on the pupil, and stimulation of the lower end no effect on the heart.

*February 25, 1901.* — Another dog. Divided left vago-sympathetic completely without separating it from the carotid. Neck bent while cutting the nerve, and for some time after.

*March 5.* — Occasionally makes sound of vomiting, although never actually vomits.

*March 6.* — Killed. The ends of the nerve are not separated at all. They lie quite in line and are united by grayish material. A casual examination might not show that the nerve had been divided, although when it is fully exposed the scar is seen. Results of stimulation the same as in the other dog operated on on the same day.

*March 8, 1901.* — Divided left vago-sympathetic with a razor, inserting an aneurism needle between the nerve and the carotid and cutting on the needle. A thin strand of nerve sheath was left uncut.

*March 16.* — The nerve was found united, with only a narrow scar and slight thickening central to the scar. The cardio-inhibitory and pupillo-dilator fibres were quite inexcitable.

In a second experiment on March 8, 1901, the left vago-sympathetic was completely divided in the same way. On March 16 the ends were seen to lie close together, and united by a narrow band of whitish material. The nerve looked as if only a nick had been made in it, although

there is no doubt that it was completely divided. Stimulation had the same effect as in the first dog operated on on March 8.

In a third dog operated on on March 8, 1901, in the same way a gap of about 12 mm. was found on March 12 between the ends. Stimulation of the lower end causes stoppage of the heart, stimulation of the upper end marked dilation of the pupil.

In another dog 6 days after section of the vago-sympathetic stimulation of the lower end caused acceleration of the heart, the inhibitory fibres having degenerated sooner than the accelerators which run in the vagus.<sup>26</sup>

These results confirm the foregone conclusion that preternaturally rapid regeneration of the divided vagus is not the explanation of the exceptional cases of survival. Another suggestion is that a sufficient number of fibres necessary for life may in these dogs, owing to some anatomical vagary, escape section when the vagi are divided. It is known that some of the fibres usually carried in the vagus may run an aberrant course, efferent cardio-inhibitory fibres, for example, being occasionally present in the rabbit's depressor. May it not be that a part of the motor fibres of the larynx and œsophagus usually running in the recurrent laryngeals may leave the main trunk by the superior laryngeals? This would explain the absence of paralysis of the glottis, and the greater efficiency of the deglutition mechanism after vagotomy, which would be sufficient to account for the more favorable position of the animal, particularly if associated with such an abnormal course of, say, a portion of the Hering-Breuer and the cardio-inhibitory fibres as permitted a sufficient proportion of these to be spared.

In connection with this question and also on account of the interest of the problem in other relations, a series of experiments was made in which one vagus was completely divided, while varying fractions of the total cross section of the other were severed, in order to determine how small a proportion must remain uncut to prevent death. J. Steiner<sup>27</sup> stated that in the rabbit the efferent fibres going to muscle (cardio-inhibitory, œsophageal, and laryngeal fibres) occupy a position on the median side of the vagus, while the afferent (pulmonary) fibres which affect the respiration are contained in a lateral bundle, which it is possible to separate with a thin instrument

<sup>26</sup> Cf. SCHIFF: *Archiv für die gesammte Physiologie*, 1878, xviii, p. 172; ARLOING: *Archives de physiologie normale et pathologique*, 1896, viii, 5e serie, p. 75.

<sup>27</sup> STEINER, J.: *Archiv für Physiologie*, 1878, p. 218.

from the median bundle. At the level of the medulla oblongata the cardio-inhibitory fibres run in the middle and lowermost rootlets of the 9th, 10th, and 11th cranial nerves, and the afferent respiratory fibres in the uppermost rootlets. Cadman<sup>28</sup> has shown that in the dog the afferent respiratory fibres and the afferent cardio-inhibitory fibres enter the bulb in the upper rootlet of the 9th, 10th, and 11th cranial nerves. The efferent cardio-inhibitory fibres leave the bulb in the lowest rootlet. In the cat, although the rootlets are more numerous, the various groups of fibres occupy similar positions. Friedenthal<sup>29</sup> has taken advantage of these facts to eliminate all the extrinsic nerves of the heart without causing death.

So far as I am aware, the precise distribution in the vagus trunk of the dog of the various functional groups of fibres is unknown. Judging from the similarity of the paths in the rootlets of the rabbit and dog, it is not very hazardous to assume that the efferent cardio-inhibitory fibres are probably mainly at the median side, and the afferent respiratory fibres mainly at the external side of the nerve. A larger or smaller part of the cross section was therefore divided, beginning either at the median or at the lateral side of the nerve, the amount actually cut being controlled by micrometric measurements on the hardened material obtained *post mortem*. Several dogs were operated on with aseptic technique. In one of these (a young male fox terrier) the vagi were slightly separated with a needle from the sympathetics in the neck. Rather more than half the cross section of the right vagus proper, beginning at the median side, was divided. The partial section of the right vagus caused no change in the pulse rate (138); the respiratory frequency slightly diminished (from 41 to 36). Then the left vagus was completely divided. The effect on the pulse rate (increase to 177) on the respiration (diminution to twelve a minute with deep, prolonged inspiration), and on the voice indicated that all the cardio-inhibitory fibres, many of the Hering-Breuer fibres, and a part of the motor supply of the glottis muscles in the right vagus had been cut. This would suggest that the cardio-inhibitory fibres lie at the median side of the nerve and the Hering-Breuer fibres at the lateral side, as Steiner says is the case in the rabbit. The wound healed by first intention. The animal recovered entirely and was killed for autopsy after 55 days. Among the symptoms in regard to which this animal

<sup>28</sup> CADMAN: *Journal of physiology*, 1900, xxvi, p. 42.

<sup>29</sup> FRIEDENTHAL: *Archiv für Physiologie*, 1902, p. 135.

in the first days after the operation conformed to the classical clinical picture of double vagotomy were the greatly accelerated pulse and the diminished respiratory frequency. A striking difference was the rapid restoration of a normal rate of respiration and a normal pulse rate. (Respiration on the following day 20, pulse rate 126; on the second day 20 and 139; on the fifth day  $22\frac{1}{2}$  and 104 respectively.) As regards the digestive tract, the animal swallowed without difficulty from the first, indicating the escape of a portion at any rate of the œsophageal motor fibres, which therefore do not, it is to be supposed, all lie at the median side of the dog's vagus, as Steiner states is the case in the rabbit. There is some possibility, perhaps, that a larger portion of gullet is provided with smooth muscle which sooner recovers its power of peristalsis in some dogs than in others. Nevertheless there was some gastro-œsophageal disturbance indicated by numerous abortive attempts at vomiting, accompanied by the characteristic sound, repeated at generally increasing intervals during the first four weeks. Actual vomiting or regurgitation, which forms so constant a symptom after total section of both vagi, was never seen. Partial aphonia was present for a while, the attempted whine or bark being shriller than usual. Frequent hawking or coughing was observed from the end of the second till the end of the fourth week, possibly excited by some irritation arising in the partially severed nerve. On the second, third, and fourth days there were several peculiar convulsive attacks, never seen, in my experience, in dogs after total vagotomy. The animal would fall down on the floor on its back, the limbs extended in spasm, with moderate opisthotonus and twitching of the eyelids. Consciousness was not lost. The anal sphincter was relaxed. The heart was decidedly slowed (to 92 a minute immediately after one attack and 96 a minute after another). The animal soon got up, but was weak on its legs for some time. The normal acceleration of the heart towards the end of inspiration was already present on the day after the operation. After total section it is not seen. Apparently a small number of vagal cardio-inhibitory fibres had been spared. The number could not have been large, since the heart after the operation was accelerated to the rate ordinarily seen after total vagotomy. It is interesting that the removal or diminution at the time of the inspiratory acceleration of the cardio-inhibitory tone maintained by this relatively small number of fibres should produce a very distinct effect. However, it has never been demonstrated that the accelerator fibres in the vagus may not be concerned in this acceleration.

Another young dog died in less than three days with the typical symptoms seen after complete section of both vagi, although somewhat less than one third of the total diameter of one vagus proper remained uncut on the external side (total diameter, 71; uncut portion, 23). On the day after the operation the pulse was 164 in the morning, 180 in the evening, and the respiration 8. Vomiting was frequent, and deglutition difficult. Aponia was present.

In other experiments the vagi were divided piecemeal and the effects of successive partial sections on the pulse and respiration observed. Thus in the experiment of January 25, 1905, in a dog under morphia and ether, the following observations were made:

	Pulse	Respiration
Both vago-sympathetics exposed . . . . .	102	25.3
	94	22.6
Cut $\frac{1}{2}$ of left v. s. from outer side . . . . .	96	21.3
Cut $\frac{2}{3}$ of left v. s. from outer side . . . . .	110	21.3
	114	21.3
Cut through left v. s. . . . .	114	17.3
	116	18.6
Cut more than $\frac{1}{3}$ of right v. s. from median side . . . . .	116	19
	116	19
Cut $\frac{2}{3}$ of right v. s. from median side . . . . .	184 <sup>30</sup>	14.6
	182 <sup>30</sup>	13.6
Cut through right v. s. . . . .	184 <sup>30</sup>	13 <sup>-</sup>
	200	12.8

Another set of experiments was made in which the vagus was stimulated above or below the level at which successive partial sections had been made, in order to study the quantitative differences in the effects produced on the pulse rate, the respiration and blood pressure by stimulation of given strength when the proportion of the total cross section of the nerve stimulated was varied.

*Experiment of February 13, 1905*, is an example. A large dog was anesthetized with ACE mixture. The left vagus was isolated from the sympathetic. The sympathetic was cut and the lower end stimulated. No effect on the heart was caused by the stimulation. The peripheral end of the isolated vagus was now stimulated (by shielded electrodes fixed

<sup>30</sup> These pulse counts are somewhat too low, as a weak beat was missed occasionally in the femoral artery, where the pulse was counted. In the last observation it was counted over the thorax.

once for all in position on the nerve) with a strength of current sufficient to stop the heart completely. Made a partial section of the vagus below the electrodes extending a little more than one-third across, beginning at the median side. Stimulated vagus with same current as before; the heart is now only slowed, not stopped. Increased the strength of the stimulus, the heart is stopped. Deepened the cut (Post mortem it was shown by hardening the nerve and measuring the total section<sup>31</sup> and the uncut portion of the section that the ratio between them was 11.8:4.8, so that about three-fifths of the fibres were severed). Stimulation with the weaker current previously used caused no effect whatever on the heart. The stronger stimulation caused standstill. Now made a second cut a little lower down on the nerve than the first, but beginning at the outer side, and dividing about one-third of the total section of the nerve. No effect on the heart is caused by stimulation with the weaker stimulus used before. Now made a third cut one-third of an inch below the second, beginning at the median side of the nerve and involving between one-fourth and one-third of the total section. Stimulation at the same position as before with the stronger stimulus caused stoppage of the heart. Deepened this cut so as to include rather more than one-half the section of the nerve. Stimulation with the stronger current causes stoppage of the heart. Deepened the cut somewhat. The stronger stimulus has now no effect on the heart. Since the various portions of the nerve are still in physical contact with each other, none of the previous inhibitory effects are to be attributed to escape of current below the incisions or to electrotonic currents. A still stronger current causes stoppage of the heart. Deepened the third cut so as to leave only between one-eighth and one-ninth of the total cross section undivided (as shown by *post mortem* measurement). Stimulation with the strongest current has *no* effect on the heart.

In some experiments one vagus or vago-sympathetic was stimulated without dividing it, and then partial sections were made above or below the point of stimulation. The other vagus was either intact or previously divided. The conflicting or conspiring effects of simultaneous excitation of efferent and afferent fibres in the nerve were sometimes analyzed in an interesting way by means of the partial sections. For instance, in the

*Experiment of January 10, 1905*, the left vago-sympathetic of a dog anaesthetized by chloretone and ether was stimulated without division, a

<sup>31</sup> By making outlines of the sections with the camera lucida, cutting out patterns from thin sheet copper and weighing them, and also by measuring various diameters with the eyepiece micrometer.

blood-pressure tracing being taken from the carotid. The relations of the nerve were not disturbed, and the other vago-sympathetic was left intact. Both with weak and strong stimulation slowing of the heart and a slight fall of blood pressure were obtained. The nerve was then partially divided below the level of the electrodes, the cut beginning at the median (vagal) side and extending fully half-way across the vago-sympathetic. Stimulation with the strong stimulus used before caused slowing of the heart and only a slight fall of pressure. The cause of the small effect on the pressure was revealed on deepening the cut, for stimulation was now without effect on the heart rate but caused a marked increase of pressure, the afferent pressor fibres being now permitted to prevail over the efferent inhibitory fibres. The left vago-sympathetic was then completely divided. The pulse was now 146, the respiration 22. Partial section of the vago-sympathetic beginning at the median side reduced these rates to 140 and 20. A further cut left the pulse rate unchanged, but reduced the respiratory rate to 18. On increasing the depth of the cut still further, the pulse rate rose to 150, the maximum seen in the experiment, even after total section of the right vago-sympathetic somewhat later, and the respiratory frequency sank to 14, the minimum rate. Although the increase of the pulse rate to the maximum might seem to indicate that all the cardio-inhibitory fibres had been divided, this was not the case, since stimulation of the nerve above the cut still caused some slowing of the heart, with a slight fall of blood pressure and stoppage of respiration. When the cut was extended still more, stimulation no longer produced any effect upon the heart, but caused a slight fall of blood pressure, after a longer latent period than the fall previously obtained, and probably due to the excitation of depressor fibres.

Good evidence was obtained in several of the experiments that while inhibitory effects can be produced on the heart by excitation of a relatively small proportion of the total number of cardio-inhibitory fibres, the threshold is raised when the proportion is reduced beyond a certain limit, and further that the effect produced is less profound and more transient. The period of recovery of the heart when it has begun to beat again is especially shortened when the number of inhibitory fibres is reduced, even when the stoppage of the heart is as prompt and its complete quiescence as prolonged as before. For this reason the fall of blood pressure caused by cardiac inhibition produced by excitation of a limited number of inhibitory fibres, even where the inhibition is complete, is less persistent than where a greater number of fibres have been excited. Thus, in the experiment of February 7, 1905, the right

vago-sympathetic of a dog anesthetized with morphia and ACE was exposed and the strength of stimulation determined which just caused stoppage of the heart. A partial section was now made below the electrodes, beginning at the median (vagal) side and extending between one-third and one-half way across the nerve. The pulse rate was 86 and the respiratory rate 32.8 before the section, 82 and 27.4 respectively after it. Stimulation of the same strength stops the heart only for an instant. It requires a stronger stimulus to cause stoppage as complete and lasting as long as that caused before section. When the peripheral end of the left vago-sympathetic was stimulated with such a strength of current as caused complete inhibition of the heart, no inhibitory effect was obtained after a section of the nerve below the position of the electrodes, beginning at the lateral (sympathetic) side and dividing everything except about one-tenth of the total cross section of the vagus proper at the median side. The existence of accelerator fibres in the vagus of the dog was clearly demonstrated in many of the experiments, and indications were observed that an effective proportion of them may escape section when all or nearly all the inhibitory fibres have been cut. For example, in the experiment just referred to, when the peripheral end of the vago-sympathetic was stimulated the heart was stopped, and when it resumed beating after stimulation was ended there was distinct acceleration, as is very commonly observed in dogs. A partial section, beginning at the median side and extending nearly one-third across the vagus proper, was now made below the level of the electrodes. Stimulation of the same strength caused the same effects as before. But when the cut was extended so as to sever about one-half of the vagus proper, the same stimulation caused only slight slowing of the heart, although quite as great an after-acceleration as before the section.

One or two similar experiments on partial stimulation of the afferent fibres in the sciatic which influence the blood pressure and the respiration were performed, although not enough to permit any very definite conclusions to be drawn. In a dog anesthetized with chlorotone and ether the central end of the sciatic was stimulated (after section of both vago-sympathetics) with strong induction shocks. The respiration was markedly quickened, then slowed, and there was a slight fall of blood pressure. The nerve was now partially divided centrally to the electrodes. Stimulation of the

same strength as before caused no change in the blood pressure, but the same respiratory effects as previous to the section. On deepening the cut and repeating the stimulation, slowing of the respiration without previous acceleration was obtained. This apparent reversal of the original effect may be due to the escape of a group of inhibitory respiratory fibres, while the fibres which accelerate the respiration have been cut. It is also conceivable, however, that the excitation of a relatively small number of afferent fibres might so affect the respiratory centre as to reduce the rate of its discharge, while stimulation of a larger number of similar fibres might increase that rate, just as stimulation of one and the same group of fibres may have different effects according to the strength of the stimulus.

#### SUMMARY.

1. After double vagotomy in dogs the ratio of the pulse rate to the respiratory rate, which is at first much increased through the quickening of the heart and slowing of respiration, tends to diminish, as time goes on, in animals which survive more than two or three days. This change is due to a fall in the pulse rate, the rate of respiration in the great majority of cases showing no tendency to increase.

2. The relative constancy of the respiratory rate after double vagotomy depends largely on the fact that in the absence of the Hering-Breuer fibres the fundamental rhythm of the respiratory centre becomes predominant.

3. This fundamental rhythm is revealed in the initial rate of respiration in resuscitation after anæmia of the brain and cervical cord before the afferent paths to the respiratory centre have been opened up. The initial rate (about four a minute) has been studied mainly in cats; but it appears to be remarkably constant not only in different individuals of the same species, but in the different mammalian species investigated (dog, cat, rabbit).

4. At a certain stage in resuscitation, after respiratory movements have returned, an artificial rhythm may be impressed upon the respiratory centre through the pulmonary vagus fibres, by inflating and deflating the lungs at a given rate. The breathing becomes exactly synchronous with the artificial respiration, and persists at the acquired rate for some time after the artificial respiration is stopped.

5. The rate of the heart when isolated from its extrinsic nerves by cerebral anæmia is relatively constant when the external conditions (for instance, the temperature and pressure of the blood) are kept constant.

6. The fatal result of double vagotomy in dogs is not obviated when a considerable interval is allowed to elapse between division of the two vagi.

7. In very rare cases dogs may recover completely after simultaneous bilateral vagotomy above the origin of the recurrent laryngeals. This result is not connected with any specially favorable situation of the ends of one or both of the divided nerves which ensures prompt regeneration. It may be due to some anatomical peculiarity.

8. When one vagus was divided completely and somewhat more than one-half of the other (beginning at the median side, and thus sparing to a great extent the Hering-Breuer fibres), a dog recovered completely. When somewhat less than one-third of the total diameter of the second vagus remained uncut (at the external side), another dog died with all the usual symptoms of complete vagotomy.

9. When one vagus is stimulated and partial sections of the nerve increasing successively in depth are made between the electrodes and the heart, evidence is obtained that up to a certain point excitation with a given strength of stimulus produces the same effect upon the heart whether all the cardio-inhibitory fibres in the nerve are stimulated, or only a certain number. Beyond this point the inhibitory effect is diminished, but can be made as great as before by increasing the strength of the stimulus. Beyond this, again, no increase in strength of stimulation suffices to cause the same inhibitory effect. When a relatively small number of cardio-inhibitory fibres is excited, the heart may stop as promptly as when all are excited, but it recovers more rapidly.

I have to thank my friend Dr. F. H. Pike for correcting the proof in my absence.

## XANTHIN AS A CAUSE OF FEVER AND ITS NEUTRALIZATION BY SALICYLATES.

By ARTHUR R. MANDEL.

[From the *Physiological Laboratory of the University and Bellevue Hospital Medical College.*]

IN a former paper the writer<sup>1</sup> showed that a constant relationship exists between the height of fever and the quantity of purin bases eliminated in the urine. The experiments were performed upon patients suffering from so-called surgical or aseptic fevers. In all the cases examined the patients were placed upon a milk diet and comparison was made between the purin elimination before and after the operation.

In one case of hernia, with primary union, no infection, being a true aseptic fever, the purin bases rose from 38 to 60 mgm. as the temperature rose from 98.9° F. to 101.0° F. Morgenbesser,<sup>2</sup> in a research from the chemical laboratory of this institution, has shown that the normal elimination of purin bases in man on a purin-free diet is between 20 and 30 mgm. daily.

In further confirmation of the discovery that fever and high purin base elimination were coincident, the writer showed that xanthin administered subcutaneously to a monkey caused a rise in temperature, and that a strong decoction of coffee caused a fibrile temperature in himself. He called attention to the fact that von Jacksch<sup>3</sup> had found an increase of purin bases in the urine of tuberculous patients. Also Benjamin<sup>4</sup> reports a case of typhoid whose urine contained the large quantity of 0.1 gm. of purin bases.

The writer can confirm Benjamin's work, for he has found as high as 64 mgm. of purin bases in the case of a typhoid patient on a milk diet.

<sup>1</sup> MANDEL: This journal, 1904, x, p. 452.

<sup>2</sup> MORGENBESSER: New York medical journal, April 14, 1906.

<sup>3</sup> VON JACKSCH: Zeitschrift für klinische Medizin, 1902, xlvii, p. 1.

<sup>4</sup> BENJAMIN: Salkowski's Festschrift, 1904, p. 61.

Further, an investigation upon the course of purin excretion in pneumonia, upon a patient on a milk diet, yielded the following results.

TABLE I.  
CASE A. K., PNEUMONIA.

Date of disease.	Average 24 hr. temp.	Purin bases.	Leucocyte count.
	deg.	mgm.	
3	103.8	54	23,000
4	104.6	56	24,000
5	103.6	67	20,000
6	103.8	58	19,000
7	104.4	62	22,000
8	103.9	64	17,000
9	99.8	57	15,000
10	98.8	48	15,000
11	99.2	42	12,000

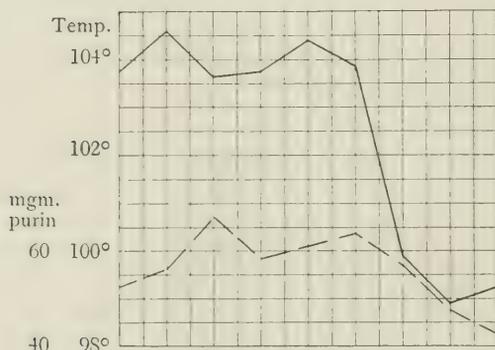


FIGURE 1. — Case A. K., Purin elimination in pneumonia.

It will be noticed that the purin excretion reaches 67 mgm. and in general follows the temperature record of the days of the experiment.

The experiment of injecting a monkey with xanthin, which was mentioned before, lost some of its significance after the publication of a description of a wide diurnal variation of temperature in this

animal.<sup>5</sup> Professor Lusk suggested that another monkey be given a constant diet, and that on certain days xanthin, and on other days xanthin and sodium salicylate, be administered.

A monkey was therefore obtained. His weight was 3.7 kgm. He was kept on a constant diet of one or two eggs, one banana, and about 200 c.c. of milk daily. When the xanthin or the salicylate was given, the banana was split and these substances were spread on the split surface, the two pieces were put together, and the banana was always greedily eaten without loss.

The following chart is characteristic of the results repeatedly obtained:

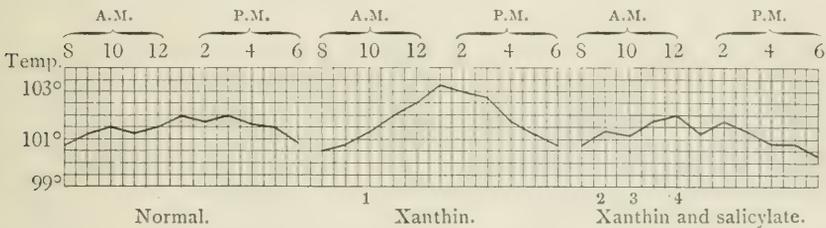


FIGURE 2.—Temperature curves in a monkey. Xanthin, 0.1 gm., was given at 1; salicylic acid, 0.1 gm., at 2; xanthin, 0.1 gm., at 3; and salicylic acid, 0.1 gm., at 4.

The first portion of the chart shows the temperature curve of a normal day. The following day administration of 0.1 gm. of xanthin caused a rise to a temperature 1.6° F. higher than had been observed on the normal day. On the third day 0.1 gm. of sodium salicylate was given at 9 A. M. and again the same at noon. At 10 A. M. 0.1 gm. of xanthin was given. It will be noticed that no rise in temperature followed, such as was the case when xanthin was given alone.

The salicylate administered by itself had no effect upon the normal curves.

The results, however, are not based upon a single experiment. Thus the average of the 1 P. M. readings on twenty-six normal days showed a temperature of 101.9° F. (38.9° C.); of fifteen readings on the days when xanthin was given the average was 103.0° F. (39.4° C.); and of six readings on days when both xanthin and salicylate were administered the average was 102.1° F. (38.9° C.).

The relationships in the average of all the experiments are exhibited in the curves on the following page.

<sup>5</sup> GOLDBRAITH and SIMPSON: *Journal of physiology*, 1903, xxx, p. xx.

It is distinctly evident that xanthin administered at 10 A. M. causes a rise in temperature which may be prevented by the simultaneous administration of sodium salicylate. It would be an interesting question to determine whether xanthin forms a chemical

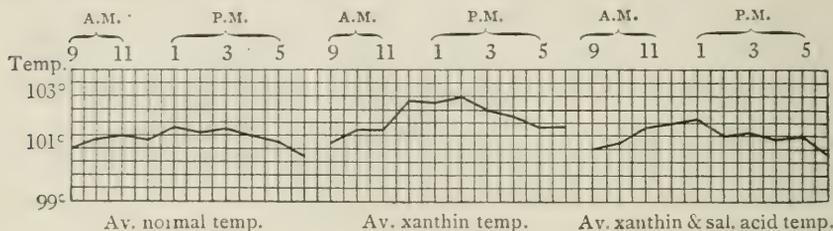


FIGURE 3.

combination with salicylic acid similar to diuretin (= caffeine + salicylic acid), thereby rendering it innocuous.

Experiments with antipyrin failed to reduce the temperature rise caused by administration of xanthin in the monkey.

Neither Professor Lusk nor the writer was able to cause any rise of temperature in himself after taking 0.4 gm. of xanthin. Larger doses would probably have been effective, but were not administered on account of the cost of the substance. It is very probable that a febrile patient would have been more susceptible to the ingestion of xanthin.

In reviewing this work it should be recalled that the writer found a considerable fall in the output of uric acid in aseptic fevers. This fall in uric acid elimination is probably in consequence of a vaso-constriction of the blood vessels of the kidney, thereby decreasing the blood supply to that organ, which is a usual accompaniment of fever.<sup>6</sup> The actual concentration of purins in the blood stream may therefore be far greater than the comparison between the normal and pathological urinary content betokens.

In surgical fevers the purins must be derived from crushed tissue, and they must enter the blood stream without previous oxidation to uric acid. In septic fevers it may well be that toxins lessen the power of some of the body's tissues, the muscles for example, to oxidize xanthin to uric acid, and hence a larger quantity than normal is thrown into the blood. In both cases these purin bases may be a considerable factor in that stimulation of cranial vaso-motor nerve centres, which is part of the syndrome of fever.

<sup>6</sup> MENDELSON: VIRCHOW'S Archiv, 1885, c, p. 274.

It is obvious from this discussion that the use in fever of purin-free milk, instead of purin-containing meat, has its scientific justification.

#### SUMMARY.

1. In fever there is a distinct relationship between the rise in temperature and the appearance in the urine of purin bases.

2. The supposition that purin bases are directly concerned in the production of febrile temperatures, is strengthened by the fact that the administration of xanthin or caffen will effect a rise of body temperature.

3. Sodium salicylate may neutralize the temperature rise produced by xanthin.

#### CORRECTION.

In Table V, page 90, the word "boiled" should read "unboiled," and "unboiled" should read "boiled," throughout the table.

## THE RELATION OF AFFERENT IMPULSES TO FATIGUE OF THE VASOMOTOR CENTRE.

BY W. T. PORTER, H. K. MARKS, AND J. B. SWIFT, JR.

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

### I.

SINCE the bulbar centre deals for the most part with streams of afferent impulses, the physiologist is interested in determining whether at any point fatigue of the nerve cells may prevent these constant stimuli from calling forth or regulating the discharges on which many vital functions depend.

To the clinician and especially the surgeon this question is of supreme importance. The life of the patient hangs on the maintenance of balance or equilibrium reactions, such as the pressure of the blood. These must be held against the violent changes in the intensity or volume of the afferent stream often witnessed in disease or injury. Apparently the vasomotor apparatus in every individual must be forearmed against afferent storms that afflict only the rare unfortunate, and him as a rule but once. Practice with increasing loads, the builder up and habitual protector of reflex mechanisms, is here impossible.

The present research attempts to determine whether this equilibrium can indeed be seriously affected by the prolonged stimulation of afferent nerves.

### II.

The animals used in this investigation were cats, rabbits, and dogs. They were anæsthetized with ether. The carotid blood pressure was written by a membrane manometer or, in some cases, by a mercury manometer.

The following stimulations were made: the central ends of the divided sciatic nerve, the brachial nerves, the posterior spinal roots,

the lumbar branches of the spinal nerves; the great splanchnic, coeliac ganglion, superior mesenteric plexus, gastric branches of the vagi, various parts of the abdominal sympathetic; the testis.

The stimuli were chiefly induction currents. In some experiments they were provided by the ordinary inductorium; in others the primary circuit was made and broken by the Ewald interrupter. The frequency and strength of the induction currents was varied in the different experiments and at times in the same experiment. When the Ewald interrupter was used, from four to six Daniell cells supplied the primary circuit. The duration of stimulation averaged about three hours. The usual precautions were taken to prevent the nerves from drying. The electrodes were not allowed to remain long at the same point upon the nerve.

### III.

The course of the investigation will appear from the following selected protocols:

*June 27, 1905.* — The spinal nerves in the lumbar region were stimulated three hours, fifty-five minutes in a strong female cat, anæsthetized with ether. Five Daniell cells supplied the primary circuit, which was interrupted by the Ewald device twelve times per ten seconds during the first hour and subsequently fifteen times per ten seconds. Stimulation began at 10.50 A.M.; at 2 P.M. the blood pressure had fallen but 20 mm. Hg.

*July 10, 1905.* — A cannula was placed as usual in the carotid artery of a cat anæsthetized with ether. There was a considerable loss of blood through a defective seam in the rubber cannula-tube. The posterior root of the IV lumbar nerve was stimulated three hours. The rate was eleven induction currents per ten seconds. The currents were recognizable but not painful when the electrodes were placed on the tongue. In spite of the prolonged stimulation following the hæmorrhage noted above and the severe operation upon the spinal cord, the blood pressure fell no more than in a control animal subjected to the same manipulations except stimulation of the nerves.

The stimulation of the posterior root of a lumbar nerve was repeated in three other cats, but there was no significant reduction of the blood pressure.

This closed the first series of experiments, nineteen in all. In none was there a significant fall of blood pressure following prolonged stimulation. As the results were negative, they were not

immediately published, although the number and character of the experiments hardly admit a doubt as to the conclusion drawn from them.

Additional evidence follows:

*November 5, 1906.* — In an etherized dog, in which the carotid blood pressure was written, repeated efforts were made to lower the blood pressure by crushing the testes, but wholly without effect.

*November 12, 1906.* — In an etherized dog the prolonged stimulation of the testes with strong and with weak tetanizing currents did not lower the carotid blood pressure, nor did repeated crushing of the testes half an hour later. The animal was then curarized, and the brachial and sciatic nerves stimulated at intervals for two hours and forty minutes, but no significant fall of blood pressure was observed.

The following year, still other experiments were made. In these, anaesthesia was obtained by dividing the brain anterior to the pons; the possible influence of ether anaesthesia was thus eliminated.

*September 6, 1907.* — The large hemispheres of an etherized cat were divided transversely above the pons by a blunt seeker introduced through a small trephine hole. The ether was then discontinued. At intervals of one or two minutes, strong currents from the inductorium were passed through the central end of the sciatic nerve for thirty seconds. This continued from 11.30 A. M. to 1.30 P. M. At the beginning the blood pressure was 80 mm.; on stimulation of the sciatic it rose to 105 mm.; two hours later the blood pressure was 60 and rose to 113 mm. on stimulation.

On September 9 a similar though shorter stimulation in a rabbit also failed to produce a significant fall in the blood pressure.

#### IV.

The numerous stimulations just described uniformly failed to give a significant fall in blood pressure, nor have we been able to find in the literature any instance in which the stimulation of a nerve produced such a fall in the normal animal.<sup>1</sup>

It is true that cases are of record which seem at first glance to contradict this statement. The following are examples:

<sup>1</sup> It need hardly be said that the temporary fall produced by the stimulation of depressor fibres is not to be classed with the abnormal sinking of the blood pressure discussed in this paper.

"After a control had been taken, in which the blood pressure was 146 mm., the animal [dog] was subjected to manipulation, operation on the skin, including extensive removal, irritation of the sciatic nerve and the nerves of the tracheal plexus, and finally opening of the abdomen and manipulation of the intestines during the period of one hour."<sup>2</sup> The blood pressure fell to 20 mm.

In another experiment,<sup>3</sup> "tearing, crushing the brachial plexus, sciatic nerve, abstracting six ounces of blood, and performing pylorotomy reduced the dog to profound shock. Respiration was irregular. The blood pressure fell to 36 mm."<sup>4</sup>

In the large class of experiments which these citations illustrate, a combination of procedures, including the stimulation of nerves, is followed by a fall in the general blood pressure. It is impossible to draw correct conclusions from such experiments because they confuse hydrostatic, chemical, and nervous phenomena.

Exposure of the intestines inevitably dilates the blood vessels in the largest vascular area in the body. The general blood pressure thereupon necessarily falls. Primarily, this is simply an hydrostatic phenomenon, identical with the fall in arterial pressure produced in a rubber and glass model of the circulation by lessening the peripheral resistance. It may indeed be very dangerous—a rabbit may be bled to death in its own portal system by dividing both splanchnic nerves—but the cause of death is anæmia of the bulbar cells; a local anæmia. The removal of large portions of the skin acts also primarily in this hydrostatic way, by dilatation of extensive vascular areas.

The escape of blood from the arteries and their capillary terminals either outside the body or into the veins produces not merely an hydrostatic fall in the general blood pressure, but if the dislocation be excessive deprives the bulbar cells of oxygen by removing large quantities of hæmoglobin. It is for this reason that animals exposed to a very low blood pressure cannot be recovered by the use of normal saline solutions. The bulbar cells are permanently injured by very brief oxygen starvation. Only when red corpuscles are present in sufficient numbers can normal saline injections rescue the asphyxiating cells by driving the hæmoglobin carriers to the bulb.

<sup>2</sup> CRILE, G. W.: Blood pressure in surgery, 1903, Experiment 45, p. 62.

<sup>3</sup> CRILE, G. W.: *Loc. cit.*, Experiment 51, p. 67.

<sup>4</sup> CRILE, G. W.: *Loc. cit.*, p. 67.

Such experiments as the two just cited afford therefore no evidence that the stimulation of the afferent nerves produced the observed fall in the blood pressure.

Another class of observations subject to a serious error of interpretation is well illustrated by a case recorded in an interesting paper by Dr. Harvey Cushing. In operating on a sarcoma, a "mass of glands in the neck had been freely exposed by the high incision and was readily enucleated. Several large branches of the brachial plexus, however, were spread out over the growth, and a secondary division of this portion consequently was necessitated. When this was done, the patient's radial pulse immediately became impalpable. It continued thready and almost imperceptible during the remainder of the operation, which was rapidly completed, and for almost twenty-four hours afterwards."<sup>5</sup>

Such occurrences are often attributed to a reflex lowering of the blood pressure through the action of afferent impulses on the vasomotor centres. Unfortunately it is very difficult to obtain in the human subject conditions sufficiently uncomplicated to withstand rigid criticism. There is reason to believe that Dr. Cushing's patient suffered primarily from reflex inhibition of the heart. An entirely parallel instance occurred recently in this laboratory in an experiment by Mr. Russell Richardson, who is studying with Dr. Porter the effect of afferent impulses upon the vasomotor centres in different vertebrates.

*November 6, 1907.*—A rat was etherized and the carotid pressure written with a membrane manometer. A small quantity (0.75 c.c.) of very dilute curare solution was injected slowly into the external jugular vein. The blood pressure was now 70 mm. The difference between diastolic and systolic pressure was about 20 mm. On stimulating the brachial nerves the individual heart beats almost disappeared from the curve, the blood pressure fell 20 mm., and the writing lever traced an almost unbroken line. On injecting saline solution, the heart improved, and the difference between diastolic and systolic pressure rose to about 15 mm. An effort was now made to stimulate the central end of the already divided sciatic nerve. When the severed nerve was gently raised upon a thread, the heart again failed and the above phenomena were repeated. Thirty-six minutes later, a saline injection was given, the heart gradually recovered, the blood pressure rose to 110 mm., and stimulation of the brachial and sciatic nerves caused a rise of about 20 mm. Hg.

<sup>5</sup> CUSHING, H.: *Annals of surgery*, 1902, xxxvi, p. 324. The experienced will not think we are attacking Dr. CRILE and Dr. CUSHING because we find useful illustrations in their protocols.

Similar occurrences were noted in other animals during curare poisoning.

Cases like Dr. Cushing's, in which reflex inhibition of the heart cannot be excluded, should not be accepted as evidence of vasomotor fatigue.

We have not been able to find reliable instances in which the stimulation of afferent nerves produced a significant fall of blood pressure in the normal animal.

## V.

Nor have we been able to find acceptable instances of vasomotor fatigue in animals not to be classed as normal.

It has sometimes been urged that vasomotor fatigue is indicated whenever the blood pressure falls upon the stimulation of the sciatic or the brachial nerves instead of rising, as it normally does. But it has long been known that certain drugs, such as curare, chloral, and strychnine, may convert the usual rise into a fall, and that this abnormal reaction may rapidly give place to the reflex rise commonly observed. Instances of this have been recorded in this laboratory in a research in which Dr. Porter and Dr. Clark have succeeded in demonstrating specific differences between the bulbar and the spinal vasomotor cells. Yet we have seen no instance in our own experiments, or in the literature, in which the blood pressure suffered more than the usual temporary fall in cases uncomplicated by inhibition of the heart, hydrostatic reduction, or anæmia of the bulb.



## FURTHER OBSERVATIONS ON THE RELATION BETWEEN BLOOD PRESSURE AND RESPIRATORY MOVEMENTS.

BY C. C. GUTHRIE AND F. H. PIKE.

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

ABOUT one year ago we<sup>1</sup> reported some experiments on the effect of changes in blood pressure upon respiratory movements. We concluded that, under the conditions of our experiments, the usual effect of any considerable increase in blood pressure is an increase in the respiratory rate with diminution in the amplitude of the movements during the period of high pressure, while a fall in pressure causes a slower rate with increased amplitude. The changes in amplitude are constant, but the changes in the rate are not always constant. We supposed that, in the production of the phenomena which we observed, there might be two factors, possibly acting in opposite directions, — one in the high blood pressure itself, tending perhaps to increase the activity of the respiratory centre, and the other the lower CO<sub>2</sub> content of the blood, since the same volume of blood now supplies but about one half of the tissues of the body, tending to decrease the activity of the centre. The pressure we believed to be the constant factor, and the CO<sub>2</sub> content of the blood to be the variable. In the absence of positive evidence we suggested that the seat of action might be either peripheral or central, but inclined to the view that it was central.

Our conclusions have recently been questioned by Eyster, Austrian, and Kingsley.<sup>2</sup> In order to determine whether our results were due largely to stimulation of afferent nerves in the region of the thoracic aorta, as these authors allege, or whether the same results could be obtained under conditions precluding such a stimulation of afferent nerves, we made some additional experiments, which we present in this paper.

<sup>1</sup> GUTHRIE and PIKE: This journal, 1906, xvi, p. 475.

<sup>2</sup> EYSTER, AUSTRIAN, and KINGSLEY: This journal, 1907, xviii, p. 413.

## TECHNIQUE OF THE EXPERIMENTS.

The animals were anæsthetized with ether, and tracheotomy performed. The respiratory tracings were taken by means of a Marey-recording tambour connected with the tracheal cannula in the manner described in our previous paper. The right carotid artery was ligated, closed with a bull-dog clamp some distance below the ligature, and a nick made in one side as for the insertion of a cannula. A sound similar to that employed by Chauveau and Marey<sup>3</sup> and by Fredericq,<sup>4</sup> fitted at the lower end with a small rubber bulb which could be expanded or relaxed at will, was now pushed down the carotid into the descending portion of the thoracic aorta. The sound was of such a calibre that it could be readily introduced into a dog's carotid, and it did not interfere materially with the circulation in the aorta when the bulb was not expanded. The circulation below the end of the sound could be restored after temporary occlusion by relaxing the bulb. All portions of the tubing connecting the tracheal cannula, tambour, and ether bottle were adjusted so as to give a good excursion of the writing-point of the tambour, and left untouched throughout the remainder of the experiment. The degree of anæsthesia was the same before and after the occlusion of the aorta, and at no time was the corneal reflex present. A tracing of the normal respiration was first taken, and then the bulb at the lower end of the sound was expanded so as to occlude the thoracic aorta. The position of the bulb was determined *post mortem*.

## THE EXPERIMENTAL RESULTS.

The results were similar in all respects to those previously reported. In all experiments the depth of the respiration decreased immediately after expanding the bulb at the lower end of the sound. The rate suffered either no change or a slight quickening during the occlusion of the aorta. After reopening the lumen of the aorta, independently of any change in the rate, there was always a marked increase in amplitude over that observed before occlusion as well as over that observed during occlusion. It is worthy of note that the same variations in the depth of the respiration occurred during

<sup>3</sup> MAREY: cited by MORAT ET DOYON, *Traité de physiologie*, Paris, 1899, iii, p. 17.

<sup>4</sup> FREDERICQ: *Archives de biologie*, 1890, x, p. 131.

the occlusion as occurred before it (Fig. 1). The respiratory rate observed in a typical experiment for twelve-second intervals before and during occlusion, and after the relaxation of the bulb, are given

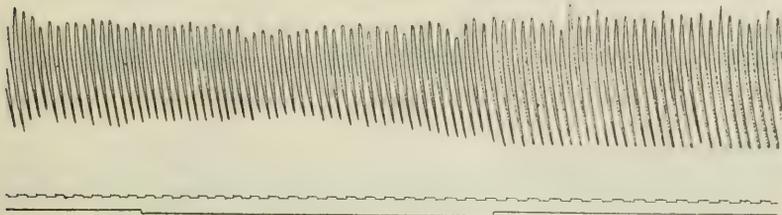


FIGURE 1.—Two fifths the original size. Showing the effect upon respiratory movements of occluding the thoracic aorta by means of a sound. The depression of the signal marks the period of occlusion and high blood pressure. Time trace in seconds. Normal respiration shown in first part of tracing. Vagi intact.

below in Table I. The position of the bulb was slightly below the level of the diaphragm.

TABLE I.

Time of observation.	Respiratory rate.	Time of observation.	Respiratory rate.
1. Before occlusion .	13 in 12 sec.	4. During occlusion .	14 in 12 sec.
2. During " .	13 in 12 sec.	5. After relaxation .	13 in 12 sec.
3. During " .	13 in 12 sec.	6. After " .	13 in 12 sec.

Essentially the same results were obtained after double vagotomy (Fig. 2).

DISCUSSION OF THE RESULTS.

Certain errors, in our opinion, crept into the work of Eyster, Austrian, and Kingsley. It has not been shown that the rate and depth of respiration are in all respects the same during artificial respiration with Brauer's<sup>5</sup> apparatus, after opening the thorax, and during normal respiration with the thorax intact. Again, the authors do not state the manner of clamping the descending aorta at the upper part of its course. It should be pointed out that this is near the place of distribution of the endings of the depressor fibres, and that compression either by a ligature or by hæmostatic forceps might well set up a stimulation of afferent nerves which would complicate or obscure the results due to changes in blood

<sup>5</sup> BRAUER: Mittheilungen aus den Grenzgebieten der Medizin und Chirurgie, 1904, xiii, art. xviii.

pressure. Indeed, the practice of clamping the aorta in this region by means of hæmostatic forceps causes so much disturbance in the cardiac rhythm that we abandoned it in our experiments on the effect of blood pressure on cardiac activity.<sup>6</sup> Thrusting a pair of hæmostatic forceps into the thoracic cavity in almost any region where they will come in contact with the lungs or diaphragm is almost certain to cause disturbances in the heart rate. We found

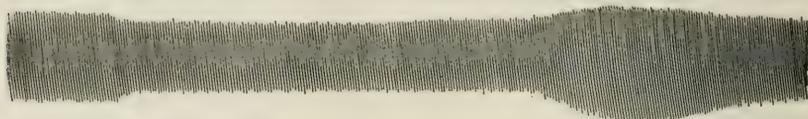


FIGURE 2.—About one-half the original size. Same as Fig. 1, except that both vagi have been divided.

that a carefully placed ligature drawn up through a glass tube with smooth ends might be tightened sufficiently to occlude the aorta well down toward the diaphragm by drawing against the end of the tube without introducing any cardiac irregularity traceable to direct stimulation of the nerves. The open thorax and the stimulation of afferent nerve endings must, therefore, be admitted as two possible sources of error.

Another element to be considered when the aorta is ligated high in the thorax, as in Eyster, Austrian, and Kingsley's experiments, is the great diminution of oxygen-consuming tissue. It is evident that the consumption of oxygen and the production of  $\text{CO}_2$  would be much less, and we should expect, as we have pointed out above, that the respiratory activity would be greatly diminished if this were the only factor in the case. That this tendency to depression should be sometimes greater than the opposite tendency towards stimulation is a reasonable supposition, particularly when the aorta is ligated high up. A diminution both in rate and amplitude is not, therefore, cause for great surprise.

It has been objected that our first method of occluding the thoracic aorta is a frequent source of error, since traction upon the ligature causes a stimulation of afferent nerves. That sufficiently

<sup>6</sup> GUTHRIE and PIKE: This journal, 1907, xviii, p. 14.

strong traction upon a ligature passed about the vertebral column, but not including the aorta, may cause enough stimulation of afferent nerves to affect the respiration, we do not doubt. But that a reasonable degree of traction, equal to the lateral pressure of the blood in the aorta plus a little more than the elastic resistance of the wall of the vessel, will necessarily do so, we are not so ready to admit, particularly when the animal is properly anesthetized, — that is, insensible to pain. But whatever the effect of such a ligature upon the afferent nerves, it is most probably avoided by the use of the aortic sound. The only sources of error due to stimulation of afferent nerves are (1) excitation of sensory nerves in the wall of the aorta at the part occluded by the sound, and (2) the stimulation of peripheral endings by the suddenly increased blood pressure in parts of the vascular system which are still open. As a matter of fact, we see little reason for distinguishing between the two. If this should prove to be a source of error in our experiments, it is obvious that it would apply also to any experiments, such as the unpublished experiments of Eyster, in which the general systemic blood pressure is raised by ligation of all or part of the cerebral arteries, and the results there stated would not, therefore, be due solely to the circulatory changes, but would be complicated by the effects of the stimulation of the afferent nerves. It has been shown also that respiration may persist for a time after the blood pressure in the circle of Willis, as measured from the peripheral end of one carotid, has fallen to the base line.

If there is no direct evidence to show that the respiratory centre is affected by changes of blood pressure *per se*, there is also no evidence to show that the increased blood flow *per se* is the sole regulating factor in respiratory activity. It is scarcely necessary to say that a crucial experiment separating definitively the effects upon the respiratory centre of blood flow from those of tension or pressure has not yet been performed. Indirect evidence,<sup>7</sup> drawn from experiments upon the heart ganglion of *Limulus*, is in favor of the independent effect of pressure upon the respiratory centre.

We have not made any measurements of the respired air during the period of high blood pressure, nor did we make any predictions as to the relative amounts of air required during periods of high blood pressure and normal pressure. It would be surprising, indeed, if an animal whose oxygen-consuming tissue was reduced to

<sup>7</sup> CARLSON: This journal, 1907, xviii, p. 149.

only one half or one third of the total tissues during occlusion of the aorta should respire as much air at this time as it required under normal conditions. But in order to show that the increased blood pressure exerts a depressing action on the respiratory centre, it is necessary to show that that portion of the tissues supplied with blood actually receive less oxygen, as measured by the total ventilation of the lungs, during the period of high blood pressure than they would receive during normal blood pressure. Eyster's statement that a diminution in the rate as well as in the amplitude of respiration occurs during the period of increased blood pressure, or that the total ventilation of the lungs is less at this time than when the pressure is normal, lacks the quantitative exactness necessary to prove his contention that the increased blood pressure depresses the activity of the respiratory centre.

In the light of the present series of experiments we are, for the present, content to abide by our original conclusions that (1) "the amplitude or force of the respiration, as indicated by the excursion of the writing-point of the tambour, usually is less during high pressure, but shows a constant augmentation when the pressure falls"; and (2) "that, under the conditions of our experiments, the usual effect of any considerable increase in blood pressure is an increase in the respiratory rate with diminished amplitude, while a fall in pressure causes a slower rate with greater amplitude." We do not believe that these results are due, in all cases, to our method of occlusion.

## FURTHER STUDIES ON THE RELATION OF THE OXYGEN SUPPLY OF THE SALIVARY GLANDS TO THE COMPOSITION OF THE SALIVA.

By A. J. CARLSON AND F. C. McLEAN.

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

**I**N a recent communication to this Journal by Carlson, Greer, and Becht,<sup>1</sup> it was shown that when, during the stimulation of the dog's chorda tympani, the blood supply to the submaxillary gland is artificially diminished to the same degree as during the stimulation of the cervical sympathetic, the percentage composition of the chorda saliva becomes identical with that of the sympathetic saliva. By greatly diminishing the oxygen supply to the gland during activity following chorda stimulation the rate of secretion is greatly diminished, and the percentage of organic solids in the saliva increased to a point equal or exceeding that of ordinary sympathetic saliva. These results are contrary to Heidenhain's observations, but in view of the fact that they were obtained constantly the conclusion seemed inevitable that the cause of the normal difference between saliva obtained by stimulation of the cranial secretory nerves and that following the stimulation of the cervical sympathetic is the difference in the vascular condition of the gland rather than a difference in the character of the cranial and sympathetic secretory nerves. The existence of true sympathetic secretory fibres to the dog's submaxillary gland was also demonstrated, and evidence adduced that on abundant oxygen supply to the salivary gland the sympathetic saliva becomes just as dilute as that obtained on stimulation of the chorda or of Jacobson's nerve.

These facts seem to render the theory of trophic secretory nerves superfluous. But, as pointed out in that report, one apparent fact still remains to be brought into line with the above results before the theory of trophic secretory nerves as stated by Heidenhain can

<sup>1</sup> CARLSON, A. J., GREER, J. R., and BECHT, F. C.: This journal, 1907, xx, p. 180.

be dispensed with entirely. Heidenhain's experiments seemed to show, namely, that the stimulation of the cervical sympathetic increases the percentage of organic solids in the subsequent chorda saliva. The data reported by Carlson, Greer, and Becht are not conclusive on this point. They show, to be sure, that a previous stimulation of the sympathetic does not invariably increase the percentage of organic solids in the subsequent chorda saliva. But some precautions necessary to render the results conclusive were not taken in those experiments, as they were carried out with a different purpose. The main purpose of the experiment now reported was the examination of Heidenhain's observations touching this question, and to determine whether this supposed effect of the sympathetic stimulation on the subsequent chorda saliva can be duplicated by previous anemia of the gland secured by other means than the stimulation of the sympathetic. We may say at the outset that we failed to confirm Heidenhain's observations. His results were due to experimental errors, as will be made evident in this report.

Further data are also required on the variations of the organic solids in the chorda saliva obtained during a single period of stimulation, a period of stimulation yielding from ten to fifteen c.c. of the saliva. It is well known, from the researches of Ludwig<sup>2</sup> and Heidenhain, that the chorda saliva grows poorer in organic solids *pari passu* with the increasing fatigue of the gland. Does this relation obtain in the case of the first five or ten c.c. of saliva yielded by the gland on chorda stimulation? It was shown by Carlson, Greer, and Becht in the previous report that after a period of gland rest the first c.c. or half c.c. of saliva is much richer in organic solids than the subsequent two or three c.c. In fact, the concentration of the organic solids in the first c.c. of chorda saliva following a period of rest is usually as great as that in ordinary sympathetic saliva. In the case of a single period of stimulation yielding five c.c. of saliva, does the same relation as regards the organic solids obtain between the fifth and the fourth c.c. as between the first and the second c.c.? This point is obviously of importance, because, if we have such a gradual decline in the percentage of organic solids during any single period of stimulation, it is necessary to use exactly the same amount of saliva, secured at exactly corresponding stages in the gland activity, when comparisons are to be made of the

<sup>2</sup> BECHER and LUDWIG: *Zeitschrift für rationelle Medizin*, 1851, N. F. i, p. 278.

percentage composition of saliva under varying conditions of the gland.

Unless otherwise stated, the same experimental methods are followed as in the work by Carlson, Greer, and Becht.

### I. THE GRADUAL DIMINUTION IN ORGANIC SOLIDS OF THE CHORDA SALIVA DURING A SINGLE PERIOD OF STIMULATION.

The data from our several experiments on this point are given in Table I. In Experiment 5 the organic solids in the second sample are less than that in the third sample, but in the other experiments there is a progressive diminution in the organic constituents during the period of secretion. In Experiments 1 and 4 the difference be-

TABLE I.

Submaxillary chorda saliva. Dog. Variations in the percentage composition of the saliva yielded in a single period of chorda stimulation with the weak interrupted current. The three samples represent the total amount of saliva yielded by the stimulation, except in Experiments 6 and 7.

No. of experiment.	Order of samples of saliva collected.	Quantity of saliva in c.c.	Solids per 100 c.c. saliva.		
			Total.	Inorganic.	Organic.
1	First	0.95	1.36	0.31	1.05
	Second	7.05	1.28	0.55	0.73
	Third	10.00	1.25	0.57	0.68
2	First	0.99	1.63	0.25	1.38
	Second	4.85	1.53	0.31	1.22
	Third	9.80	1.37	0.51	0.86
3	First	1.17	1.58	0.23	1.35
	Second	5.00	1.40	0.38	1.02
	Third	9.85	1.33	0.48	0.85
4	First	1.04	1.34	0.32	1.02
	Second	4.80	1.23	0.52	0.71
	Third	9.00	1.05	0.36	0.69
5	First	1.02	1.49	0.34	1.15
	Second	4.92	1.21	0.55	0.66
	Third	9.75	1.45	0.60	0.80
6	First	1.10	2.07	....	....
	Second	4.82	1.85	0.25	1.60
	Third	5.10	1.55	0.30	1.25
7	First	1.05	2.00	....	....
	Second	4.68	2.10	0.36	1.74
	Third	5.00	1.84	0.59	1.25

tween the second and third sample is very slight, however. In Experiments 2, 3, 6, and 7 the difference is considerable.

This gradual diminution in the organic solids during a single period of stimulation is, at least in part, independent of the rate of secretion. According to Heidenhain, the stronger the stimulation of the chorda, and therefore the greater the rate of secretion, the greater the percentage of organic solids in the saliva, other things being equal. Even if this is a fact, it cannot be the cause of the difference between the first c.c. and the next two or three c.c. of saliva in our experiments, because by varying the strength of stimulation the first c.c. may be secured at a slower rate than the following sample, and yet the first sample is richer in organic constituents. When the strength of the stimulation is so graduated that the gland is to yield only fourteen or fifteen c.c. of the saliva in that period, the last five c.c. will necessarily be secreted at a slower rate than the first or second samples. But the same difference in the percentage of organic solids of the second and third samples appears even when the strength of the stimulation is varied so that the rate of secretion remains nearly constant. This was done in Experiments 6 and 7, Table I.

It is not within the scope of this report to consider the cause of this variation. The rate of diminution of the organic solids appears in some cases to be too great to be due solely to actual diminution in preformed secretion granules in the gland cells, although this is in all probability one of the factors. Now, inasmuch as the composition of the chorda saliva varies from one drop to another during any single period of stimulation, and furthermore, since its composition varies with the length of the intervals of rest between any two periods of stimulation, it is evident that comparisons between the composition of samples of saliva from the same gland may lead to faulty conclusions unless these facts are taken into consideration. It is necessary that the gland have equal periods of rest preceding the periods of activity, that the oxygen supply remain the same on stimulation, and that comparisons be made between the same quantities of saliva secured during corresponding stages of the period of gland activity.

## II. THE INCREASE IN THE ORGANIC CONSTITUENTS OF PILOCARPIN PAROTID SALIVA ON STIMULATION OF THE CERVICAL SYMPATHETIC AND ON ARTIFICIALLY DIMINISHING THE OXYGEN SUPPLY.

The work of Carlson, Greer, and Becht on the relation between the blood supply to the gland and the composition of the saliva was confined to the submaxillary gland of the dog and the cat. No work was done on the parotid gland. There is this difference between the submaxillary and the parotid gland of the dog, the cat, and the rabbit, that the cervical sympathetic always causes secretion from the former, but none at all or at the most a barely perceptible secretion from the latter. Yet we know, from the work of Heidenhain and others, that by stimulation of the sympathetic in the neck during the stimulation of Jacobson's nerve or during the secretion of the parotid under pilocarpin, the flow of saliva is diminished and the percentage of organic solids contained in it greatly increased. Heidenhain considers this fact as a most striking evidence of the presence of trophic secretory fibres to the parotid gland in the cervical sympathetic; and so it is, to be sure, unless it can be shown that this effect is not due to the diminished blood supply to the gland caused by the sympathetic stimulation. Heidenhain found, apparently, that diminished blood supply does not alter the composition of the saliva, and most of his experiments touching this point were made on the parotid gland.

There is little doubt that what is true for the submaxillary gland is also true for the parotid; and the results of Carlson, Greer, and Becht on the former show that typical sympathetic saliva is yielded on chorda stimulation, provided the oxygen supply to the gland is greatly reduced. We decided, however, to repeat the experiments on the parotid, partly in hope of learning how Heidenhain came to get only negative results. As Jacobson's nerve is rather difficult to isolate for direct stimulation, we made use of intravenous injection of pilocarpin for producing gland activity.

Care was taken, in collecting the saliva, to secure pure or true samples. When the change is made from the normal pilocarpin saliva to the pilocarpin saliva secreted during sympathetic stimulation or during venous or arterial occlusion, it is not enough to remove the normal saliva from the collecting cannula, but it is also necessary to discard the first two or three drops yielded under the

new conditions, as there is at the start at least that quantity of normal saliva remaining in the passages of the gland from the preceding activity.

TABLE II.

Parotid saliva following intravenous injection of pilocarpin. The effect on the percentage composition of stimulation of the cervical sympathetic (Nos. 1-4) and of diminution of the oxygen supply to the gland by occlusion of the gland veins or the gland arteries (Nos. 5-10).

No. of experiment.	Animal.	Character of the pilocarpin saliva.	Solids per 100 c.c. saliva.		
			Total.	Inorganic.	Organic.
1	Cat	1. Normal	3.23	0.62	2.61
		2. During symp. stim.	4.61	0.17	4.44
2	Dog	1. Normal	0.53	0.08	0.45
		2. During symp. stim.	2.66	0.10	2.56
3	Rabbit	1. Normal	1.06	0.46	0.60
		2. Piloc. sal. during symp. stim.	2.28	0.32	1.90
4	Rabbit	1. Normal	1.22	0.42	0.80
		2. During symp. stim.	2.22	0.35	1.87
5	Cat	1. Normal	2.44	0.33	2.11
		2. Gl. vein occl.	3.02	0.17	2.85
6	Cat	1. Normal	2.48	0.57	1.91
		2. Gl. vein occl.	2.73	0.51	2.20
7	Cat	1. Normal	2.88	0.26	2.62
		2. R. & L. carotids & R. vert. occl.	5.08	0.20	4.88
8	Cat	1. Normal	3.02	0.45	2.57
		2. R. & L. carotids & R. vert. occl.	4.89	0.42	4.47
9	Cat	1. Normal	2.91	0.45	2.46
		2. R. & L. carotids & R. vert. occl.	2.94	0.52	2.42
10	Cat	1. Normal	2.73	0.33	2.40
		2. R. & L. carotids & R. vert. occl.	4.00	0.25	3.75

Stimulation of the cervical sympathetic during pilocarpin secretion diminishes the rate of secretion and increases the percentage of organic solids in the saliva. This is true for the cat, the dog, and the rabbit. The relative increase of the organic solids varies. Our results are given in Experiments 1 to 4, Table II. Some of the analyses reported by Heidenhain show a relatively greater increase.

An examination of Experiments 5 to 10, Table II, shows that this

effect of the sympathetic stimulation is duplicated by diminishing the oxygen supply to the gland. In Experiments 5 and 6 the oxygen supply was diminished by occlusion of the gland vein; in the rest of the experiments the greatly diminished blood supply to the gland was secured by ligation of the innominate and the left carotid, leaving the left vertebral unobstructed. This ligation usually reduces the amount of blood passing through the parotid to a degree equal to that on stimulation of the sympathetic with a moderately strong interrupted current.

In Experiment 9 there was no appreciable slowing of the secretion during the ligation of the arteries, as compared to the normal rate previous to the ligation, and in that particular experiment the two samples of saliva exhibit practically no difference in percentage composition. It is probable that the degree of diminution of the blood supply to the gland in this case was not sufficient. In all the other experiments the saliva secreted during the diminished blood supply is much richer in the organic and slightly poorer in the inorganic solids, just as is the case with the sympathetic saliva in Experiments 1 to 4. The venous occlusion appears to produce less change than the arterial obstruction.

These results on the parotid and the previous results on the submaxillary gland render it highly probable that the relation between the oxygen supply to the gland and the percentage composition of the saliva holds true for all the salivary glands of mammals. The only explanation that we can offer of Heidenhain's negative results is that he did not diminish the oxygen supply to the same degree that obtains on sympathetic stimulation, or perhaps did not take sufficient care in removing the normal saliva from collecting canula and gland before securing the sample of saliva secreted during the period of diminished blood supply.

### III. PREVIOUS STIMULATION OF CERVICAL SYMPATHETIC DOES NOT INCREASE THE PERCENTAGE OF ORGANIC SOLIDS IN THE SUBSEQUENT CHORDA OR PILOCARPIN SALIVA.

1. Heidenhain found that the saliva secreted on stimulation of the cranial secretory nerves after a previous stimulation of the cervical sympathetic is richer in organic solids than the normal saliva. This he attributes to the actual increase in the organic materials in the cells destined to pass into the saliva because of the stimulation

of trophic secretory fibres in the sympathetic. This observation of Heidenhain does not appear to have been subjected to criticism, and seems even to-day to be generally accepted as correct.

We know that partial anemia of the gland increases the percentage of organic solids in the chorda saliva by diminishing the secretion of water and inorganic salts. It does not appear from Heidenhain's account that he allowed a sufficient period to elapse between the cessation of the sympathetic stimulation and the beginning of the chorda stimulation to assure the return to the normal vascular conditions in the gland. The first part of the chorda saliva may therefore have been secreted under conditions of diminished oxygen supply. Care must also be taken to discard all of the sympathetic saliva remaining in the gland from the previous stimulation. These precautions were taken in our experiments. A period of from five to seven minutes was allowed to elapse between the sympathetic and the chorda stimulation or between the sympathetic stimulation and the collection of the sample of normal pilocarpin saliva in the case of the parotid gland. It is hardly necessary to state that the sympathetic saliva was removed from the collecting cannula, and the first three or four drops of chorda or pilocarpin saliva discarded, as being secreted during the sympathetic stimulation.

Our results are given in Table III. Of the sixteen experiments thirteen are on the dog's submaxillary, one on the dog's parotid, and two on the rabbit's parotid. The data in Experiments 5 to 12 are taken from the paper by Carlson, Greer, and Becht already referred to. Those experiments were made with another end in view; but as most of the above-stated precautions were taken in collecting the samples, these analyses are available for this comparison. Experiments 1 to 4 and 13 to 16 were made purposely to test Heidenhain's observation.

An examination of Table III reveals the fact that in three cases (Nos. 2, 8, and one series of No. 12) the chorda saliva following the sympathetic stimulation is slightly richer in organic solids than that preceding it. In four cases (Nos. 3, 4, 9, 14) the percentage of organic constituents is identical in the two samples, while in the remaining eight experiments the saliva following the sympathetic stimulation is poorer in organic solids than that preceding it.

It is evident from these results that stimulation of the sympathetic does not increase the organic solids in the subsequent chorda saliva, if time is given for the gland to recover from the anemia

TABLE III.

The effect of previous stimulation of the cervical sympathetic on the percentage composition of chorda submaxillary saliva and pilocarpin parotid saliva. In Experiment 13 corresponding samples of chorda saliva were collected from the right gland, only the left sympathetic being stimulated. A period of about five minutes was allowed between the cessation of the sympathetic and the beginning of the chorda stimulation. The first two or three drops of chorda or pilocarpin saliva following stimulation of the sympathetic were discarded as being sympathetic saliva.

Number of experiment.	Duration of stim. of the cervical sympathetic.	Chorda saliva.	Solids per 100 c.c. saliva.		
			Total.	Inorganic.	Organic.
1	8 min.	1. Normal	1.32	0.30	1.02
		2. After symp.	1.01	0.25	0.76
2	7 min.	1. Normal	1.23	0.32	0.91
		2. After symp.	1.26	0.25	1.01
3	7 min.	1. Normal	0.83	0.17	0.66
		2. After symp.	0.91	0.29	0.62
4	8 min.	1. Normal	0.97	0.26	0.71
		2. After symp.	1.03	0.31	0.72
5	About 10 min.	1. Normal	2.12	0.29	1.83
		2. After symp.	1.21	0.25	0.96
6	About 10 min.	1. Normal	1.85	0.43	1.42
		2. After symp.	1.63	0.40	1.23
7	About 10 min.	1. Normal	2.21	0.52	1.69
		2. After symp.	2.01	0.55	1.45
8	About 10 min.	1. Normal	1.08	0.24	0.84
		2. After symp.	1.15	0.26	0.89
9	About 10 min.	1. Normal	1.16	0.22	0.94
		2. After symp.	1.17	0.26	0.91
10	About 10 min.	1. Normal	1.46	0.22	1.24
		2. After symp.	1.30	0.27	1.03
11	About 10 min.	1. Normal	1.66	0.55	1.11
		2. After symp.	1.45	0.74	0.71
12	About 10 min.	1. Normal	1.51	0.51	1.00
		2. After symp.	1.67	0.43	1.24
13	30 min.	3. After 2d symp. stim.	1.12	0.53	0.59
		1. Normal.	(1.38)	(0.27)	(1.10)
		2. After symp. stim. L. side	(1.21)	(0.31)	(0.90)
		3. After 2d period of symp. stim. L. side	(1.06)	(0.36)	(0.70)
	R.				
	L.				
14 Dog's parotid	45 min.	1. Piloc. sal. normal	0.53	0.008	0.46
		2. After symp. stim.	0.61	0.017	0.43
15 Rabbit's parotid	25 min.	1. Piloc. sal. normal	1.06	0.46	0.60
		2. After symp. stim.	0.66	0.43	0.23
16 Rabbit's parotid	25 min.	1. Piloc. sal. normal	1.22	0.42	0.80
		2. After symp. stim.	0.50	0.40	0.10

prior to stimulation of the chorda or the injection of the pilocarpin. There is not the slightest basis for the trophic nerve theory in the after effects of sympathetic stimulation. In the three cases where we obtained positive results the difference is not great enough to justify any conclusions. On the other hand, the organic solids may be considerably scantier in the chorda saliva after a previous stimulation of the sympathetic. The cause of this appears to us quite obvious. The stimulation renders the gland greatly anemic, and in consequence interferes, in all probability, with the processes of building up during gland rest the organic material that passes into the gland during activity. And, secondly, in case the sympathetic stimulation actually causes the gland to secrete, the gland is of course more exhausted after the stimulation than before it; and we know now that the percentage of organic solids in the saliva diminishes gradually during a single period as well as during successive periods of activity.

IV. PARTIAL ANEMIA OF THE GLAND DOES NOT INCREASE THE PERCENTAGE OF ORGANIC SOLIDS IN THE SUBSEQUENT CHORDA SALIVA UNLESS THE CHORDA STIMULATION IS BEGUN BEFORE THE GLAND HAS RECOVERED FROM THE ANEMIA.

Further evidence that the increase in saliva solids, in case the stimulation of the sympathetic is immediately followed by excitation of chorda, is due to the partial anemia of the gland, is secured by the effect on the chorda saliva obtained immediately after a partial occlusion of the gland artery or the gland vein. Our experiments on this point are given in Table IV. Partial anemia of the submaxillary gland was again produced by occlusion of the main gland vein, by partial occlusion of the gland artery directly, or, in the dog, by temporary ligation of both carotids and both vertebrales.

In the first six experiments the chorda stimulation was begun immediately on release of the gland veins or the occluded arteries. In each case the chorda saliva then secured is more concentrated than that preceding the interference with the gland circulation. The analyses in the case of the first two experiments go to show that this increase is in the organic constituents. Under these experimental conditions the gland is necessarily anemic during the initial part of the secretion, and as a consequence we have the typical chorda saliva of anemia.

In Experiments 7 and 8 a period of five to six minutes was allowed to elapse between the restoration of the normal gland circulation and the beginning of the chorda stimulation. Under these

TABLE IV.

The effect on the percentage composition of saliva of previous anemia of the gland by venous or arterial occlusion. In Experiments 1-6 the chorda stimulation began immediately on restoration of the normal blood supply to the gland. In 7 and 8 the stimulation was begun five minutes after restoration of the circulation.

No. of experiment.	Chorda saliva.	Solids per 100 c.c. saliva.		
		Total.	Inorganic.	Organic.
1 Cat's subm. gland	1. Normal	0.64	0.17	0.47
	2. After 15 min. partial occl. sub. artery	0.98	0.32	0.66
2 Cat's subm. gland	1. Normal	0.95	0.29	0.66
	2. After 20 min. occl. of the main subm. vein.	0.99	0.25	0.74
3 Dog's subm. gland	1. Normal	1.83	....	....
	2. After 10 min. partial occl. of gland artery	2.73	....	....
4 Dog's subm. gland	1. Normal	2.38	....	....
	2. After 10 min. occl. of main gl. vein	2.47	....	....
5 Dog's subm. gland	1. Normal	3.08	....	....
	2. After 10 min. occl. of both carotids and vertebrales	3.30	....	....
6 Dog's subm. gland	1. Normal	2.73	....	....
	2. After 10 min. occl. of both carotids and vertebrales	3.66	....	....
7 Dog's subm. gland	1. Normal	1.92	0.55	1.37
	2. After 10 min. occl. of both carotids and vertebrales	1.92	0.45	1.47
8 Dog's subm. gland	1. Normal	1.85	0.25	1.60
	2. After 15 min. occl. of both carotids and vertebrales	1.69	0.53	1.16

conditions the saliva following the anemia is either of the same or of a less concentration in organic solids than the normal.

The conclusion is therefore evident that the effect of sympathetic stimulation on the percentage composition of the subsequent saliva secured by stimulation of the cranial secretory nerves can be duplicated by producing partial anemia of the gland prior to such stimulation. This effect is in each case due to the gland anemia at the beginning of the gland activity, and does not appear when the condition of anemia no longer obtains.

## SUMMARY.

During any single period of activity of the submaxillary gland produced by direct stimulation of the chorda tympani there is usually a gradual diminution in the percentage of organic solids in the saliva. This gradual diminution in the organic solids is, at least under some conditions, independent of the rate of secretion.

In the case of the parotid gland of the dog, the cat, and the rabbit, diminution of the oxygen supply diminishes the secretion rate and increases the percentage of organic constituents of the pilocarpin saliva. This relation has been previously demonstrated for the submaxillary chorda saliva of the dog and the cat. It is probably true for the salivary glands of all mammals.

Stimulation of the cervical sympathetic during pilocarpin activity of the parotid gland retards the rate of secretion and increases the percentage of organic constituents in the saliva. Both of these actions of the sympathetic are duplicated by diminution of the oxygen supply to the active gland by means of venous occlusion or obstruction of the arteries. It is therefore probable that the change in the parotid pilocarpin saliva produced by the sympathetic stimulation is due to the vaso-constriction and not to the stimulation of trophic secretory nerves.

Stimulation of the cervical sympathetic does not increase the percentage of organic solids in the subsequent saliva obtained by stimulation of the cranial secretory nerves or by the injection of pilocarpin. Such an increase is evident in case the stimulation of the cranial secretory nerves follows immediately on cessation of the sympathetic stimulation. This effect is due to the partial anemia of the gland from the sympathetic vaso-constrictor action, and does not appear when time is given for the normal vascular condition to be re-established in the gland. These effects of the sympathetic stimulation are duplicated by diminishing the oxygen supply to the gland by means of venous occlusion or obstruction of the arteries.

These facts, together with the results obtained by Carlson, Greer, and Becht, render the Heidenhain theory of trophic secretory nerves to the salivary glands untenable. Still, the salivary glands may be supplied with "trophic" nerve fibres acting in a way quite different from that conceived by Heidenhain. The atrophy of the

salivary glands following section of the cranial secretory nerves indicates that the life of these cells, and therefore the building up of secretion material, is dependent on impulses reaching them through these nerves. We probably have "gland tone," analogous to "muscle tone," sustained by impulses from the ordinary secretory nerves.

## HYDROLYSIS OF AMANDIN FROM THE ALMOND.<sup>1</sup>

BY THOMAS B. OSBORNE AND S. H. CLAPP

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

THE chief part of the protein substance of the almond (*Prunus amygdalus var. dulcis*) consists of a globulin to which the name amandin has been given. An investigation of the proteins of the almond made in this laboratory<sup>2</sup> gave no evidence of the presence of more than one globulin in this seed. Amandin is characterized by its high content of nitrogen, about 19 per cent, and by the large proportion of nitrogen which it yields as ammonia on hydrolysis.

In preparing the amandin which was used for this hydrolysis we received the assistance of Mr. I. F. Harris, to whom we wish to express our thanks.

The almonds were freed from their outer coating by immersing them for an instant in hot water and from the greater part of their oil by pressure. The remainder of the oil was removed by petroleum benzine and the meal ground to a fine powder.

The oil-free meal was then extracted with one tenth saturated ammonium sulphate solution, the extract filtered clear, and enough crystals of ammonium sulphate dissolved in it to bring its concentration up to four tenths saturation. The precipitate thus produced was filtered out, dissolved in dilute sodium chloride brine, and the solution, after filtering perfectly clear, was dialyzed for several days. The amandin was thus precipitated for the most part as a somewhat gummy and coherent mass. This, when washed with water and with alcohol, dehydrated with absolute alcohol and dried over sulphuric acid, formed a snow-white powder.

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

<sup>2</sup> OSBORNE and CAMPBELL: Journal American Chemical Society, 1896, xviii, p. 609.

Five hundred grams of this amandin, equal to 457.2 gm. moisture and ash-free, were suspended in a mixture of 500 c.c. of water and 500 c.c. of hydrochloric acid of specific gravity 1.19 and warmed on the water bath until solution was nearly effected. The hydrolysis was then made complete by boiling the solution in the oil bath for fourteen and a half hours.

A preliminary removal of glutaminic acid, performed in the usual manner, yielded 83.64 gm. of the hydrochloride, or 67 gm. of the free acid. The filtrate from glutaminic acid hydrochloride was then concentrated under reduced pressure very sharply, and the residue esterified with alcohol and dry hydrochloric acid gas according to the directions of Emil Fischer.

After liberating the free esters from the hydrochlorides and shaking out with ether, the aqueous layer was freed from inorganic salts and the esterification repeated.

The combined esters were then distilled under diminished pressure with the following result:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	80°	0.8 mm.	14.32 gm.
II	92°	0.8 "	19.94 "
III	100°	0.70 "	53.05 "
IV	134°	0.70 "	50.79 "
V	150°	0.70 "	50.14 "
VI	200°	0.70 "	42.89 "
Total . . . . .			231.13 gm.

The undistilled residue weighed 68 gm.

**Fraction I.**— This fraction yielded 1.10 gm. of the hydrochloride of glycocoll ethyl ester. The melting-point was 144°.

*Chlorine*, 0.2866 gm. subst., gave 0.2925 gm. AgCl.

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

Found . . . . . = Cl 25.23 " "

The filtrate was added to the glycocoll filtrate from Fraction II.

**Fraction II.**— The esters were saponified with boiling water, the solutions evaporated to dryness under reduced pressure, and the residue extracted with boiling alcohol to remove the proline.

The insoluble portion was re-esterified and the glycocoll brought to separation as the hydrochloride of the ethyl ester. The yield

was 3.30 gm. By fractional crystallization of the free amino acids from water and from water and alcohol, there were further obtained from Fraction II 1.50 gm. of leucine, 6.45 gm. of alanine, 0.75 gm. of substance having the percentage composition of amino-valerianic acid and a fraction of perfectly definite appearance, which on analysis gave results agreeing closely for a mixture of equal parts of leucine and amino-valerianic acid.

*Carbon and hydrogen*, 0.1221 gm. subst., gave 0.2368 gm. CO<sub>2</sub> and 0.1087 gm. H<sub>2</sub>O.

Calculated for equal molecules of leucine and amino-valerianic acid =  
C 53.12 ; H 9.66 per cent.

Found . . . . = C 52.89 ; H 9.88 per cent.

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

*Carbon and hydrogen*, 0.1542 gm. subst., gave 0.2294 gm. CO<sub>2</sub> and 0.1163 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.45 ; H 7.86 per cent.

Found . . . . = C 40.57 ; H 8.37 " "

The amino-valerianic acid was analyzed as follows:

*Carbon and hydrogen*, 0.1027 gm. subst., gave 0.1931 gm. CO<sub>2</sub> and 0.0901 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.28 ; H 9.40 per cent.

Found . . . . = C 51.28 ; H 9.75 " "

The preparation of amino-valerianic acid seemed to be homogeneous under the microscope, but, owing to the small amount of substance at our disposal, we are unable to offer any real chemical evidence of the existence of this substance in the protein.

**Fraction III.**— This fraction was saponified and the proline extracted in the usual way. The part remaining undissolved in alcohol yielded 19.04 gm. of very nearly pure leucine.

*Carbon and hydrogen*, 0.4203 gm. subst., gave 0.4840 gm. CO<sub>2</sub> and 0.2154 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96 ; H 9.92 per cent.

Found . . . . = C 54.93 ; H 9.95 " "

The substance decomposed at about 298°.

In the filtrate from leucine no definite substance could be isolated. The proline extracts of Fractions II and III were united.

The substance was converted to the copper salt, and the lævo separated from the racemic with boiling absolute alcohol. The yield of air-dry racemic proline copper was 2.61 gm., while of the amorphous copper salt of lævo-proline there were obtained 11.98 gm. dried at 110°. The racemic proline copper salt was analyzed as follows:

*Water*, 0.1163 gm. subst. (air-dried), lost 0.0124 gm. H<sub>2</sub>O at 110°.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu · 2 H<sub>2</sub>O = H<sub>2</sub>O 10.99 per cent.

Found . . . . . = H<sub>2</sub>O 10.66 “ “

*Copper*, 0.1030 gm. subst., gave 0.0278 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 21.81 per cent.

Found . . . . . = Cu 21.56 “ “

The lævo-proline was identified as the characteristic phenylhydantoine. The melting-point was 142°-143°.

*Carbon and hydrogen*, 0.1332 gm. subst., gave 0.3244 gm. CO<sub>2</sub> and 0.0672 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.67; H 5.57 per cent.

Found . . . . . = C 66.42; H 5.60 “ “

**Fraction IV.**—The ester of phenylalanine was removed in the usual manner by shaking out with ether. The yield of phenylalanine hydrochloride was 2.94 gm. As from Fractions V and VI there were further obtained 11.35 gm. of the hydrochloride, the total yield of free phenylalanine from amandin was 11.71 gm., or 2.53 per cent. The substance was identified as the copper salt.

*Copper*, 0.1133 gm. subst., gave 0.0227 gm. CuO.

Calculated for C<sub>13</sub>H<sub>20</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 16.24 per cent.

Found . . . . . = Cu 16.01 “ “

From Fraction IV there were further obtained 4.55 gm. of aspartic acid as the barium salt and 12.74 gm. of air-dry copper aspartate. From this fraction no glutaminic acid could be isolated as the hydrochloride.

**Fraction V.**—There were further obtained from this fraction 6.97 gm. of aspartic acid as the barium salt, 1.06 gm. of glutaminic acid hydrochloride, and 15.25 gm. of air-dry copper aspartate. The latter substance, crystallized in the tyrosine-like bundles of needles and dried in the air, gave the following analysis:

*Copper*, 0.1381 gm. subst., gave 0.0396 gm. CuO.

*Nitrogen*, 0.2100 gm. subst., required 1.12 c.c. 5/7 N-HCl.

Calculated for  $C_4H_5O_4NCu \cdot 4\frac{1}{2} H_2O = Cu$  23.07; N 5.08 per cent.

Found . . . . . = Cu 22.91; N 5.33 " "

The aspartic acid from the barium salt reddened but did not decompose at 300°.

*Carbon and hydrogen*, 0.2600 gm. subst., gave 0.3458 gm. CO<sub>2</sub> and 0.1292 gm. H<sub>2</sub>O.

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

Found . . . . . = C 36.26; H 5.51 " "

**Fraction VI.** — The aqueous layer of this fraction, remaining after the removal of phenylalanine ester with ether, was saponified with baryta in the usual way. After prolonged standing no barium aspartate had separated. The barium was accordingly removed and the concentrated solution separated 14.16 gm. of pure glutaminic acid. The decomposition point was at about 198°–199°.

*Carbon and hydrogen*, 0.1840 gm. subst., gave 0.2759 gm. CO<sub>2</sub> and 0.1064 gm. H<sub>2</sub>O.

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

Found . . . . . = C 40.89; H 6.42 " "

There were further obtained from this fraction 3.4 gm. of glutaminic acid hydrochloride, while no copper aspartate could be obtained.

#### TYROSINE.

Fifty grams of amandin, moisture and ash free, were boiled with a mixture of 150 gm. sulphuric acid and 300 c.c. of water for ten hours. After removing the sulphuric acid with an equivalent quantity of baryta, the filtrate and washings were concentrated to small volume, and the crystalline substance that separated on standing was filtered out, washed well with water, and recrystallized. The tyrosine thus obtained in characteristic needles weighed 0.5635 gm. equal to 1.12 per cent.

The solution from which the tyrosine separated, together with the mother liquor from the recrystallization, was concentrated and a second separation obtained. This was dried and treated with

glacial acetic acid. All was dissolved, and no more tyrosine could be obtained from the hydrolysis solution.

*Nitrogen*, 0.5630 gm. subst., required 4.3 c.c. 5/7 N-HCl.

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

Found . . . . = N 7.64 " "

#### HISTIDINE.

Fifty grams of moisture and ash free amandin were boiled for ten hours with a mixture of 150 gm. sulphuric acid and 300 c.c. of water, and the bases isolated according to the direction of Kossel and Patten. The solution of the histidine was made up to 300 c.c. and nitrogen determined in an aliquot part of it.

*Nitrogen*, 10 c.c. solution required 0.58 c.c. 5/7 N-HCl = 0.0058 gm. N = 0.1740 gm. N in 300 c.c. = 0.6438 gm. histidine, or 1.29 per cent.

Another determination, made with the same amount of amandin boiled for fourteen hours with the addition of 15 gm. sodium chloride,<sup>3</sup> gave a little higher result, namely, 1.87 per cent.

*Nitrogen*, 20 c.c. solution, required 1.69 c.c. 5/7 N-HCl = 0.0169 gm. N = 0.2535 gm. N in 300 c.c. = 0.9379 gm. histidine = 1.87 per cent.

The remainder of the histidine was converted into the dichloride, but, owing to a loss, its identity was not established.

#### ARGININE.

The solution from this second hydrolysis, containing the arginine, was made up to 1000 c.c.

*Nitrogen*, 20 c.c. solution, required 3.68 c.c. 5/7 N-HCl = 0.0368 gm. N = 1.840 gm. N in 1000 c.c. = 5.9189 gm. arginine = 11.85 per cent.

The rest of the arginine was identified as the copper nitrate double salt.

*Water*, 0.2223 gm. subst., air dry, lost 0.0217 gm.  $H_2O$  at  $110^\circ$ .

Calculated for  $C_{12}H_{23}O_4N_3Cu(NO_3)_2 \cdot 3 H_2O = H_2O$  9.16 per cent.

Found . . . . . =  $H_2O$  9.76 " "

\* Cf. HART, *Zeitschrift für physiologische Chemie*, 1901, xxxiii, p. 348.

*Copper*, 0.1983 gm. subst. (dried at 110°), gave 0.0292 gm. CuO.

Calculated for  $C_{12}H_{23}O_4N_6Cu(NO_3)_2 = Cu$  11.87 per cent.

Found . . . . . = Cu 11.77 " "

#### LYSINE.

The lysine in the two hydrolysis solutions was converted into the picrate, of which 0.5845 gm. was obtained in the solution to which the sodium chloride had been added and 0.8833 gm. in the solution without this salt. This is equivalent to 0.2277 gm. and 0.3441 gm. lysine respectively, or 0.46 and 0.70 per cent. of the amandin.

*Nitrogen*, 0.4260 gm. subst., required 7.9 c.c. 5/7 N-HCl (Kjeldahl-Jodlbauer).

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

Found . . . . . = N 18.54 " "

The results of this hydrolysis were the following:

	Per cent.		Per cent.
Glycocoll . . . . .	0.51	Serine . . . . .	?
Alanine . . . . .	1.40	Tyrosine . . . . .	1.12
Valine . . . . .	0.16	Arginine . . . . .	11.85
Leucine . . . . .	4.45	Histidine . . . . .	1.58
Proline . . . . .	2.44	Lysine . . . . .	0.70
Phenylalanine . . . . .	2.53	Ammonia . . . . .	3.70
Aspartic acid . . . . .	5.42	Tryptophane . . . . .	present
Glutaminic acid . . . . .	23.14 <sup>4</sup>		59.00

<sup>4</sup> OSBORNE and GILBERT: This journal, 1906, xv, p. 350.

## HYDROLYSIS OF THE PROTEINS OF MAIZE, ZEA MAYS.<sup>1</sup>

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

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

THE seeds of maize or Indian corn, *Zea Mays*, like those of the other cereals, contain a very small proportion of protein soluble in water or neutral saline solutions, a relatively large amount of protein soluble in strong alcohol, and a considerable quantity insoluble in all neutral solvents but soluble in very dilute alkaline and acid solutions.

An investigation of the proportions of these several proteins was made some years ago in this laboratory,<sup>2</sup> and it was found that the sample of yellow corn meal then examined contained 5 per cent of zein soluble in strong alcohol, 3.15 per cent of protein soluble only in alkaline or acid solutions, and 0.45 per cent of globulins, albumins, and proteoses. The composition and properties of these different proteins have been extensively described by Chittenden and Osborne.<sup>3</sup>

Owing to the very small amount of globulins and albumins, no attempt was made to prepare any of these for hydrolysis, but the zein and alkali soluble proteins were prepared in quantity from freshly harvested, high-protein, white maize, which was kindly sent to us by Professor Hopkins of the University of Illinois. We wish to here express our high appreciation of Professor Hopkins' kindness in furnishing so large a quantity of these valuable seeds.

After the seeds were dried until they became hard, they were ground to a fine powder in the laboratory mill and then extracted

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

<sup>2</sup> OSBORNE, *Journal American Chemical Society*, 1897, xix, p. 525.

<sup>3</sup> CHITTENDEN and OSBORNE, *American chemical journal*, 1891, xiii, pp. 327 and 385; 1892, xiv, p. 20.

with cold 85 per cent (by volume) alcohol as long as anything was removed. The voluminous extract was filtered perfectly clear and concentrated under reduced pressure to a small volume. The clear syrup that remained, after cooling, was poured in a very fine stream into about eight volumes of distilled ice water, containing a very little sodium chloride in order to promote the separation of the zein. The zein, which separated as a flocculent precipitate, soon united to a coherent plastic mass. This was filtered out on cloth, washed superficially with water, and again dissolved in 95 per cent alcohol to a syrup. This solution was then shaken out repeatedly with petroleum benzine until all the yellow coloring matter was removed, together with most of the fat.

The perfectly water-clear solution of the zein was then poured into eight volumes of cold water, containing a very little sodium chloride. The zein that separated was then dried and ground, yielding a fine powder that was entirely colorless.

The meal that had been extracted with alcohol until nothing more could be obtained from it was then extracted with a large quantity of 0.20 per cent sodium hydroxide solution, the extract filtered perfectly clear and treated with very dilute hydrochloric acid until the dissolved protein separated as a flocculent precipitate. This was then filtered out, again dissolved in the dilute sodium hydroxide solution, the resulting solution filtered perfectly clear, and the protein precipitated as before. After washing with water, this precipitate was digested several successive times with fresh quantities of strong alcohol as long as any zein was removed, and when dehydrated with absolute alcohol and dried, formed a dusty powder entirely free from color.

This preparation represents the residual protein of the seed after all that is soluble in alcohol has been removed. Of its individuality as a protein substance we of course know nothing, for it has heretofore been impossible to obtain any considerable quantity of it in a state of even approximate purity as respects admixture with non-protein substances. This preparation contains more nitrogen than the five other preparations that we have previously made, and probably contains but a small amount of non-protein matter. As to whether it consists of a single protein substance or is a mixture, we can say nothing, for we have no evidence whatever on this point. We have, nevertheless, made this preparation for hydrolysis because it represents a large part of the protein of the seed and it is im-

portant to know how its products of hydrolysis compare with those of zein.

In addition to the preparations just described we have made similar ones from the "gluten-meal," a by-product of the glucose works. For this meal we wish to thank Mr. H. C. Humphrey of the Corn Products Refining Company. The material was sent to us in cold weather in a moist state, just as it came from the filter press. It was at once frozen solid and kept in that condition until we dried it rapidly at a low temperature.

This meal contains about 50 per cent of protein, a large part of which is zein. The method of preparation of the proteins from this meal was essentially the same as that described for the corn meal. The products obtained were used for some of the separate determinations to be described later, as the quantity of the preparation from the corn meal was insufficient for all. Where the "gluten" preparations were used special mention will be made of the fact, although we have no doubt that the two products are entirely alike.

#### HYDROLYSIS OF ZEIN.

Five hundred and sixty-five grams of zein, equal to 487.2 gm. moisture, ash, and fat free, were suspended in a mixture of 565 c.c. of water and 565 c.c. of hydrochloric acid of specific gravity 1.19, and heated for ten hours at 100°. The hydrolysis was then made complete by boiling the solution for sixteen hours in a bath of oil.

A preliminary removal of glutaminic acid hydrochloride, performed in the usual manner, yielded 69.80 gm. freed from ammonium chloride, or 55.92 gm. of free glutaminic acid, which is 10.9 per cent of the protein. The substance melted at about 202°–203°.

*Carbon and hydrogen*, 0.1607 gm. subst. gave 0.2413 gm. CO<sub>2</sub> and 0.0953 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . = C 40.95; H 6.58 " "

The filtrate from the glutaminic acid hydrochloride was then concentrated very sharply under strongly reduced pressure, and the residue esterified with alcohol and dry hydrochloric acid gas in the usual way.

After liberating the free esters with sodium hydroxide and potassium carbonate, as described by Emil Fischer in the original hydro-

lysis of casein, and shaking out with ether, the aqueous layer was made strongly acid with hydrochloric acid, freed from inorganic salts, and the esterification repeated. The combined esters were divided into the following fractions by distillation under diminished pressure:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	70°	12 mm.	11.24 gm.
II	92°	12 "	25.38 "
III	a	100°	12 "
	b	94°	2 "
	c	94°	1 "
	d	105°	0.92 "
IV	a	145°	0.92 "
	b	170°	0.92 "
	c	186°	0.92 "
Total . . . . .			304.97 gm.

The undistilled residue weighed 84 gm.

**Fraction I** — From this fraction no glycooll could be brought to separation as the hydrochloride of the ethyl ester. The fraction consisted essentially of alanine, of which 3.13 gm. were obtained.

**Fraction II.** — This fraction was saponified with boiling water, the solution of the amino acids evaporated to dryness under reduced pressure, and the proline extracted with boiling absolute alcohol. The part remaining undissolved was then examined for glycooll, but no crystals of the ethyl ester hydrochloride could be obtained.<sup>4</sup>

By systematic fractionation of the free amino acids from water and from water and alcohol, there were obtained from this fraction 3.36 gm. of leucine and 5.52 gm. of alanine, while the presence of valine could not be established. The leucine gave the following analysis:

*Carbon and hydrogen*, 0.1105 gm. subst. gave 0.2224 gm. CO<sub>2</sub> and 0.0983 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 54.89; H 9.88 " "

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

<sup>4</sup> We were, furthermore, unable to detect this substance in the ether distilled from the esters on the water bath.

Carbon and hydrogen, 0.1496 gm. subst. gave 0.2237 gm. CO<sub>2</sub> and 0.1066 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.45; H 7.86 per cent.

Found . . . . = C 40.78; H 7.91 " "

**Fraction III.** — The esters of this fraction were boiled with five volumes of water for six hours, after which time the solution had ceased to react alkaline to litmus. The leucine that had separated was then filtered off, and the filtrate rapidly evaporated to dryness under strongly reduced pressure. The large quantity of proline present was then dissolved out with boiling absolute alcohol, and the insoluble portion dissolved in water and systematically fractionated. The yield of leucine was 87.75 gm.

Carbon and hydrogen, 0.1790 gm. subst. gave 0.3610 gm. CO<sub>2</sub> and 0.1593 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 55.00; H 9.89 " "

The substance decomposed at about 298°.

In the filtrate from the leucine there were further isolated 2.04 gm. of alanine and 1.4 gm. of valine. To isolate the latter substance it was found necessary to have recourse to racemization. The fraction containing the valine was accordingly heated in the autoclave with excess of baryta for twenty-four hours at 175°. After removing the barium exactly with sulphuric acid, a preparation was readily obtained, which not only gave closely agreeing results on analysis, but which under the microscope looked perfectly homogeneous.

Carbon and hydrogen, 0.1444 gm. subst. gave 0.2721 gm. CO<sub>2</sub> and 0.1246 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.28; H 9.40 per cent.

Found . . . . = C 51.38; H 9.58 " "

The remainder of the substance was then coupled with phenylisocyanate in alkaline solution and the hydantoic acid recrystallized from water. The substance separated in the characteristic plates, which, heated side by side with a similar preparation from phaseolin,<sup>5</sup> melted simultaneously with the latter at 161° (corr.).

Carbon and hydrogen, 0.1331 gm. subst. gave 0.2974 gm. CO<sub>2</sub> and 0.0820 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>16</sub>O<sub>3</sub>N<sub>2</sub> = C 61.02; H 6.78 per cent.

Found . . . . = C 60.94; H 6.84 " "

<sup>5</sup> OSBORNE and CLAPP: This journal, 1907, xviii, p. 301.

The phenyl-isocyanate derivative was then converted to the anhydride by dissolving in strong hydrochloric acid and concentrating on the water bath. The prisms of the hydantoine melted on recrystallizing from ether and petroleum ether at  $120.5^{\circ}$  (corr.), while the preparation from phaseolin<sup>6</sup> melted at  $122.5^{\circ}$  (corr.), whereas Slimmer<sup>7</sup> found, in the case of the synthetic  $\alpha$ -amino-iso-valerianic acid,  $163.5^{\circ}$  (corr.) for the phenyl-isocyanate derivative and  $124^{\circ}$ – $125^{\circ}$  (corr.) for the hydantoine.

The proline extracts of Fractions II and III were united. The solutions were evaporated to dryness under reduced pressure, and the residue taken up in boiling absolute alcohol. After prolonged standing 1.24 gm. of substance, melting at  $250^{\circ}$ , had separated, the identity of which was not established.

The filtrate was then slightly concentrated and the proline precipitated with ether, as a slightly colored crystalline mass, which dried in vacuum over sulphuric acid to constancy, weighed 31.99 gm.

On redissolving in absolute alcohol, the substance separated in the characteristic prisms melting at about  $203^{\circ}$ – $205^{\circ}$ .

*Carbon and hydrogen*, 0.2045 gm. subst. gave 0.3889 gm.  $\text{CO}_2$  and 0.1472 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_5\text{H}_9\text{O}_2\text{N} = \text{C } 52.18$ ;  $\text{H } 7.83$  per cent.

Found . . . . =  $\text{C } 51.86$ ;  $\text{H } 8.00$  " "

For the strict identification the proline was converted to the copper salt, and the latter substance separated into the lævo and racemic by boiling with absolute alcohol. The undissolved racemic salt separated from water in the characteristic plates.

*Water*, 0.1506 gm. subst. (air dry) lost 0.0164 gm.  $\text{H}_2\text{O}$  at  $110^{\circ}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2\text{Cu} \cdot 2 \text{H}_2\text{O} = \text{H}_2\text{O } 10.99$  per cent.

Found . . . . . =  $\text{H}_2\text{O } 10.89$  " "

*Copper*, 0.1294 gm. subst. (dried at  $110^{\circ}$ ) gave 0.0351 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2 \text{Cu} = \text{Cu } 21.81$  per cent.

Found . . . . . =  $\text{Cu } 21.67$  " "

The amorphous copper salt of lævo proline was converted to the phenyl-hydantoine. The substance crystallized from water in the characteristic prisms melting at  $143^{\circ}$ .

<sup>6</sup> OSBORNE and CLAPP: This journal, 1907, xviii, p. 301.

<sup>7</sup> SLIMMER, MAX D.: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 403.

*Nitrogen*, 0.1897 gm. subst. required 2.5 c.c. 5/7 N-HCl.

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

Found . . . . . = N 13.17 " "

**Fraction IV.**—From this fraction phenylalanine was removed as the ester in the usual way. The yield of the hydrochloride of phenylalanine was 29.11 gm. The free phenylalanine decomposed at about  $270^\circ$  and gave the following analysis:

*Carbon and hydrogen*, 0.1150 gm. subst. gave 0.2773 gm.  $CO_2$  and 0.0699 gm.  $H_2O$ .

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

Found . . . . . = C 65.76; H 6.75 " "

The aqueous layer was saponified by warming with an excess of baryta on the water bath. The yield of aspartic acid isolated as the barium salt was 5 gm.

*Carbon and hydrogen*, 0.2571 gm. subst. gave 0.3426 gm.  $CO_2$  and 0.1252 gm.  $H_2O$ .

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

Found . . . . . = C 36.34; H 5.41 " "

The filtrate from the barium aspartate was freed quantitatively from barium, concentrated under reduced pressure, and saturated with hydrochloric acid gas. The yield of glutaminic acid, which separated after prolonged standing at  $0^\circ$ , was 20.37 gm. The free glutaminic acid decomposed at about  $201^\circ$ – $202^\circ$  with effervescence to a clear oil. From this fraction there were further isolated 3.94 gm. of air-dry copper aspartate, while no serine was obtained.

*Copper*, 0.1135 gm. subst. (air dry) gave 0.0330 gm.  $CuO$ .

*Nitrogen*, 0.5275 gm. subst. (air dry) required 2.7 c.c. 5/7 N-HCl.

Calculated for  $C_4H_5O_4NCu \cdot 4\frac{1}{2} H_2O = Cu$  23.07; N 5.08 per cent.

Found . . . . . = Cu 23.23; N 5.12 " "

#### THE RESIDUE AFTER DISTILLATION.

The residue remaining after distillation of the esters weighed 84 gm. It was saponified with baryta and the glutaminic acid separated as the hydrochloride in the usual manner. The yield of glutaminic acid hydrochloride was 14.32 gm., which makes the

total yield of free glutaminic acid obtained on this hydrolysis 83.71 gm., or 16.32 per cent of the protein, while by the direct method 18.28 per cent was obtained.

#### GLUTAMINIC ACID.

Osborne and Gilbert<sup>8</sup> found 16.87 per cent of glutaminic acid in zein, but as the amount of protein available for that determination was only 32 gm. we have repeated the determination with the following results: 100 gm. equal to 91.6 gm. moisture, ash, and fat free zein from the "gluten-meal" was heated on a water bath for two and a half hours with 200 c.c. of hydrochloric acid sp. gr. 1.19, and then boiled in an oil bath for twelve hours. After concentrating the hydrolysis solution to about two thirds its original volume it was saturated with hydrochloric acid gas and allowed to stand for some days on ice. There were thus obtained 20.9 gm. of glutaminic acid hydrochloride equal to 16.74 gm. glutaminic acid, or 18.28 per cent.

#### TYROSINE.

Kutscher<sup>9</sup> has stated that zein yields 10.06 per cent of tyrosine. We have made every effort to isolate all the tyrosine possible from our solutions, but have obtained barely one third as much as Kutscher. Although we are convinced that tyrosine still remained in our solutions, we have obtained no evidence that the amount that defied separation was more than relatively small in proportion to that which did separate.

As several unusual observations were made in the course of our efforts to separate the last traces of tyrosine, we will give a brief description of them.

Three hundred grams of the zein from the "gluten meal" were hydrolyzed with 900 gm. of sulphuric acid and 1800 gm. of water by heating on the water bath for ten hours and then boiling in an oil bath for ten hours longer. After removing the sulphuric acid with an equivalent quantity of baryta and thoroughly washing the barium sulphate, the filtrate and washings were concentrated to about 2500 c.c. and cooled. The substance that separated, when

<sup>8</sup> OSBORNE and GILBERT: *This journal*, 1906, xv, p. 333.

<sup>9</sup> KUTSCHER, *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 111.

washed with cold water and thoroughly dried at  $100^{\circ}$ , weighed 22.8 gm., I. The filtrate and washings were concentrated to about 1200 c.c. and similarly yielded 19.88 gm. of II. On further concentration 12 gm. of III were obtained and 16.27 gm. of IV. The filtrate from IV, when concentrated to a syrup, gave 27.75 gm. of V, from which the thick mother liquor could not be very thoroughly sucked out.

The total yield of substances that were thus separated in a solid state capable of removal by filtration was about one third of the hydrolyzed zein. Small parts of the five fractions were then treated with glacial acetic acid in which IV and V were completely soluble at the room temperature, II and III were mostly soluble, while I contained much that did not dissolve. The whole of Fractions I, II, and III were therefore treated with an abundant quantity of glacial acetic acid, the residue filtered out, washed with absolute alcohol, and when dried at  $100^{\circ}$  weighed 8.27 gm. (crude tyrosine A). The acetic acid solution filtered from A was freed from acetic acid as completely as possible by repeatedly evaporating under diminished pressure with water and with alcohol and then subjected to extensive fractional crystallization. The less soluble fractions, which closely resembled nearly pure leucine, when recrystallized gave no Millon's reaction, whereas, although the most soluble fractions gave strong Millon's reactions, no tyrosine could be obtained from them in characteristic crystals. All the fractions which gave Millon's reaction were set aside for further examination.

Fraction IV was dissolved in water, the solution treated with bone-black and subjected to fractional crystallization. There was obtained 9.35 gm. of the original 16.27 gm. which did not give Millon's reaction; but the second fraction, which was very soluble in water, gave a Millon's reaction, while the filtrate from this, on concentration to a syrup and adding alcohol, gave a gummy deposit which would not harden under alcohol and gave a strong Millon's reaction.

A similar fractional recrystallization of V yielded no fractions which did not give a strong Millon's reaction.

The thick syrup that had been separated from V was then treated with alcohol, and on long standing a product separated that could be sucked out but contained so much mother liquor that it could not be weighed. This was redissolved, its solution treated with bone-black and concentrated, and 8.8 gm. of substance obtained which gave a moderately strong Millon's reaction.

The fractions thus far obtained which gave a Millon's reaction were united, dissolved in a considerable quantity of 5 per cent sulphuric acid, and treated, as long as a precipitate formed, with a 20 per cent solution of phosphotungstic acid dissolved in 5 per cent sulphuric acid. After washing the precipitate with a dilute solution of phosphotungstic acid in 5 per cent sulphuric acid, these two acids were removed from the filtrate and washings with an excess of baryta and the excess of baryta with an equivalent quantity of sulphuric acid.

On concentrating and cooling the solution and subjecting it to fractional crystallization, 3.78 gm. of tyrosine were obtained in characteristic crystals suitable for weighing. Although long-continued and persistent effort was made to bring more tyrosine to separation, no more than traces could be obtained. Occasionally small fractions crystallized out in which a few needles that looked like tyrosine could be seen under the microscope, but no weighable quantity could be separated. As before, the tyrosine accumulated, so far as could be judged from the Millon's reaction, in the most soluble fractions. So extensive and thorough was this fractionation that two fractions were obtained, weighing 0.50 and 1.10 gm., equal to 0.57 per cent of the zein, which, after a single recrystallization, were found to be pure serine.

The crystals, which had the characteristic form and sweet taste of serine, browned at about  $215^{\circ}$  and melted with effervescence to a brownish mass at about  $240^{\circ}$ .

*Carbon and hydrogen*, 0.2644 gm. subst. gave 0.3317 gm.  $\text{CO}_2$  and 0.1623 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_3\text{H}_7\text{O}_3\text{N} = \text{C } 34.29$ ;  $\text{H } 6.67$  per cent.

Found . . . . =  $\text{C } 34.22$ ;  $\text{H } 6.82$  " "

Further in the filtrate from the serine 8.5 gm. substance was obtained which crystallized in needles, had the composition of alanine, and decomposed at about  $285^{\circ}$ .

The preparation gave a somewhat too high carbon determination.

*Carbon and hydrogen*, 0.2226 gm. subst. gave 0.3338 gm.  $\text{CO}_2$  and 0.1614 gm.  $\text{H}_2\text{O}$ .

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

Found . . . . =  $\text{C } 40.89$ ;  $\text{H } 8.05$  " "

The crude tyrosine A, when recrystallized from water, yielded 5.47 gm. of nearly pure tyrosine. The substance in the filtrate,

which still contained some tyrosine, was added to the solution that was treated with phosphotungstic acid, as already described. The total quantity of tyrosine thus obtained in weighable form was 9.25 gm. As this was obtained from 274.77 gm. of moisture, ash, and fat free zein, it is equal to 3.37 per cent.

Another determination of tyrosine was made from the same preparation of zein with a similar result. A quantity weighing 100 gm., equal to 91.6 gm. moisture, ash, and fat free zein, from the "gluten-meal," were heated with a mixture of three times its weight of sulphuric acid and six times its weight of water for two hours in a water bath and boiled for twelve hours in an oil bath. The hydrolysis solution was then diluted with water, the sulphuric acid removed with an equivalent quantity of baryta, and the barium sulphate boiled out four times with water and washed very thoroughly. The filtrate and washings were then concentrated to about 500 c.c. under diminished pressure, with the addition of barium carbonate, filtered from the barium carbonate, and the latter boiled out with water and thoroughly exhausted with boiling water.

The filtrate and washings from the barium carbonate were concentrated to crystallization, and, after standing over night, the product that separated was filtered out, washed with cold water, and dried. It weighed 4.2 gm. This was dissolved in about 800 c.c. of boiling water, the solution decolorized with a little bone-black, the latter extracted thoroughly with boiling water, and the solution concentrated to crystallization. After twenty-four hours 2.95 gm. of tyrosine separated. The filtrate on further concentration yielded 0.3 gm. more, making 3.25 gm. of tyrosine, or 3.55 per cent.

#### ARGININE, HISTIDINE, AND LYSINE.

A quantity of the "gluten-meal" weighing 100 gm. was heated for three hours on the water bath with three times its weight of sulphuric acid and six times its weight of water and then boiled for twelve hours on an oil bath. The products of hydrolysis were worked up for bases according to the method of Kossel and Patten with the following results:

The solution containing the histidine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. sol. required 1.26 c.c. 5/7 N-HCl = 0.0126 gm. N = 0.0630 gm. N in 500 c.c. = 0.2322 gm. histidine, or 0.24 per cent.

The solution of the arginine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 50 c.c. sol. required 1.61 c.c. 5/7 N-HCl = 0.0161 gm. N = 0.1610 gm. in 500 c.c. = 0.5000 gm. arginine, or, allowing for the solubility of the arginine silver, 0.6180 gm., or 0.67 per cent.

The filtrate from the first silver precipitate of arginine and histidine was examined carefully for lysine according to the method of Kossel and Kutscher, but none was found.

As the results of these determinations were so low, we repeated them, using Kossel's later method for separating histidine from arginine, which depends on heating the neutral or slightly acid solution of the silver salt with barium carbonate.<sup>10</sup>

One hundred grams of zein from the "gluten-meal" were hydrolyzed as before, and, after removing the sulphuric acid, the solution containing histidine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. solution required 2.14 c.c. 5/7 N-HCl = 0.0214 gm. N = 0.1070 gm. N in 500 c.c. = 0.3589 gm. histidine = 0.43 per cent.

The solution containing the arginine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. solution required 6.1 c.c. 5/7 N-HCl = 0.0610 gm. N = 0.305 gm. N in 500 c.c. = 0.9477 gm. arginine. Adding 0.1180 gm. for the solubility of the arginine silver gives 1.0657 gm., or 1.16 per cent.

These results are somewhat higher than those first obtained, but fall decidedly below those published by Kossel and Kutscher,<sup>11</sup> namely, histidine 0.81, arginine 1.85 per cent.

#### HYDROLYSIS OF THE ALKALI-SOLUBLE PROTEIN.

The preparation used for this hydrolysis was that made from the seeds ground in the laboratory. Unfortunately the amount of this material made it necessary to use much less of this protein for the hydrolysis than we would have used had our supply been greater.

<sup>10</sup> Cf. WEISS, *Zeitschrift für physiologische Chemie*, 1907, lii, p. 107.

<sup>11</sup> KOSSEL and KUTSCHER: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 165.

Two hundred and fifty grams of the protein (containing ash equal to 1.45 per cent, moisture equal to 10.63 per cent, ether-soluble substance equal to 0.81 per cent) were suspended in a mixture of 250 c.c. of water and 250 c.c. of hydrochloric acid of specific gravity 1.19 and warmed at 100° for about five hours. The hydrolysis solution was then boiled in the oil bath for eighteen and a half hours.

A preliminary removal of glutamic acid yielded 23.66 gm. of the free acid, or 10.87 per cent of the protein.

Carbon and hydrogen, 0.1654 gm. subst. gave 0.2484 gm. CO<sub>2</sub> and 0.0916 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . = C 40.95; H 6.15 " "

The substance melted at about 202°.

The filtrate from glutamic acid hydrochloride was then freed from water by evaporation under reduced pressure and the residue esterified precisely as in the case of zein. By distillation under diminished pressure the following fractions were obtained:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	85°	12.00 mm.	15.62 gm.
II	95°	5.00 "	19.47 "
III	80°	2.00 "	17.73 "
IV	110°	0.70 "	15.73 "
V	200°	0.36 "	37.19 "
Total . . . . .			105.74 gm.

The undistilled residue weighed 39 gm.

**Fraction I.** — This fraction yielded 1.00 gm. of the hydrochloride of glycocoll ethyl ester. The melting-point was 144°.

Chlorine, 0.2390 gm. subst. gave 0.2411 gm. AgCl.

Calculated for C<sub>4</sub>H<sub>10</sub>O<sub>2</sub> NCl = Cl 25.45 per cent.

Found . . . . . = Cl 24.94 " "

The filtrate from the glycocoll was added to the corresponding filtrate of Fraction II.

**Fraction II.** — From this fraction there were obtained 2.6 gm. of leucine, while the presence of neither glycocoll nor alanine could

be definitely established. The fraction further contained a not inappreciable quantity of proline which was worked up conjointly with that from Fractions III and IV. The leucine gave the following analysis:

*Carbon and hydrogen*, 0.1304 gm. subst. gave 0.2621 gm. CO<sub>2</sub> and 0.1180 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 54.82; H 10.05 " "

**Fraction III and IV.** — From Fractions III and IV there were further isolated 10.98 gm. of leucine.

*Carbon and hydrogen*, 0.1266 gm. subst. gave 0.2541 gm. CO<sub>2</sub> and 0.1167 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 54.74; H 10.23 " "

The proline extracts from Fractions II, III, and IV were united. By concentrating somewhat under reduced pressure and precipitating with ether, the proline was obtained as a somewhat colored crystalline mass, which proved to be readily soluble in alcohol. After drying to constancy over sulphuric acid, it weighed 10.86 gm. For identification the phenyl-hydantoin of the lævo modification was employed.

*Carbon and hydrogen*, 0.1504 gm. subst. gave 0.3674 gm. CO<sub>2</sub> and 0.0792 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.67; H 5.56 per cent.

Found . . . . = C 66.62; H 5.85 " "

The melting-point was 143°.

**Fraction V.** — From this fraction there were isolated by the usual method 4.63 gm. of phenylalanine hydrochloride, 0.60 gm. of aspartic acid as the barium salt, and 1.58 gm. of air-dry copper aspartate, while no glutaminic acid hydrochloride was obtained. The phenylalanine gave, on boiling with dilute sulphuric acid and potassium bichromate, the characteristic odor of phenylacetaldehyde, but the analysis indicated a considerable admixture.

*Carbon and hydrogen*, 0.1667 gm. subst. gave 0.3922 gm. CO<sub>2</sub> and 0.1031 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N = C 65.45; H 6.66 per cent.

Found . . . . = C 64.16; H 6.87 " "

The aspartic acid reddened but did not decompose at 300°.

*Carbon and hydrogen*, 0.0966 gm. subst. gave 0.1288 gm. CO<sub>2</sub> and 0.0515 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>4</sub>N = C 36.09; H 5.26 per cent.

Found . . . . = C 36.36; H 5.92 " "

#### TYROSINE.

Fifty grams of the protein, equal to 43.55 gm. moisture, ash, and fat free, were hydrolyzed by boiling in an oil bath for twelve hours with a mixture of 150 gm. sulphuric acid and 300 c.c. of water. After removing the sulphuric acid with baryta the hydrolysis solution was concentrated until crystallization began and then allowed to stand for twenty-four hours. The substance that had separated was filtered out, washed with cold water, and recrystallized. There were thus obtained 1.51 gm. of tyrosine in the characteristic needles, equal to 3.44 per cent. By similar treatment of a preparation from the "gluten-meal" there were obtained from 100 gm. equal to 86.4 gm. moisture, ash, and fat free, 3.30 gm. recrystallized tyrosine equal to 3.82 per cent.

*Nitrogen*, 0.2340 gm., dried at 110°, required 1.82 c.c. 5/7 N-HCl.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>3</sub>N = N 7.73 per cent.

Found . . . . = N 7.78 " "

#### HISTIDINE.

The solution, washings, and mother liquor, from which the tyrosine first described had separated, were concentrated and worked up for bases according to the method of Kossel and Patten. The solution of the histidine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. solution, required 7.09 c.c. 5/7 N-HCl = 0.0709 gm. N = 0.3545 gm. N in 500 c.c. = 1.3063 gm. histidine, or 3.00 per cent.

The histidine was converted into the dichloride. The characteristic prisms decomposed at about 230° and gave on warming a pronounced biuret reaction.

## ARGININE.

The solution of the arginine was made up to 1000 c.c. and nitrogen determined in it.

*Nitrogen*, 50 c.c. solution, required 4.84 c.c. 5/7 N-HCl = 0.0484 gm. N = 0.968 gm. in 1000 c.c. = 3.0047 gm. arginine. Adding 0.072 gm. for the solubility of silver arginine gives 3.0767 gm., or 7.06 per cent.

The arginine was identified as the copper nitrate double salt.

*Water*, 0.2893 gm. subst., air dry, lost 0.0296 gm. H<sub>2</sub>O at 110°.

Calculated for C<sub>12</sub>H<sub>28</sub>O<sub>4</sub>N<sub>8</sub>Cu (NO<sub>3</sub>)<sub>2</sub> · 3 H<sub>2</sub>O = H<sub>2</sub>O 9.16 per cent.

Found . . . . . = H<sub>2</sub>O 10.23 " "

*Copper*, 0.1822 gm. subst., dried at 110°, gave 0.0267 gm. CuO.

Calculated for C<sub>12</sub>H<sub>28</sub>O<sub>4</sub>N<sub>8</sub>Cu (NO<sub>3</sub>)<sub>2</sub> = Cu 11.87 per cent.

Found . . . . . = Cu 11.71 " "

## LYSINE.

The lysine was isolated as the picrate, of which there were obtained 3.27 gm. equal to 1.2730 gm. of lysine, or 2.93 per cent. The lysine picrate gave the following determination of nitrogen:

*Nitrogen*, 0.0980 gm. subst. gave 16.8 c.c. moist N<sub>2</sub> at 28° and 756.6 mm.

Calculated for C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>N<sub>2</sub> · C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>N<sub>3</sub> = N 18.67 per cent.

Found . . . . . = N 18.77 " "

## GLUTAMIC ACID.

Fifty grams, equal to 43.09 gm. ash, moisture, and fat free protein, were hydrolyzed with 200 c.c. of hydrochloric acid sp. gr. 1.11 by boiling on an oil bath for fifteen hours. The solution was concentrated to about 75 c.c., saturated with hydrochloric acid gas, and after standing on ice for some time the substance that had separated was filtered out and recrystallized after decolorizing with animal charcoal. The weight of the glutamic acid hydrochloride thus obtained was 6.85 gm. after deducting the ammonium chloride which it contained. This is equal to 5.48 gm. glutamic acid, or 12.72 per cent.

## TRYPTOPHANE.

A qualitative test for tryptophane with glyoxylic acid gave a strong reaction for this substance.

## AMMONIA.

One gram protein equal to 0.8711 gm. moisture, ash, and fat free substance was hydrolyzed by boiling for eight hours with 50 c.c. hydrochloric acid, during which time the solution was finally concentrated to about 3 c.c. The residual solution was then taken up in about 300 c.c. water and distilled with magnesium oxide. The ammonia that was liberated was equivalent to 1.52 c.c. 5/7 N—HCl equal to 0.0152 gm. N, or 2.12 per cent of ammonia.

The results of these hydrolyses are given in the following table:

	Zein. per cent.	Alkali soluble Protein. per cent.		Zein. per cent.	Alkali soluble Protein. per cent.
Glycocoll . . .	0.00	0.25	Serine . . . .	0.57	not isolated
Alanine . . . .	2.23	not isolated	Tyrosine . . . .	3.55	3.78
Valine . . . .	0.29	not isolated	Arginine . . . .	1.16	7.06
Leucine . . . .	18.60	6.22	Histidine . . . .	0.43	3.00
Proline . . . .	6.53	4.99	Lysine . . . .	0.00	2.93
Phenylalanine . . . .	4.87	1.74	Ammonia . . . .	3.61 <sup>12</sup>	2.12
Aspartic acid . . . .	1.41	0.63	Tryptophane . . . .	0.00 <sup>13</sup>	present
Glutamic acid . . . .	18.28	12.72	Total . . . .	61.53	45.44

These figures show that zein, like the other alcohol soluble proteins, is characterized by yielding a very small percentage of arginine and histidine, no lysine, and much ammonia and proline. The proportion of glutamic acid is much less than that found in the other alcohol soluble proteins, hordein and gliadin, while the proportion of leucine is very much greater. Unfortunately the amount of the alkali soluble protein which was available for hydrolysis was too small to enable us to obtain satisfactory results for quantitative comparison. It is interesting, however, to note that those amino acids which are lacking in zein are all present in notable proportions in this protein, so that the mixture of the proteins as they occur in this seed yields all of the amino acids usually obtained from protein substances.

<sup>12</sup> OSBORNE and HARRIS: *Journal American Chemical Society*, 1903, xxv, p. 323.

<sup>13</sup> OSBORNE and HARRIS, *Ibid.*, 1903, xxv, p. 853.

## THE HYDROLYSIS OF GLIADIN FROM RYE.<sup>1</sup>

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

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

ALCOHOL extracts from rye flour a protein substance which very closely resembles gliadin obtained from wheat flour under similar conditions. The gliadin from rye has been the subject of extensive study in this laboratory,<sup>2</sup> and a strict comparison in respect to composition and reactions has been made between it and the gliadin from wheat without revealing any differences. This comparison has now been supplemented by a determination of the proportion of the several decomposition products which the gliadin of rye yields when hydrolyzed. The material for this hydrolysis was obtained by extracting rye flour, ground in this laboratory, with cold 75 per cent (by volume) alcohol, concentrating the perfectly clear extract under reduced pressure to a syrup, and precipitating the gliadin by pouring the solution into ice water. The precipitate thus obtained was redissolved in 85 per cent (by volume) alcohol, and the solution again poured into several volumes of ice water. The gliadin that separated was then washed with distilled water, redissolved in 85 per cent alcohol, and the clear solution precipitated by pouring in a thin stream into a large volume of absolute alcohol. After dehydrating by long digestion with absolute alcohol, the gliadin was dried over sulphuric acid, ground to a fine powder, and moisture, ash, and ether soluble matter determined in it.

Four hundred and fifteen grams of this preparation, equal to 362.67 gm. moisture, ash, and fat free, were suspended in a mixture of 415 c.c. of water and 415 c.c. of hydrochloric acid of sp. gr. 1.19. After warming for three hours at 100°, the hydrolysis solution was boiled in a bath of oil for eighteen and a half hours.

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

<sup>2</sup> OSBORNE: *Journal of the American Chemical Society*, 1895, xvii, p. 429.

After concentrating to about two thirds of the original volume, the solution was saturated with hydrochloric acid gas and allowed to stand at 0° for four days.

The precipitate of glutaminic acid hydrochloride when recrystallized from strong hydrochloric acid and freed from ammonium chloride weighed 124.44 gm., equivalent to 99.68 gm. of free glutaminic acid, or 28.24 per cent of the protein.

The filtrate from the glutaminic acid hydrochloride was freed from water as completely as possible by evaporating under reduced pressure, and the residue esterified with alcohol and dry hydrochloric acid gas, as often described.

The esters were liberated and shaken out with ether, and the aqueous layer made strongly acid with hydrochloric acid, freed from inorganic salts, and the esterification repeated according to the usual procedure.

By distillation under diminished pressure the following fractions were obtained:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	100°	18 mm.	14.61 gm.
II	70°	0.38 "	47.44 "
III	100°	0.50 "	42.87 "
IV { A	170°	0.35 "	54.30 "
	B	200°	0.43 "
Total	. . . . .	. . . . .	169.43 gm.

The undistilled residue weighed 68 gm.

**Fraction I.**— From this fraction and from the ether distilled from the esters on the water bath at atmospheric pressure, there were obtained 0.87 gm. of pure glycocoll ester hydrochloride, which is equivalent to 0.47 gm. of glycocoll, or 0.13 per cent of the protein. The melting-point was 144°. The remainder of Fraction I consisted essentially of alanine, of which 3.6 gm. were isolated.

*Carbon and hydrogen*, 0.1011 gm. subst. gave 0.1502 gm. CO<sub>2</sub> and 0.0756 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.45 ; H 7.86 per cent.

Found . . . . = C 40.52 ; H 8.31 " "

**Fraction II.**— This fraction yielded by the customary methods 10.54 gm. of leucine and 1.08 gm. of alanine, while no valine was obtained.

The large quantity of proline contained in Fraction II was worked up conjointly with that from Fraction III. No glycooll could be brought to separation in this fraction as the ethyl ester hydrochloride.

**Fraction III.**—The yield of leucine was 11.71 gm. The substance decomposed at about  $298^{\circ}$  and gave the following analysis:

*Carbon and hydrogen*, 0.1755 gm. subst. gave 0.3524 gm.  $\text{CO}_2$  and 0.1575 gm.  $\text{H}_2\text{O}$ .

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

Found . . . . =  $\text{C } 54.76$ ;  $\text{H } 9.97$  " "

The proline from Fractions II and III, when freed as completely as possible from substances insoluble in absolute alcohol and dried to constancy over sulphuric acid, weighed 34.67 gm. By redissolving in absolute alcohol the substance separated in the characteristic prisms melting at about  $206^{\circ}$ .

*Carbon and hydrogen*, 0.1760 gm. subst. gave 0.3348 gm.  $\text{CO}_2$  and 0.1307 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_5\text{H}_9\text{O}_2\text{N} = \text{C } 52.18$ ;  $\text{H } 7.83$  per cent.

Found . . . . =  $\text{C } 51.88$ ;  $\text{H } 8.24$  " "

For identification the substance was converted to the copper salt and the lævo separated from the racemized with absolute alcohol in the usual way.

The racemic copper salt crystallized from water in the characteristic plates containing two molecules of water-of-crystallization.

*Water*, 0.1365 gm. subst. (air dry) lost 0.0146 gm.  $\text{H}_2\text{O}$  at  $110^{\circ}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2\text{Cu} \cdot 2 \text{H}_2\text{O} = \text{H}_2\text{O } 10.99$  per cent.

Found . . . . . =  $\text{H}_2\text{O } 10.86$  " "

*Copper*, 0.1177 gm. subst. dried at  $110^{\circ}$ , gave 0.0318 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2\text{Cu} = \text{Cu } 21.81$  per cent.

Found . . . . . =  $\text{Cu } 21.59$  " "

The lævo proline was converted to the characteristic phenylhydantoine. The melting-point was  $142^{\circ}$ .

*Carbon and hydrogen*, 0.2716 gm. subst. gave 0.6604 gm.  $\text{CO}_2$  and 0.1401 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2 = \text{C } 66.67$ ;  $\text{H } 5.57$  per cent.

Found . . . . =  $\text{C } 66.31$ ;  $\text{H } 5.72$  " "

**Fraction IV.**—The yield of phenylalanine hydrochloride from this fraction was 11.62 gm. The free substance was employed for the analysis.

*Carbon and hydrogen*, 0.1347 gm. subst. gave 0.3226 gm. CO<sub>2</sub> and 0.0833 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N = C 65.45; H 6.66 per cent.

Found . . . . = C 65.32; H 6.87 “ “

There were further isolated from this fraction 0.87 gm. of pure aspartic acid as the barium salt. The substance crystallized in the characteristic form, and reddened but did not decompose at 300°. Unfortunately no elementary analysis was obtained, as the preparation was lost. The filtrate from the barium aspartate was freed from barium and examined for glutamic acid. There were isolated 9.89 gm. of the hydrochloride. The free substance decomposed at about 203°.

*Carbon and hydrogen*, 0.2687 gm. subst. gave 0.4049 gm. CO<sub>2</sub> and 0.1524 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . = C 41.09; H 6.29 “ “

In the filtrate from the glutamic acid hydrochloride no copper salt of aspartic acid could be obtained.

There was further isolated from Fraction IV 0.2 gm. of pure serine. The substance browned at about 215° and decomposed at about 238°, with effervescence, to a brownish mass.

*Carbon and hydrogen*, 0.1212 gm. subst. gave 0.1536 gm. CO<sub>2</sub> and 0.0790 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>3</sub>N = C 34.29; H 6.67 per cent.

Found . . . . = C 34.56; H 7.24 “ “

#### TYROSINE.

Fifty grams of rye gliadin, equal to 42.98 gm. moisture, fat, and ash free, were hydrolyzed by heating with a mixture of 150 gm. sulphuric acid and 300 c.c. water for two and a half hours on a water bath and boiling for twelve hours on an oil bath. After removing the sulphuric acid with an equivalent quantity of baryta and boiling out the barium sulphate several times with water the solution was concentrated to crystallization. After standing over night the substance that had separated was recrystallized from

water, and 0.51 gm. of tyrosine in characteristic needles was obtained.

*Nitrogen*, 0.2045 gm. subst. required 1.63 c.c.  $5/7$  N—HCl = 0.0163 gm. N.

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

Found . . . . = N 7.91 " "

#### HISTIDINE, ARGININE, AND LYSINE.

Fifty grams of the rye gliadin, equal to 47.37 gm. moisture, fat, and ash free, were hydrolyzed and the bases determined as Kossel and Patten direct. The solution of the histidine was made up to 500 c.c. and nitrogen determined in 100 c.c. of it.

*Nitrogen*, 100 c.c. solution required 1.00 c.c.  $5/7$  N—HCl = 0.0100 gm.

N = 0.0500 gm. N in 500 c.c. = 0.1843 gm. histidine, or 0.39 per cent.

The solution containing the arginine was made up to 1000 c.c. and nitrogen determined in 50 c.c. of it.

*Nitrogen*, 50 c.c. solution required 1.50 c.c.  $5/7$  N—HCl = 0.0158 gm. N =

0.3160 gm. N in 1000 c.c. = 0.9809 gm. arginine. Adding 0.0720 gm.

for solubility of silver arginine gives 1.0529 gm. arginine, or 2.22 per cent.

The results of this hydrolysis are given in the following table, and for comparison are also given those which we have obtained with the other alcohol soluble proteins:

	Gliadin, Rye per cent	Gliadin, Wheat per cent	Hordein, Barley per cent	Zein, Maize per cent
Glycocoll . . . .	0.13	0.02	0.00	0.00
Alanine . . . .	1.33	2.00	0.43	2.23
Valine . . . .	not isolated	0.21	0.13	0.29
Leucine . . . .	6.30	5.61	5.67	18.60
Proline . . . .	9.82	7.06	13.73	6.53
Phenylalanine . . . .	2.70	2.35	5.03	4.87
Aspartic acid . . . .	0.25	0.58	not isolated	1.41
Glutaminic acid . . . .	33.81	37.33	36.35	18.28
Serine . . . .	0.06	0.13	not isolated	0.57
Tyrosine . . . .	1.19	1.20	1.67	3.55
Arginine . . . .	2.22	3.16	2.16	1.16
Lysine . . . .	0.00	0.00	0.00	0.00
Histidine . . . .	0.39	0.61	1.28	0.43
Ammonia . . . .	5.11	5.11	4.87	3.61
Tryptophane. . . .	present	present	present	0.00
Cystine . . . .	not determined	0.45	not determined	not determined
Total . . . .	64.31	65.81	71.32	61.53

The agreement between the analyses of the gliadin from wheat and rye is so close that the conclusion that differences exist between the preparations from these two seeds is not justified. Between hordein, zein, and gliadin, however, such distinct differences exist that, taken in connection with the differences in ultimate composition and properties, there can be no question that these are distinctly different proteins. These hydrolyses show that the alcohol-soluble proteins of the cereals form a distinctly characterized group which differ from all the other protein substances thus far analyzed. These differences are especially shown in their high content of proline, glutamic acid, and ammonia, and their low content of arginine and histidine and absence of lysine.<sup>3</sup> Zein is especially worthy of note, as it lacks glycocoll, lysine, and tryptophane, which are obtained from nearly all the other proteins.

<sup>3</sup> Cf. KOSSEL and KUTSCHER, *Zeitschrift für physiologische Chemie*, 1900, **xxx**i, p. 165.

## FURTHER DATA REGARDING THE CONDITION OF THE VASOMOTOR NEURONS IN "SHOCK."

BY W. T. PORTER AND W. C. QUINBY.

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

### I.

IN 1903 the writers pointed out that the quantitative method of Porter and Beyer<sup>1</sup> might be used to determine the condition of the vasomotor neurons in the symptom complex termed shock. The depressor nerve is afferent to the bulbar vasomotor centre. The fall in blood pressure produced by stimulating this nerve can be measured with exactness and its percentile value recorded. If this percentile value be as great, or almost as great, in shock as in the normal state, it is certain that the vasomotor cells concerned in the reaction are not "exhausted" or "depressed."

In the experiments reported very briefly in 1903 "the normal fall of blood pressure produced by stimuli of uniform intensity applied to the central end of the depressor nerve was measured in the rabbit and the cat. In the same animals shock was then brought on and the measurements repeated." It was found that the percentile fall in the blood pressure obtained during shock was little, if any, less than that obtained before shock appeared.

A fuller report has been reserved until certain studies of vasomotor reflexes carried on in this laboratory should be, at least in part, completed. These studies confirm our former statements.

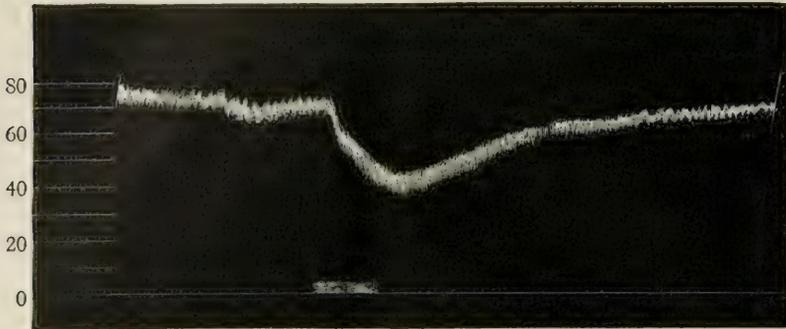
### II.

The methods employed in the experiments of 1903 and the results obtained are shown in the following protocols.

<sup>1</sup> W. T. PORTER and H. G. BEYER: This journal, 1900, iv, pp. 283-299.

Hour.	Operative procedures.	Rectal temperature.	Carotid blood pressure.		
			Before stimulation.	During stimulation.	Fall.
EXPERIMENT, SEPTEMBER 15, 1903.					
9.05	Rabbit tracheotomized.		mm. Hg.	mm. Hg.	per cent.
9.45	Left depressor nerve stimulated . . . . .	39	64	48	25
	Stimulation of depressor nerve . . . . .	..	66	48	27
	Stimulation of depressor nerve . . . . .	..	66	52	21
10.10	Sciatic nerves laid bare.				
10.15	Boiling water to hind limbs.				
10.25	Left leg thoroughly burned in Bunsen flame. The blood pressure rose.				
10.35	The other leg was burned.				
11.00	Depressor stimulation . . . . .	39	80	46	43
10.05	Depressor stimulation . . . . .	..	76	47	38
11.15	Opened abdomen; withdrew intestines; burned loops with saturated zinc sulphate and then with concentrated nitric acid.				
11.35	Depressor stimulation . . . . .	..	45	30	33
EXPERIMENT, SEPTEMBER 16, 1903.					
9.30	Spinal cord of rabbit exposed at I lumbar vertebra				
10.00	Stimulation of depressor nerve . . . . .	..	60	43	28
		..	60	42	30
		..	63	42	33
10.20	Stimulation of depressor nerve . . . . .	38	76	40	47
10.25	Thoroughly painted both hind limbs with concentrated nitric acid.				
11.15	No fall in blood pressure . . . . .	37			
	Section of spinal cord in lumbar region. Stimulation of central end of cord caused the blood pressure to rise.				
	Depressor stimulation . . . . .	..	38	25	24
	Depressor stimulation . . . . .	..	40	27	35
11.45	Injected 60 c.c. normal saline solution into external jugular vein; without effect on the blood pressure.				
11.48	Stimulation of depressor nerve . . . . .	..	35	25	29
EXPERIMENT, SEPTEMBER 24, 1903.					
9.00	Rabbit tracheotomized. Both vagicut. Blood pressure 80				
9.15	Stimulated depressor nerve . . . . .	38	67	36	46
9.20	Exposed intestines.				
9.30	Ligated mesenteric artery. Applied concentrated nitric acid to intestine; blood pressure rises.				
9.40	Stimulation of mesenteric nerves at mesenteric artery causes a fall followed by a rise in blood pressure.				
3.25	Rectal temperature 26°. No anæsthetic for many hours.				
	Stimulation of depressor nerve . . . . .	26	53	30	43
	Stimulation of depressor nerve . . . . .	..	53	30	43
4.50	Stimulation of depressor nerve . . . . .	..	40	22	45
5.16	Stimulation of depressor nerve . . . . .	25	35	23	34

In all these experiments the clinical signs of shock were present: the blood pressure was very low, the temperature was subnormal, the heart beat weak and often irregular, and the irritability of the nervous

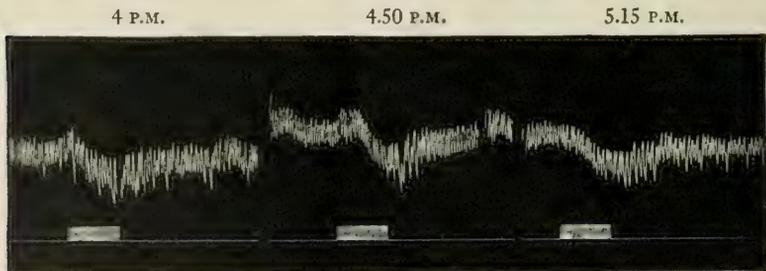


9.15 A.M. Rectal temperature, 38°. Speed, one centimetre in 24 seconds.

FIGURE 1.— The stimulation of the depressor nerve in the rabbit causes the blood pressure to fall 46 per cent (from 65 to 35 mm. Hg). Both vagi have been cut. Compare with Fig. 2 from the same rabbit about eight hours later.

system apparently much reduced, so that the animal required no anæsthetic in spite of the most serious injuries.

The conclusion that the vasomotor cells are not exhausted in the symptom complex termed shock is confirmed by the stimulation of



Rectal temperature, 26°. Speed, one centimetre in 24 seconds.

FIGURE 2.— From the same rabbit as Fig. 1, after lying about eight hours with exposed intestines painted with nitric acid. For more than two hours the rectal temperature had been 11° C. below normal. Stimulation of the depressor nerve lowered the blood pressure 43, 45, and 34 per cent respectively. The last figure will be 41 per cent if the measurement be taken from the lowest point in the curve.

the brachial, sciatic, and depressor nerves after gross injuries of the brain.<sup>2</sup> It is further confirmed by the analysis of 765 blood-pressure records obtained by stimulating these nerves after injuries to the

<sup>2</sup> W. T. PORTER and T. A. STOREY: This journal, 1907, xviii, pp. 181-199.

brain, section of the spinal cord, exposure and mechanical injury of the abdominal viscera, application of nitric acid and zinc sulphate to the peritoneum, cauterization of the skin of the limbs, hemorrhage, and section of the splanchnic nerves.<sup>3</sup>

### III.

In reflecting upon these experiments it is necessary to consider, first of all, whether the condition produced in the experimental animal was really shock. As to the symptoms, or rather the signs, of shock, there is general agreement, and it should be easy to determine how far the condition here produced is open to criticism.

Clinicians usually state that the fall in blood pressure is the most significant as well as the most constant symptom of shock. Opinion as to the extent to which the blood pressure must fall to bring the case within the category of shock can be gained by taking the average of the observations made by a clinician who has experimented upon this subject. In the first fifty pages of a recent treatise<sup>4</sup> are recorded twenty-eight experiments on dogs in which the blood pressure at the beginning of the experiment averaged 132 mm., while the blood pressure after shock is said to be present averaged 57 mm. Hg.<sup>5</sup> It is evident that in our experiments the blood pressure was even lower than that usually taken to be symptomatic of shock.

Again, it is sometimes urged that in shock the blood pressure falls instead of rising on stimulation of afferent nerves. This abnormal reaction was observed in several of our experiments.<sup>6</sup>

Finally, it may be objected, by those who are not well versed in experimental physiology, that the symptoms of shock in the cat or rabbit cannot have the diagnostic value of the identical symptoms in man, because of the differences between man and these lower animals. These differences are marked indeed, but they should not be made the

<sup>3</sup> W. T. PORTER: This journal, 1907, xx, pp. 399-405.

<sup>4</sup> G. W. CRILE: Blood pressure in surgery, 1903.

<sup>5</sup> The initial blood pressure was mentioned in nineteen instances and the blood pressure during shock in twenty-five.

<sup>6</sup> Even a fall would indicate that the vasomotor cells were not exhausted, though it would point to a disturbance of their normal equilibrium. This fall, however, often occurs when the blood pressure is at the normal level and when signs of shock are absent. \* It can be produced by strychnine, chloral, or curare.

basis of a hasty generalization. It is conceded that skilled movements, for example, are much more highly developed in man and in the anthropoid apes than in such animals as the rabbit and cat. But experience suggests that the maintenance of blood pressure, like the respiration, belongs to those fundamental functions that are singularly alike in all the higher animals. As this point is vital to the application of our experiments, we are glad to present the conclusions reached in a comparative study of the blood pressure about to be published by Dr. Porter and Mr. Richardson. The same electrical stimulus was applied to the sciatic and the brachial nerves in the dog, cat, rabbit, guinea pig, rat, and hen, and the rise in blood pressure recorded. It is found that the reaction is quantitatively very similar in these widely separated types. In the cat, rabbit, rat, and hen it is, in fact, identical. As the difference in structure between the cat and the hen, for example, is greater than the difference between the cat and man, it would seem safe to conclude that the vasomotor reactions in man are essentially like those in other high mammals.<sup>7</sup>

There can indeed be no question that the experimental animals in the several investigations reported from this laboratory exhibited the clinical picture termed shock, and unquestionably the hundreds of measurements we have now collected are evidence enough that the vasomotor cells in these animals were neither exhausted nor depressed.

#### IV.

It will be noted that this paper deals with the symptoms of shock rather than with shock itself. The distinction is important. The symptoms of shock are a clinical entity about which there can be little dispute; shock, on the contrary, is a pathological state, the data of which are at present hypothetical.

The hypothesis which constitutes the hitherto generally accepted definition of shock declares that the vasomotor cells are depressed, exhausted, or inhibited by excessive stimulation of afferent nerves; the fall in blood pressure and the accompanying symptoms are the result of this depression. The experiments cited in this paper demonstrate that the vasomotor cells are not thus depressed or inhibited, and

<sup>7</sup> It is noteworthy that of all these animals the dog is found to be the least adapted for vasomotor studies.

experiments published in the last number of this journal<sup>8</sup> show that excessive stimulation of afferent nerves does not materially lessen the blood pressure. The present hypothetical basis of shock is thus removed.

The thoughtful reader will hardly quarrel with this conclusion; he will remember that there is as yet no evidence that either the respiration or the temperature can long be altered by afferent impulses.

<sup>8</sup> W. T. PORTER, H. K. MARKS, and J. B. SWIFT, JR.: This journal, 1907, xx, pp. 444-449.



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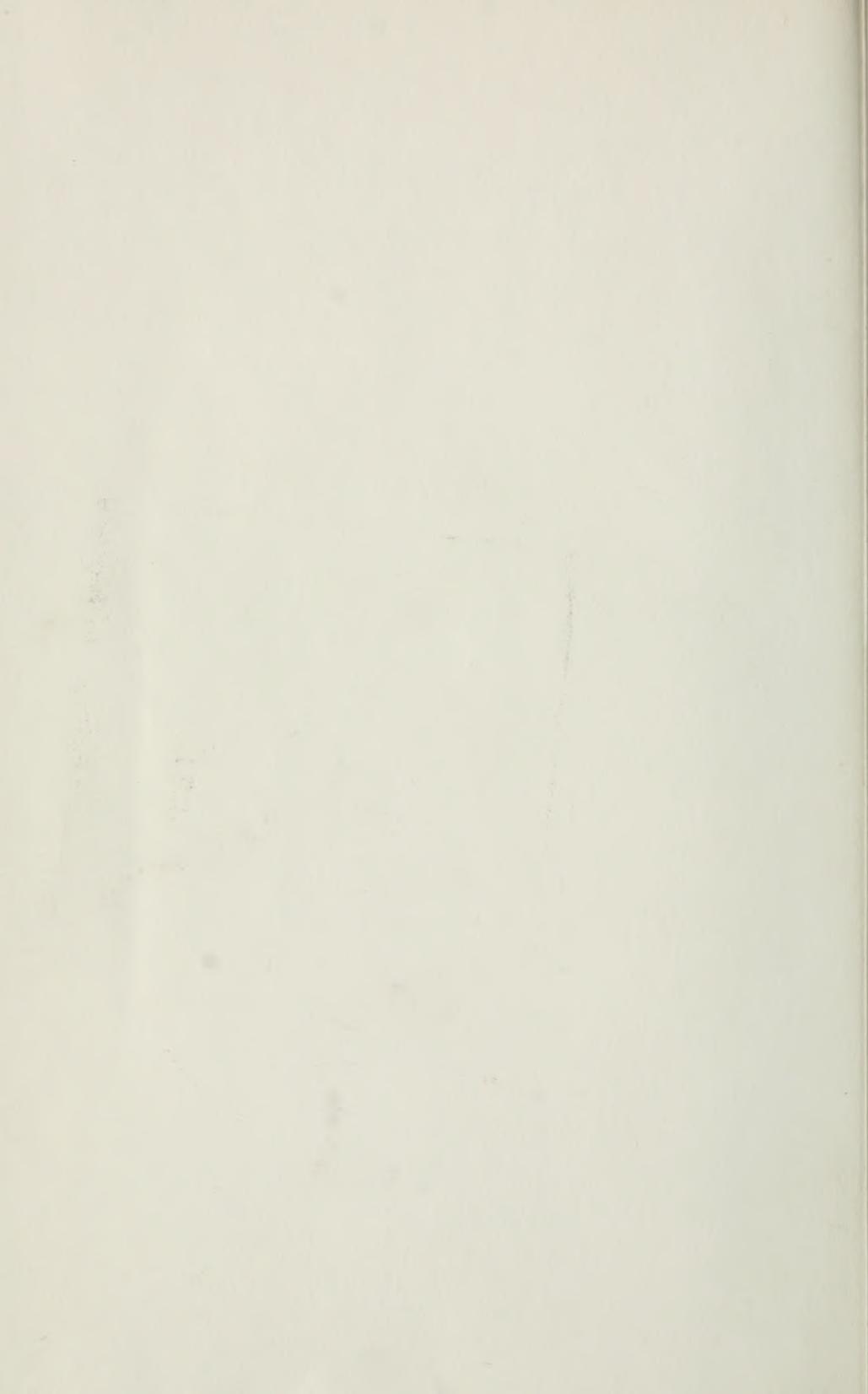
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